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The Editorial Board kindly informs that since 2014 *Nowiny Lekarskie* has been renamed to *Journal of Medical Science*.

The renaming was caused by using English as the language of publications and by a wide range of other organisational changes. They were necessary to follow dynamic transformations on the publishing market. The Editors also wanted to improve the factual and publishing standard of the journal. We wish to assure our readers that we will continue the good tradition of *Nowiny Lekarskie*.

You are welcome to publish your basic, medical and pharmaceutical science articles in *Journal of Medical Science*.

Ethical guidelines

The Journal of Medical Science applies the ethical principles and procedures recommended by COPE (Committee on Conduct Ethics), contained in the Code of Conduct and Best Practice Guidelines for Journal Editors, Peer Reviewers and Authors available on the COPE website: https://publicationethics.org/resources/guidelines

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ORIGINAL PAPER

JMS Journal of Medical Science

Two-Stage Operations in Patients with Acute Right-sided Colonic Obstruction: a 15-year Single Institution Experience

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ABSTRACT

Aim. The aim of the study was to review the characteristics, surgical outcomes, complications, and long-term outcomes of two-stage operations for acute right-sided colonic obstruction (RSCO) in a single institution.
Summary background data. Although patients with acute RSCO can be treated by resection of the tumor with a primary anastomosis, longer procedure times and bigger wounds can result in more mortality and complications. A two-stage operation by diverting loop ileostomy was another surgical option. However, the outcomes of two-stage operations are lacking.
Material and methods. The retrospective study reviewed the patients who underwent emergency surgery for acute RSCO in a tertiary center from 2004-2018. First-stage operations other than diverting loop ileostomy, incomplete obstructions that could be treated medically, or pathologies other than adenocarcinomas were excluded. Perioperative data such as first and second operations, operative times, lengths of stay, harvested lymph nodes, and any complications were included. We assessed overall survival (OS) and disease-free survival (DFS) for the oncologic outcomes.

Results. Sixty-nine patients were included. Seven patients had surgical complications related to ileostomy. Three of them died within 30 days of first admission. Thirty-one patients received a second-stage operation by right hemicolectomy. None had anastomosis leakage or 30-day mortality. Only 9.7% of patients had fewer than 12 harvested lymph nodes. One-year OS was 34% in the patients who received only ileostomy, and 89% in those who received two-stage operations (P < 0.001). Among 26 patients who underwent curative resection of tumor, 3-year DFS was 61.5%.
Conclusions. A two-stage operation is associated with low mortality and morbidity rates in an emergency setting. The subsequent right hemicolectomy can harvest more lymph nodes than emergency resection. Long-term survival benefits can be expected.

Introduction

Colorectal cancer (CRC) is a very common disease. It is the third most commonly diagnosed malignancy worldwide, with 1.8 million new cases and almost 861,000 deaths in 2018 according to the World Health Organization GLOBOCAN database. The incidence of CRC in eastern Asia in 2018 was 3.06% [1]. Although most patients with CRC have no symptoms and are diagnosed as a result of screening, 7–47% present with colonic obstruction as the first diagnosis [2]. Among these cases, about 20–30% patients are of right-sided CRC [3,4]. For such cases, surgical options include resection of the tumor with a primary anastomosis with or without a temporary proximal diversion, resection without an anastomosis and with an end colostomy, or proximal diversion with a colostomy to stabilize the patient, followed by elective definitive resection at a second operation and, occasionally, self-expandable metallic stent (SEMS) placement.

Because of the lower bacterial counts [5] and better blood supply to the small intestine [6], most patients with right-sided colonic obstruction can be treated by resection of the tumor with a primary anastomosis [5,6]. A research compared the surgical interventions and outcomes for patients with right-sided colonic obstruction. Surgical interventions in the research including resection with primary anastomosis, resection with primary anastomosis and loop ileostomy resection without anastomosis, defunctioning ileostomy, and by-pass. Postoperative mortality and morbidity were similar between patients who underwent an ileostomy at initial surgical stage and those who underwent colectomy with primary anastomosis [7]. However, these patients are commonly old, often have some comorbidities and a period of poor nutrient intake. Longer procedure times and bigger wounds can result in more complications. Even the anastomosis itself can have leakage rates of 4.2–10% [8,9] which may lead to the need for further surgery and increase patient mortality [8,10,11].

Resection without an anastomosis avoids the risk of anastomotic leakage, but the procedure takes more time than two-stage procedures such as SEMS placement and diverting loop ileostomy. According to ASCO resource-stratified guideline, diverting ostomy was recommended in patients with late-stage colorectal cancer associated with obstruction from primary tumor or from peritoneal metastases [12]. To minimize morbidity and mortality, most patients with a right-sided colonic obstruction receive diverting loop ileostomy as the first-stage emergency operation in our hospital. The aim of this study is to review the characteristics, surgical outcomes, complications, and outcomes of such cases.

Material and methods

Patients

From January 2004 to December 2018, all patients who underwent emergency surgery for acute obstructive right-sided CRC in the Tri-Service General Hospital, Taipei, Taiwan, were reviewed retrospectively. The right colon was defined as including the proximal two-thirds of the transverse colon, the ascending colon, and cecum by abdominal and pelvic computed tomography (CT) scans. Acute obstruction was defined based on clinical findings (abdominal pain, bloating, nausea or vomiting, and absence of flatus and/or bowel movement) and CT findings (tumor obstruction with proximal colon and/or small bowel dilatation). Emergency surgery was defined

as the need to receive surgical intervention within 24 h of admission.

First-stage operations other than diverting loop ileostomy, incomplete obstructions that could be treated medically, or pathologies other than adenocarcinomas were excluded.

This study was reviewed and approved by the Tri-Service General Hospital institutional review board for human subjects (No. 1–108–05–038).

Procedures

Patient characteristics such as age, gender, American Society of Anesthesiologists (ASA) score, body mass index, and comorbidities were recorded. All operations were performed by seven colorectal surgeons in our tertiary referral hospital. Clinical stage was determined by preoperative CT scans.

The surgical approach was a joint decision between the surgeons and the oncologists. Decision making depended on location of the tumor, patient factors, surgeon's expertise, and the available resources [13]. To complicated cases, they would be discussed in Tri-Service General Hospital Cancer Committee, which was composed of multidisciplinary teams.

Surgery of diverting loop ileostomy was performed with steps of making a transverse incision at the right lateral border of rectus abdominis muscle, dividing the anterior rectus sheath, rectus abdominis muscle and posterior sheath, delivering the terminal ileum into the wound outside the peritoneal cavity, forming a small hole at the omentum and the mesenteric border of the terminal ileum, opening the terminal ileum, and matured to the skin.

Another group of patients received a second operation of right hemicolectomy for resection of the tumor. Further right hemicolectomy could be performed as the colon without distension and the patient's general condition keeping stabilized [14]. The optimal time interval between diverting ileostomy and right hemicolectomy was decided by the surgeon.

Pathology stage was reported by pathologists according to the seventh edition of the American Joint Committee on Cancer. Perioperative data included first and second operations, first and second operative times, first and second lengths of stay, and any complications. Overall survival (OS) was determined by the patient's status at the last visit. Disease-free survival (DFS) was defined by the length of time the patient survived after right hemicolectomy without evidence of cancer recurrence at follow-up.

Endpoint

The primary endpoints in the study included mortality, surgical complications, first and second lengths of stay, and the length of time the patient survived after right hemicolectomy without evidence of cancer recurrence.

Statistical analysis

Quantitative data are reported as medians and 25^{th} and 75^{th} percentiles. Categorical data are reported as absolute numbers and percentages. Patients who received only ileostomy and ileostomy plus right hemicolectomy were divided into two groups. For these cases, quantitative data were analyzed using the Mann–Whitney nonparametric *U* test. Categorical data were compared using chi-squared or Fisher's exact tests, as appropriate. OS and DFS were estimated using the Kaplan–Meier method. Log-rank analysis was used to determine statistical significance. A P value < 0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics for Windows (Version 25.0; IBM Corp., Armonk, NY, USA).

Results

In total, 69 of 70 patients receiving emergency diverting loop ileostomy over the 15 years of the study were included in the analysis; one patient who underwent exploratory laparotomy with right hemicolectomy was excluded. The median age of the patients was 77 years (25^{th} and 75^{th} percentiles: 64.5 and 83.5 years, respectively). Thirty-nine (56%) patients were male. Forty-six patients had an ASA score of III-V. Fifty of the 69 cases had major comorbidities. The median operation time for diverting ileostomy was 54 min (42.5 and 77.5 min), and the median length of stay was 9 days (7 and 15.5 days; **Table 1**).

Among the 69 patients, 31 received a second operation for resection of the tumor (**Figure 1**). **Table 2** shows the characteristics of the two groups of patients. The median age of the patients who received right hemicolectomy

Table 1. Characteristics

	Patients (n = 69) at 1st admission
Age(years)	77(64.5;83.5)
Male(%)	39(56.5)
BMI(kg/m^2) (n = 57*)	22.1(19.6;24.4)
ASA class**	
I-II	23
III-IV	45
V	1
Clinical stage	
I	0
II	12
III	23
IV	34
Comorbidities	50
HCVD	32
DM	17
CAD	6
Renal disease	8
TB	2
COPD	4
Old CVA	10
Arrhythmia	9
Parkinsonism	4
Other cancer	4 (HCC; Sqcc of penis; Prostate Ca; Breast Ca)
Operation time of ileostomy (mins)	54(42.5;77.5)
30-day Mortality	3
Length of stay	9(7;15.5)

* 11 patients' BMI couldn't be counted.
BMI – Body mass index, ASA class – American Society of Anesthesiologists Classification, HCVD – Hypertensive cardiovascular disease, DM – Diabetes mellitus, CVA – Cerebrovascular accident, CAD – Coronary artery disease, HCC – Hepatocellular carcinoma, Sqcc – Squamous cell carcinoma, Ca – Cancer.

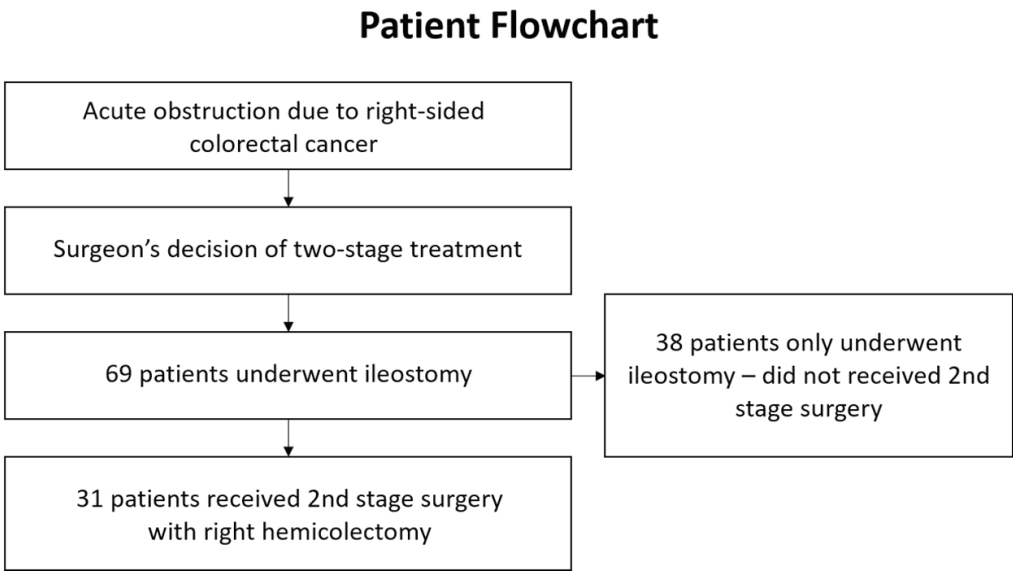

Figure 1. Among the 69 patients, 31 received a second operation for resection of the tumor. The other 38 patients received only ileostomy.

Table 2. Characteristics between two groups of patients.

	Only ileostomy (n = 38)	Ileostomy+right hemicolectomy (n = 31)	*P* value
Age (y)	80.5 (75;88.25)	68 (59;78)	<0.001
Male (%)	20 (52.6)	19 (61.3)	0.470
BMI (kg/m^2)	21.1 (19.3;24.0) (n = 29)	22.9 (20.6;24.9) (n = 28)	0.127
ASA class at 1st admission			0.156
I-II	10	13	
III-IV	28	17	
V	0	1	
Clinical stage			0.017
II	3	9	
III	11	12	
IV	24	10	
Pathologic stage			
II	N/A	8	
III	N/A	13	
IV	N/A	10	
Mean number of harvested LNs	N/A	17 (13;21)	
Comorbidities			
HCVD	16	16	
DM	9	8	
CAD	2	4	
Renal disease	3	5	
TB	1	1	
COPD	0	4	
Old CVA	4	6	
Arrhythmia	4	5	
Parkinsonism	3	1	
Other cancer	2 (Prostate Ca; Breast Ca)	2 (Sqcc of penis; HCC)	0.465
Operation time for ileostomy (mins)	55.5 (43;79.5)	51 (39;77)	
Operation time for right hemicolectomy (mins)	N/A	236 (199.75;274.25)*	0.039
Length of stay(1st) (days)	10.5 (7;21)	8 (6;12)	
Length of stay(2nd) (days)	N/A	9.5 (7;13.25)*	

* One of the patients underwent right hemicolectomy at other hospital.
BMI – Body mass index, ASA class – American Society of Anesthesiologists Classification, LNs – Lymph nodes, HCVD – Hypertensive cardiovascular disease, DM – Diabetes mellitus, CAD – Coronary artery disease, TB – Tuberculosis, COPD – Chronic obstructive pulmonary disease, CVA – Cerebrovascular accident, Ca – Cancer, Sqcc – Squamous cell carcinoma, HCC – Hepatocellular carcinoma.

was 68 years (25th and 75th percentiles: 59 and 78 years, respectively), which was significantly younger than those who received only ileostomy ($P < 0.001$). The patients who received only ileostomy had more advanced clinical cancer stages ($P < 0.017$) and longer hospital stays for ileostomy (medians: 10.5 vs. 8 days; $P < 0.039$). However, no significant difference was found in the operation time for ileostomy between the two groups (55.5 vs. 51 mins). Most patients in both groups had major comorbidities.

Seven patients had surgical complications related to ileostomy, including ileostomy prolapse, parastomal hernia, pneumonia, and 30-day Mortality. Six of these received only ileostomy. The other patient who suffered an ileostomy prolapse subsequently received right hemicolectomy. Three of the patients died within 30 days of first admission. All of these patients had clinical stage IV colon cancer. Two of the patients died from nosocomial pneumonia, and the other one died from acute myocardial infarction (**Table 3**).

For patients who received a second operation, only three of them had surgical complications related to right hemicolectomy; none had anastomosis leakage or 30-day mortality (**Table 4**).

The mean follow-up time was 8.37 ± 1.14 months in the patients who received only diverting loop ileostomy, and 109.11 ± 13.42 months in those who received two-stage operations. Dur-

Table 3. Surgical complications of ileostomy.

	Only ileostomy (38)	Ileostomy+right hemicolectomy (31)
Parastomal hernia	1	0
Ileostomy prolapse	1	1
Pneumonia	1	0
30-day Mortality	3	0

Table 4. Surgical complications of right hemicolectomy (n = 31).

Pneumonia	1
Wound infection	1
Hernia	1
Anastomosis leakage	0
30-day Mortality	0

ing follow-up, 19 of 38 patients who received only diverting ileostomy and seven of 31 who received two-stage operations died. The median survival time was 7.51 months for patients who received only ileostomy; this end point was not reached for the other group of patients. One-year OS was 34% vs. 89%, respectively, between the two groups (*P* < 0.001; **Figure 2**).

Thirty-one patients received a second-stage operation by right hemicolectomy (four by laparoscopic surgery). In three of the patients, fewer than 12 lymph nodes were harvested. The mean number of harvested lymph nodes was 17 (13 and 21, respectively). Comparing tumor stages II, III, and IV, the 1-year OS rates were 100%, 90.9%, and 87.5%, respectively, and the 3-year OS rates were 100%, 70.1%, and 62.5%, respectively. No significant difference was seen in OS between the two groups (*P* = 0.211); however, a trend was observed (**Figure 3**). Among the patients, 26 underwent curative resection of tumor and nine had a tumor recurrence. Three-year DFS was 61.5%. The median DFS was not reached during the follow-up period (**Figure 4**). Comparing tumor stages II, III, and IV, the 3-year DFS rates were 100%, 57.8%, and 20%, respectively. DFS was significantly different between patients with stage II and IV tumors (*P* = 0.017; **Figure 5**).

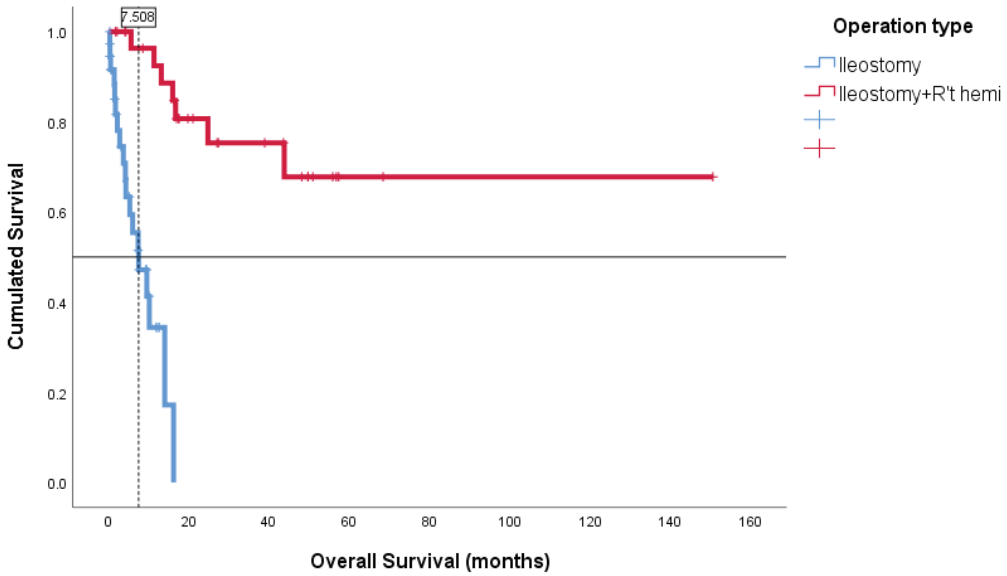

Figure 2 The median survival time was 7.51 months for patients who received only ileostomy; One-year OS was 34% in the patients who received only ileostomy, and 89% in those who received two-stage operations (*P* < 0.001). R't hemi – right hemicolectomy.

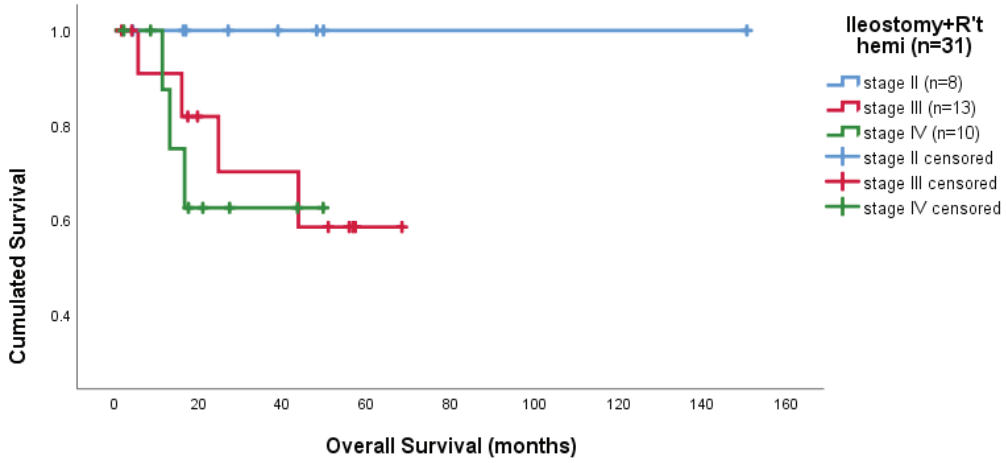

Figure 3. Comparing tumor stages II, III, and IV, no significant difference was seen in OS between the two groups (*P* = 0.211). R't hemi – right hemicolectomy.

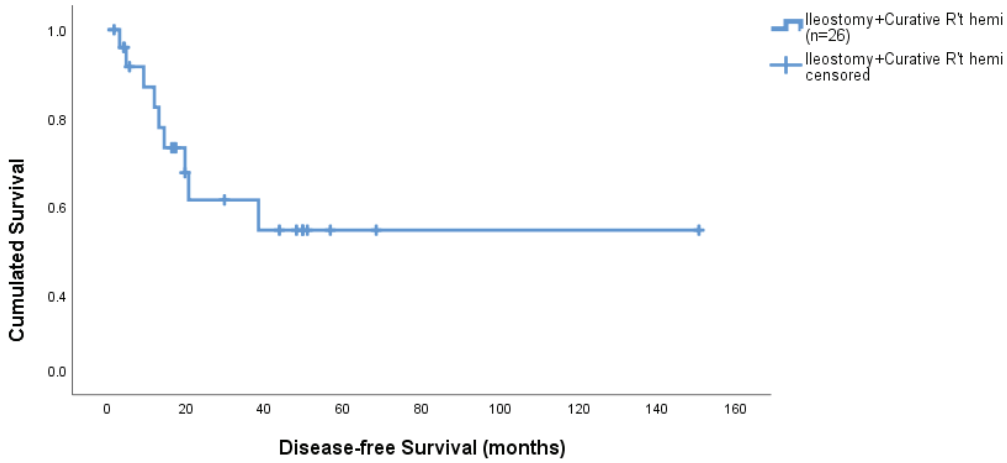

Figure 4. Three-year DFS of ileostomy and curative right hemicolectomy group patients was 61.5%, n = 26. R't hemi – right hemicolectomy.

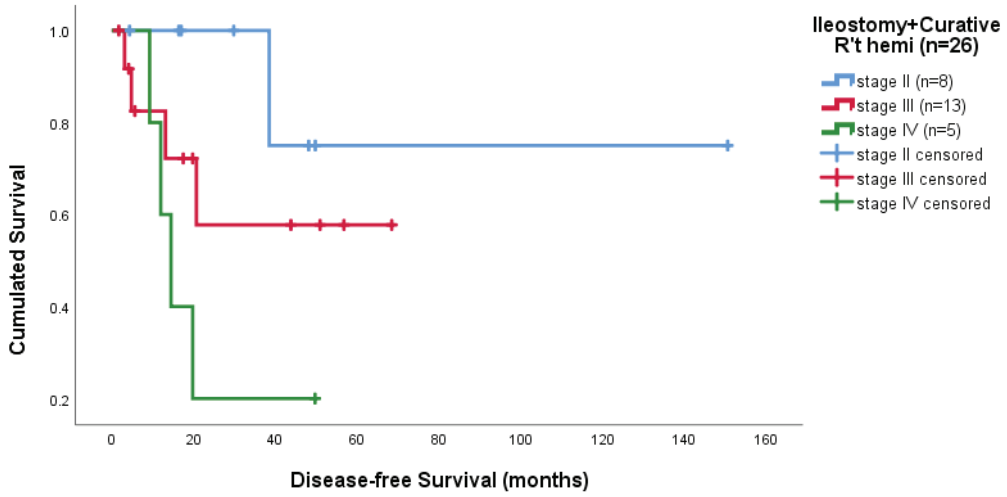

Figure 5. Comparing tumor stages II, III, and IV, DFS was significantly different between patients with stage II and IV tumors (*P* = 0.017). R't hemi – right hemicolectomy.

Discussion

Elderly patients are known to have high rates of emergency colorectal surgery for tumor obstructions or perforations [15]. The median age of the patients in our study was 77 years, consistent with the literature. Patients who have obstructive CRCs also have more comorbidities and higher ASA tumor scores than those who have nonobstructive CRCs [2,16]. In the present study, 46 of the 69 patients had ASA tumor scores of III-V at first admission, and 50 had major comorbidities. These data indicate the fragility of our patients.

Although some reports have advocated the benefits of palliative resection of the primary colon tumor for patients with unresectable metastatic CRCs, this remains controversial [17–19]. In the present study, 38 of the 69 patients received only ileostomy. It is not surprising that most of these patients had an unresectable metastatic CRC. Fourteen patients in this series had clinical stage II or III CRCs and did not receive curative treatment. The reasons for this included major comorbidities, poor performance status, incurable second cancers, and old age.

Unlike left-sided tumor obstructions, acute tumor obstructions in the right colon are usually treated by resection with a primary ileocolic anastomosis. However, the reported leakage rate is 4.3–16.4%, which could lead to death in some patients [2,3,20,21]. In the literature, the mortality rate for emergency surgery for proximal colon tumor obstruction ranges from 10.1 to 14.7% [3,21,22]. Although our patients had higher ASA tumor grades and more comorbidities compared with those in other reports [3,22], the surgical complications rate, including the mortality rate for first-stage operations, was extremely low. There are some possible reasons for this. First, we avoided prolonged operation times in emergency settings. The median operation time for an ileostomy was <1 h, which is shorter than that needed for resection of the obstructed tumor. Second, to make an ileostomy, we only need to create a small incision. Compared with the long midline incisions needed for laparotomy, an incision at the lower right quadrant of the abdomen is less painful. Third, we did not perform an anastomosis, meaning that we avoided the risk of anastomotic leakage, which could lead to the need for another operation, prolong the length of hospital stay, and increase the mortality rate.

The surgical complications of the second operation in this study were minor. No anastomotic leakage or 30-day mortality was found, even though 18 of 31 tumors had ASA scores of III or IV at the first operation. The patients could build up their nutrition, stabilize vital signs, and have better circulation during the interval between operations. All of these factors probably improved the outcomes of the second operations.

Emergency resection for an obstructing CRC can make it difficult to harvest a sufficient number of lymph nodes because of the dilated proximal bowel and limited surgical field. In the literature, 19.3–19.8% of patients had inadequate numbers of harvested lymph nodes [2,23]. This could lead to tumor understaging and poorer prognosis, especially among elderly patients [24,25]. In our study, 31 patients received two-stage operations. Less than 10% of patients had fewer than 12 harvested lymph nodes, and 74% had lymph node invasion, which is higher than that in previous reports [2,26]. Although the case number was small, four patients received laparoscopic right hemicolectomy with D3 lymphadenectomy at the second operation. Better oncological and cosmetic outcomes can be expected compared with emergency laparotomy.

It is not surprising that the patients who received only diverting loop ileostomy had significantly shorter OS. Even though 24 of 38 patients had a clinical stage IV CRC, their OS was shorter than that reported previously [27]. This result could be explained by the old age and multiple comorbidities of these patients. The patients who received two-stage operations had longer OS. Although five patients who had unresectable metastatic CRCs received a right hemicolectomy, the OS showed no significant difference among patients with stage II, III, or IV tumors. This result may imply the benefit of resecting primary tumors, even though the case number was small [17].

The patients who received curative two-stage operations had better DFS than that reported in the literature [28]. This may be explained by our use of elective curative surgery and adequate numbers of harvested lymph nodes. However, some patients who were too weak to undergo further surgery were excluded. Thus, the long-term

survival benefit of two-stage operations remains to be proven by further prospective studies.

Self-expandable metal stent (SEMS) used for obstructive CRCs as a bridge to surgery help avoid the need for emergency surgery and reduce the risk of postoperative complications and mortality. It seems to have the same benefits as diverting loop ileostomy and avoids the creation of a stoma. However, SEMS insertion for right-sided tumor obstruction is associated with a higher technical failure rate than that for left-sided tumor obstruction [29,30]. Once perforation occurs, the patients' oncologic outcome will be significantly worse.31 It must be noted that the stent procedure can increase the numbers of circulating tumor cells by compressing the tumor, and this is related to worse oncologic outcomes [32,33].

Our study had several limitations. First, it was a retrospective cohort analysis, and all cases were from a single tertiary center, which could have introduced bias in this 15-year series. Second, most of the patients with an acute right-sided colonic obstruction in our hospital received two-stage operations. Thus, we could not compare the outcomes between emergency resection and two-stage operations in our institute. Third, only 69 patients were included in our study, and this small sample size might have caused some bias.

Conclusions

Although a two-stage operation by diverting loop ileostomy is not usually the first procedure considered for acute right-sided colonic obstruction because of the need to create a temporary stoma, it is associated with low mortality and morbidity rates in an emergency setting. Diverting loop ileostomy associates with few oncologic adverse outcomes, and moreover, the subsequent right hemicolectomy harvests a large number of lymph nodes. Long-term survival benefits can be expected from this approach.

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Conflict of interest statement
The authors declare no conflict of interest.

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References

1. The Global Cancer Observatory. 2019. Available at: https://gco.iarc.fr/today/data/factsheets/cancers/10_8_9-Colorectum-fact-sheet.pdf.
2. Faucheron JL, Paquette B, Trilling B, Heyd B, Koch S, Mantion G. Emergency surgery for obstructing colonic cancer: a comparison between right-sided and left-sided lesions. Eur J Trauma Emerg Surg. 2018;44:71-7.
3. Aslar AK, Ozdemir S, Mahmoudi H, Kuzu MA. Analysis of 230 cases of emergent surgery for obstructing colon cancer--lessons learned. J Gastrointest Surg. 2011;15:110-9.
4. Pisano M, Zorcolo L, Merli C, Cimbanassi S, Poiasina E, Ceresoli M, Agresta F, Allievi N, Bellanova G, Coccolini F, Coy C, Fugazzola P, Martinez CA, Montori G, Paolillo C, Penachim TJ, Pereira B, Reis T, Restivo A, Rezende-Neto J, Sartelli M, Valentino M, Abu-Zidan FM, Ashkenazi I, Bala M, Chiara O, De' Angelis N, Deidda S, De Simone B, Di Saverio S, Finotti E, Kenji I, Moore E, Wexner S, Biffl W, Coimbra R, Guttadauro A, Leppaniemi A, Maier R, Magnone S, Mefire AC, Peitzmann A, Sakakushev B, Sugrue M, Viale P, Weber D, Kashuk J, Fraga GP, Kluger I, Catena F, Ansaloni L. 2017 WSES guidelines on colon and rectal cancer emergencies: obstruction and perforation. World J Emerg Surg. 2018;13:36.
5. Lyra A, Forssten S, Rolny P, Wettergren Y, Lahtinen SJ, Salli K, Cedgard L, Odin E, Gustavsson B, Ouwehand AC. Comparison of bacterial quantities in left and right colon biopsies and faeces. World J Gastroenterol. 2012;18:4404-11.
6. Cuffy M, Abir F, Audisio RA, Longo WE. Colorectal cancer presenting as surgical emergencies. Surg Oncol. 2004;13:149-57.
7. Mege D, Manceau G, Beyer-Berjot L, Bridoux V, Lakkis Z, Venara A, Voron T, Brunetti F, Sielezneff I, Karoui M; AFC (French Surgical Association) Working Group. Surgical management of obstructive right-sided colon cancer at a national level results of a multicenter study of the French Surgical Association in 776 patients. Eur J Surg Oncol. 2018 Oct;44(10):1522-1531.
8. Gessler B, Eriksson O, Angenete E. Diagnosis, treatment, and consequences of anastomotic leakage in colorectal surgery. International journal of colorectal disease. 2017;32:549-56.
9. Nors J, Sommer T, Wara P. Leakage Rate After Laparoscopic Ileocolic Intracorporeal Anastomosis. J Laparoendosc Adv Surg Tech A. 2018;28:1287-93.
10. Vasiliu EC, Zarnescu NO, Costea R, Neagu S. Review of Risk Factors for Anastomotic Leakage in Colorectal Surgery. Chirurgia (Bucharest, Romania: 1990). 2015;110:319-26.
11. Nasiri S, Mirminachi B, Taherimehr R, Shadbakhsh R, Hojat M. The Effect of Omentoplasty on the Rate of Anastomotic Leakage after Intestinal Resection: A Randomized Controlled Trial. The American Surgeon. 2017;83:157-61.
12. Chiorean, E. Gabriela, et al. Treatment of patients with late-stage colorectal cancer: ASCO resource-strat-

ified guideline. JCO Global Oncology 6; 2020: 414-438.
13. Rajan R, Clark DA. Current management of large bowel obstruction: a narrative review. Ann Laparosc Endosc Surg 2022;7:23.
14. Gainant, A. Emergency management of acute colonic cancer obstruction. Journal of Visceral Surgery. 2012, 149.1: e3-e10.
15. Basili G, Lorenzetti L, Biondi G, Preziuso E, Angrisano C, Carnesecchi P, Roberto E, Goletti O. Colorectal cancer in the elderly. Is there a role for safe and curative surgery? ANZ J Surg. 2008;78:466-70.
16. Finan PJ, Campbell S, Verma R, MacFie J, Gatt M, Parker MC, Bhardwaj R, Hall NR. The management of malignant large bowel obstruction: ACPGBI position statement. Colorectal Dis. 2007 Oct;9 Suppl 4:1-17.
17. Ishihara S, Nishikawa T, Tanaka T, Tanaka J, Kiyomatsu T, Kawai K, Hata K, Nozawa H, Kazama S, Yamaguchi H, Sunami E, Kitayama J, Sugihara K, Watanabe T. Benefit of primary tumor resection in stage IV colorectal cancer with unresectable metastasis: a multicenter retrospective study using a propensity score analysis. Int J Colorectal Dis. 2015 Jun;30(6):807-12.
18. Ishihara S, Hayama T, Yamada H, Nozawa K, Matsuda K, Miyata H, Yoneyama S, Tanaka T, Tanaka J, Kiyomatsu T, Kawai K, Nozawa H, Kanazawa T, Kazama S, Yamaguchi H, Sunami E, Kitayama J, Hashiguchi Y, Sugihara K, Watanabe T. Prognostic impact of primary tumor resection and lymph node dissection in stage IV colorectal cancer with unresectable metastasis: a propensity score analysis in a multicenter retrospective study. Ann Surg Oncol. 2014;21:2949-55.
19. Lee WS, Baek JH, Kang JM, Choi S, Kwon KA. The outcome after stent placement or surgery as the initial treatment for obstructive primary tumor in patients with stage IV colon cancer. Am J Surg. 2012;203:715-9.
20. Lee YM, Law WL, Chu KW, Poon RT. Emergency surgery for obstructing colorectal cancers: a comparison between right-sided and left-sided lesions. J Am Coll Surg. 2001;192:719-25.
21. Frago R, Biondo S, Millan M, Kreisler E, Golda T, Fraccalvieri D, Miguel B, Jaurrieta E. Differences between proximal and distal obstructing colonic cancer after curative surgery. Colorectal Dis. 2011;13:e116-22.
22. Biondo S, Pares D, Frago R, Marti-Rague J, Kreisler E, De Oca J, Jaurrieta E. Large bowel obstruction: predictive factors for postoperative mortality. Diseases of the colon and rectum. 2004;47:1889-97.
23. Chin CC, Wang JY, Changchien CR, Huang WS, Tang R. Carcinoma obstruction of the proximal colon cancer and long-term prognosis--obstruction is a predictor of worse outcome in TNM stage II tumor. International journal of colorectal disease. 2010;25:817-22.
24. Babcock BD, Aljehani MA, Jabo B, Choi AH, Morgan JW, Selleck MJ, Luca F, Raskin E, Reeves ME, Garberoglio CA, Lum SS, Senthil M. High-Risk Stage II Colon Cancer: Not All Risks Are Created Equal. Ann Surg Oncol. 2018;25:1980-5.
25. Reha J, Mukkamalla SKR, Rathore R, Somasundar PS. Adequate lymph node evaluation in the elderly is associated with improved survival in patients with stage I-III colon cancer: A validation study using the National Cancer Data Base. Eur J Surg Oncol. 2018;44:148-56.
26. Yang Z, Wang L, Kang L, Xiang J, Peng J, Cui J, Huang Y, Wang J. Clinicopathologic characteristics and outcomes of patients with obstructive colorectal cancer. J Gastrointest Surg. 2011;15:1213-22.
27. Arnold D, Lueza B, Douillard JY, Peeters M, Lenz HJ, Venook A, Heinemann V, Van Cutsem E, Pignon JP, Tabernero J, Cervantes A, Ciardiello F. Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomized trials. Ann Oncol. 2017;28:1713-29.
28. Ji WB, Kwak JM, Kang DW, Kwak HD, Um JW, Lee SI, Min BW, Sung NS, Kim J, Kim SH. Clinical benefits and oncologic equivalence of self-expandable metallic stent insertion for right-sided malignant colonic obstruction. Surg Endosc. 2017;31:153-8.
29. Yoon JY, Jung YS, Hong SP, Kim TI, Kim WH, Cheon JH. Clinical outcomes and risk factors for technical and clinical failures of self-expandable metal stent insertion for malignant colorectal obstruction. Gastrointest Endosc. 2011;74:858-68.
30. Cho YK, Kim SW, Lee BI, Lee KM, Lim CH, Kim JS, Chang JH, Park JM, Lee IS, Choi MG, Choi KY, Chung IS. Clinical outcome of self-expandable metal stent placement in the management of malignant proximal colon obstruction. Gut Liver. 2011;5:165-70.
31. Sloothaak DA, van den Berg MW, Dijkgraaf MG, Fockens P, Tanis PJ, van Hooft JE, Bemelman WA, collaborative Dutch Stent-In study g. Oncological outcome of malignant colonic obstruction in the Dutch Stent-In 2 trial. The British journal of surgery. 2014;101:1751-7.
32. Maruthachalam K, Lash GE, Shenton BK, Horgan AF. Tumour cell dissemination following endoscopic stent insertion. The British Journal of Surgery. 2007;94:1151-4.
33. Steinert EM, Schwartz RH, Singh NJ. At low precursor frequencies, the T-cell response to chronic self-antigen results in anergy without deletion. Eur J Immunol. 2012;42:2875-80.

ORIGINAL PAPER

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Comparison of the effect of betanin on STAT3, STAT5, and KAP1 proteins in HepG2 and THLE-2 cells

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ABSTRACT

Background. Several studies suggest that the pleiotropic properties of betanin may interfere with different signaling pathways. Our previous studies on human hepatocytes showed that betanin activated the nuclear factor erythroid-2-related factor 2 (Nrf2) signaling pathway. To further understand the exact mechanism of action of betanin, we evaluated its effect on the levels of signal transducers and activators of transcription (STATs) and KRAB domain-associated protein 1 (KAP1) in hepatoma cells (HepG2) and normal human hepatocytes (THLE-2).
Material and methods. HepG2 and THLE-2 cells were treated with 2 or 10 μM betanin for 72 h. The levels of STAT3, STAT5a, STAT5b, and KAP1 proteins in cytosolic and nuclear fractions were assessed by Western blot.
Results. At a concentration of 10 μM, betanin significantly decreased the levels of STAT3, STAT5a, and STAT5b proteins in the nuclear fraction of HepG2 cells. On the other hand, no significant changes in the levels of STAT proteins were observed in THLE-2 cells. In HepG2 cells, betanin at both tested doses increased the level of KAP1. In contrast, in THLE-2 cells, betanin at a dose of 10 μM decreased the nuclear level of KAP1.
Conclusions. Betanin modulated the levels of STAT3, STAT5, and KAP1 proteins, especially in hepatoma cells. Thus, it may be considered a potential therapeutic agent for the treatment of hepatoma.

Introduction

Hepatocellular carcinoma (HCC) is the major form of primary liver cancer. It is one of the most serious human cancers, the pathogenesis of which involves continuous hepatocyte death, inflammatory cell infiltration, and compensatory liver regeneration. Understanding the molecular signaling pathways that induce or mediate these

processes during liver carcinogenesis is essential for identifying novel therapeutic targets for this disease [1].

According to epidemiological studies, diet and physical activity influence the incidence of certain types of cancers, including liver cancer, and preventive measures should be taken in the early stages of these diseases [2]. Some prophylactic measures include a diet enriched with natural substances that can inhibit or reverse tumor development. For example, beetroot, a vegetable commonly included in the human diet, has many health-promoting properties. Besides antioxidant activity, it exhibits anti-inflammatory and detoxifying effects [3]. Many of the properties of beetroot are attributed to betalains, especially betanin. Betanin is widely used in the food industry as a coloring agent in fruit yogurt, ice cream, and cosmetic care products [4] and well-known for its anti-inflammatory and hepatoprotective functions in human cells. It has been reported that betanin inhibited cell proliferation in hepatoma cancer cells HepG2 [5] and ovarian cancer cell line PA-1 [6] and showed pro-apoptotic action in human lung cancer cell lines [7], U87MG human glioma cells [8], and oral squamous cancer cells SCC131 and SCC4 [9]. Another mechanism of the anticancer action of betanin is the modulation of signaling pathways. Betanin activated nuclear factor erythroid-2-related factor 2 (Nrf2) signaling pathway in hepatoma cancer cells HepG2 [10]. Additionally, the activation of NF-κB/PI3K/Akt signaling pathway was observed in oral squamous cancer cells SCC131 and SCC4 [9]. Since epidemiological studies indicate that liver cancer still has a poor prognosis, intensive research is needed to develop new therapeutic solutions. One promising therapeutic strategy for the treatment of liver cancer is interfering with other signaling pathways.

Literature data highlight increased expression of signal transducers and activators of transcription (STATs) in liver tumors [11]. The upregulation of STAT signaling pathways promotes tumor growth and survival, due to the inhibition of apoptosis as well as increased cell proliferation, migration, and invasion [12].

The STATs family comprises seven members – STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. These cytoplasmic transcription factors mediate signal transduction from various growth factors and cytokines to the nucleus [11]. Recent studies also indicate that STATs may interfere with the functions of KRAB domain-associated protein 1 (KAP1) [13–15], which promotes cell proliferation and metastatic progression in different cancers, including HCC [16].

Therefore, to further explore the mechanism of action of betanin, we assessed the effect of betanin on other signaling pathways such as STAT3, STAT5a, and STAT5b, as well as their dependence on KAP1 protein, in hepatoma cells (HepG2) and normal human hepatocytes (THLE-2).

Materials and methods

Chemicals

Betanin, dimethyl sulfoxide (DMSO), Tris, antibiotic solution (10^4 U penicillin, 10 mg streptomycin, 25 µg amphotericin B), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and trypsin were supplied by Sigma-Aldrich (USA). Bronchial Epithelial Cell Growth Basal Medium (BEGM) and Bullet Kit were purchased from Lonza/Clonetics Corporation (USA). Primary antibodies against STAT3 (sc-483), STAT5a (sc-1081), STAT5b (sc-1656), β-Actin (sc-7210), lamin (sc-20680), and secondary alkaline phosphatase labeled antibodies were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Primary antibody against KAP1 (ab10484) was obtained from Abcam (Cambridge, UK). The protein molecular weight marker was supplied from EURx (Gdańsk, Poland).

Cell Culture

The hepatocellular carcinoma cells – HepG2 (ATCC HB 8065, USA) were cultured in DMEM with 10% FBS and 1% antibiotic solution and grown under standard conditions (37°C, 5% CO_2). Normal human hepatocytes THLE-2 (ATCC CRL-2706, USA) were maintained in BEGM supplemented with Bullet Kit and 5 ng/mL EGF, 70 ng/mL phosphoethanolamine, and 10% FBS (37°C, 5% CO_2). After the 24 hours of initial incubation, the cells (1×10^6 cells per 100 mm culture dish) were treated with 2 or 10 µM of betanin, incubated for a further 72h, and harvested. Control cells were treated with 0.1% DMSO. The dimethyl sulfoxide 100 mM stock solutions of betanin were used and stored at –20°C.

The doses of betanin were selected based on the MTT viability assay, which was carried out in our previous research [10].

Nuclear and Cytosolic Fractions Preparation

The subcellular extracts from HepG2 and THLE-2 cells were prepared using the Nu-clear/Cytosol Fractionation Kit (BioVision Research, Mountain-View, CA, USA) according to the manufacturer's protocol. Protein concentration was assessed using the Lowry method, and the samples were stored at −80°C for further analysis.

Western Blot Analysis

Nuclear fractions for STAT3, STAT5a, STAT5b, KAP1, lamin, or cytosolic fractions for STAT3, STAT5a, STAT5b, KAP1, β-Actin protein detection, were separated on 10% SDS-PAGE slab gels. β-Actin and lamin were used as a loading control. The amount of cytosolic and nuclear fractions was 100 µg of protein per well. Proteins were transferred to the nitrocellulose Immobilon P membrane (Millipore, Bedford, MA, USA). After blocking for 2 hours with 10% skimmed milk, proteins were probed with rabbit anti-STAT3, rabbit anti-STAT5a, mouse anti-STAT5b, rabbit anti-β-Actin, rabbit anti-lamin (Santa Cruz Biotechnology, Dallas, TX, USA), rabbit anti-KAP1 (Abcam, Cambridge, UK) antibodies (dilution 1:1000). The alkaline phosphatase-labeled anti-mouse IgG and anti-rabbit IgG (dilution 1:5000) were used as the secondary antibodies. Bands were visualized using the AP Conjugate Substrate Kit NBT/BCIP (BioRad Laboratories, Hercules, CA, USA). The amount of immunoreactive products in each lane was determined using the ChemiDoc Imaging System (BioRad Laboratories, Hercules, CA, USA). Values were calculated as relative absorbance units (RQ) per mg of protein and expressed as a percentage of the control.

Statistical Analysis

GraphPad Instat version 3.10 (GraphPad Software, San Diego, CA, USA) was used to perform statistical analysis. The data are shown as the means ± SEM. To assess the significance of the differences in the evaluated parameters, one–way ANOVA with Dunnett's post hoc test was performed with the significance level of $p < 0.05$.

Results

Effect of betanin on STAT3 protein level in HepG2 and THLE-2 cells

The level of STAT3 protein in cytosolic and nuclear fractions of HepG2 and THLE-2 cells was investigated by Western blot using a specific antibody against this protein. As shown in **Figure 1**, HepG2 cells incubated with either of the tested doses of betanin showed no significant changes in the cytosolic level of STAT3 protein. However, an ~19% reduction in the nuclear STAT3 protein level was observed in these cells after treatment with betanin at the dose of 10 µM.

In THLE-2 cells, both cytosolic and nuclear levels of STAT3 protein remained unchanged following treatment with betanin at both tested doses.

Effect of betanin on STAT5a and STAT5b protein levels in HepG2 and THLE-2 cells

Western blot analysis revealed a significant increase in the cytosolic level of STAT5a protein in HepG2 tumor cells under the influence of 10 µM betanin (see **Figure 2**).

In contrast, an opposite trend of changes was observed in the nuclear levels of STAT5a and STAT5b proteins. At a dose of 10 µM, betanin significantly decreased (by 20–22%) the nuclear levels of both tested STAT5 isoforms in HepG2 cells (see **Figures 2** and **3**).

Similar to STAT3, no significant changes in both cytosolic and nuclear levels of STAT5a and STAT5b proteins were observed in THLE-2 cells after incubation with betanin at any of the tested doses (see **Figures 2** and **3**).

Effect of betanin on KAP1 protein level in HepG2 and THLE-2 cells

As shown in **Figure 4**, a significant increase in the nuclear level of KAP1 protein was noted in HepG2 cells after incubation with 2 or 10 µM betanin (by 38% and 52%, respectively), compared to the cells treated with DMSO.

However, in THLE-2 cells, a significant decrease in the nuclear KAP1 protein level was observed after treatment with betanin at a dose of 10 µM.

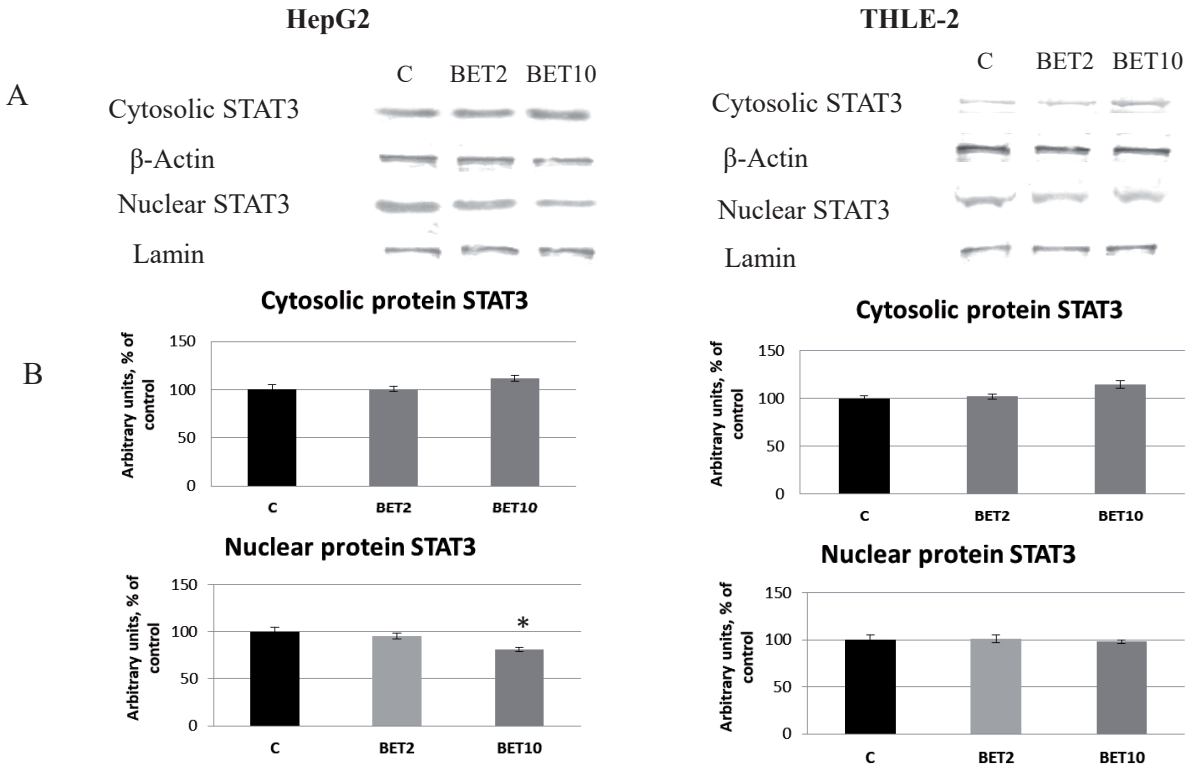

Figure 1. The effect of betanin on the level of the STAT3 protein in HepG2 and THLE-2 cells. A. Representative immunoblots showing the cytosolic and nuclear levels of STAT3 protein. B. Data (mean±SEM) from three separate experiments in comparison to the control cells set to 100%. Asterisk (*) above the bar indicates statistically significant differences from the control group, $p < 0.05$.

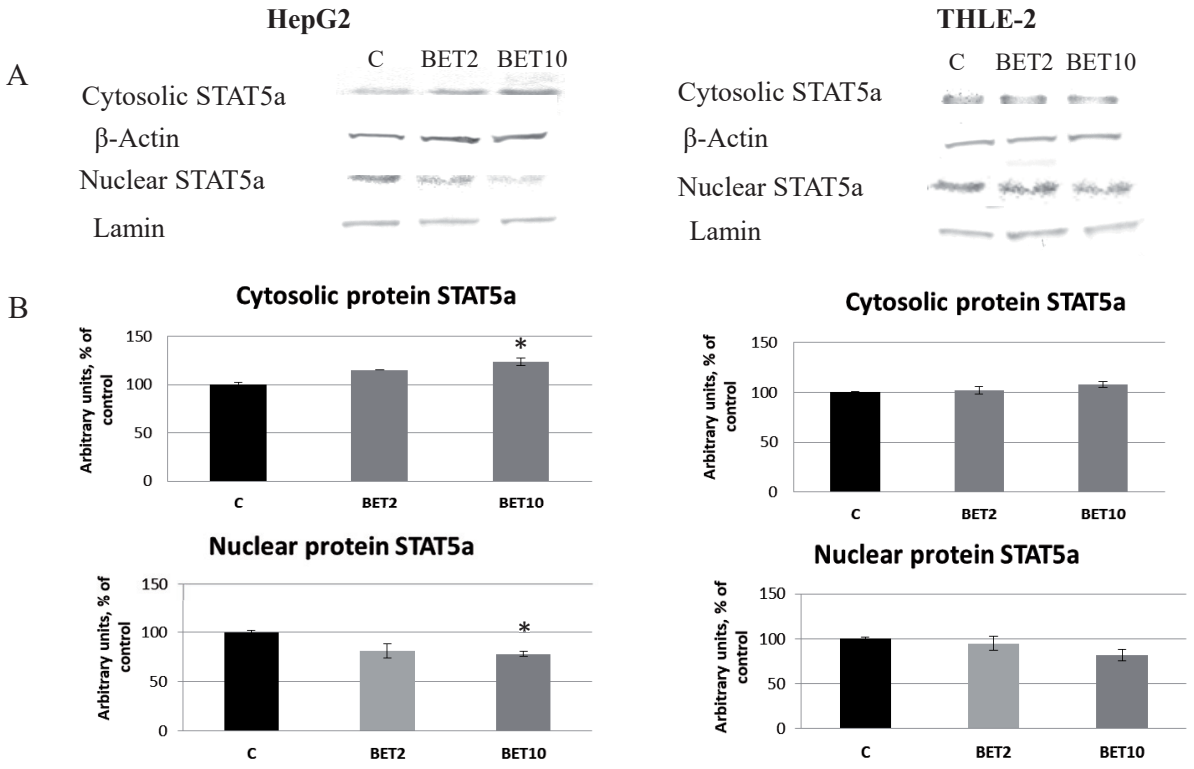

Figure 2. The effect of betanin on the level of the STAT5a protein in HepG2 and THLE-2 cells. A. Representative immunoblots showing the cytosolic and nuclear levels of STAT5a protein. B. Data (mean±SEM) from three separate experiments in comparison to the control cells set to 100%. Asterisk (*) above the bar indicates statistically significant differences from the control group, $p < 0.05$.

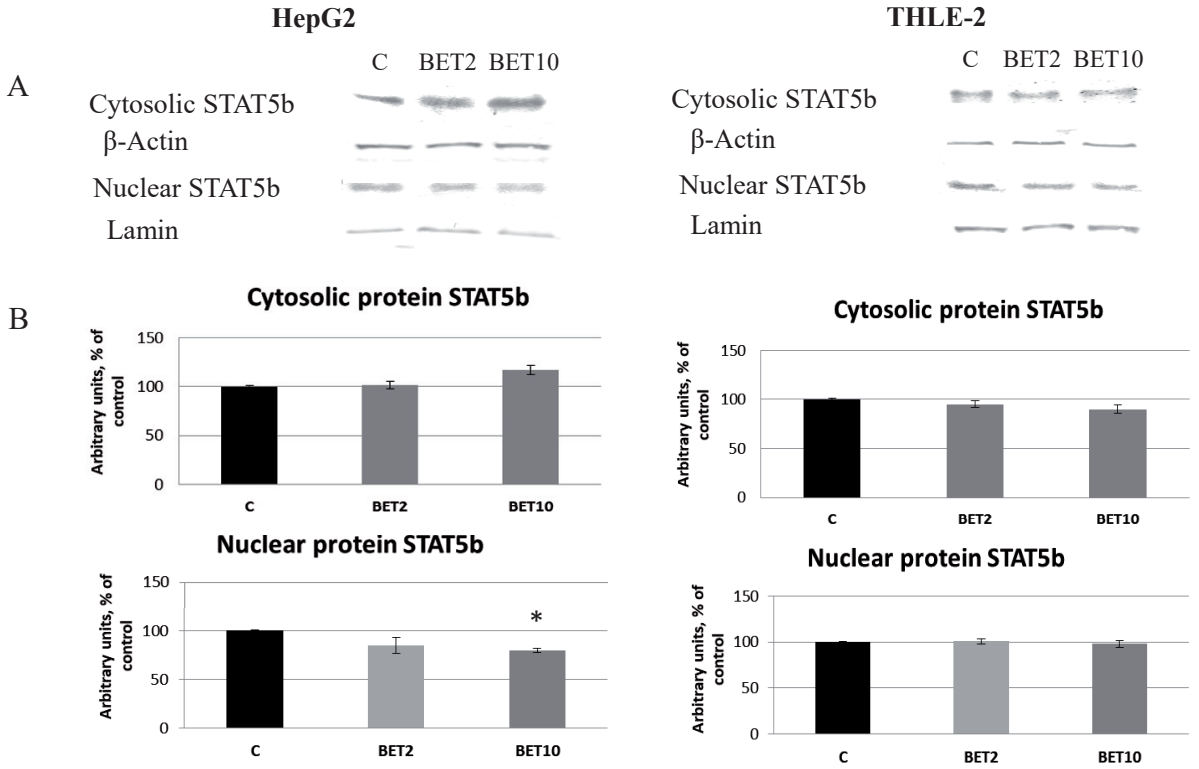

Figure 3. The effect of betanin on the level of the STAT5b protein in HepG2 and THLE-2 cells. A. Representative immunoblots showing the cytosolic and nuclear levels of STAT5b protein. B. Data (mean±SEM) from three separate experiments in comparison to the control cells set to 100%. Asterisk (*) above the bar indicates statistically significant differences from the control group, $p < 0.05$.

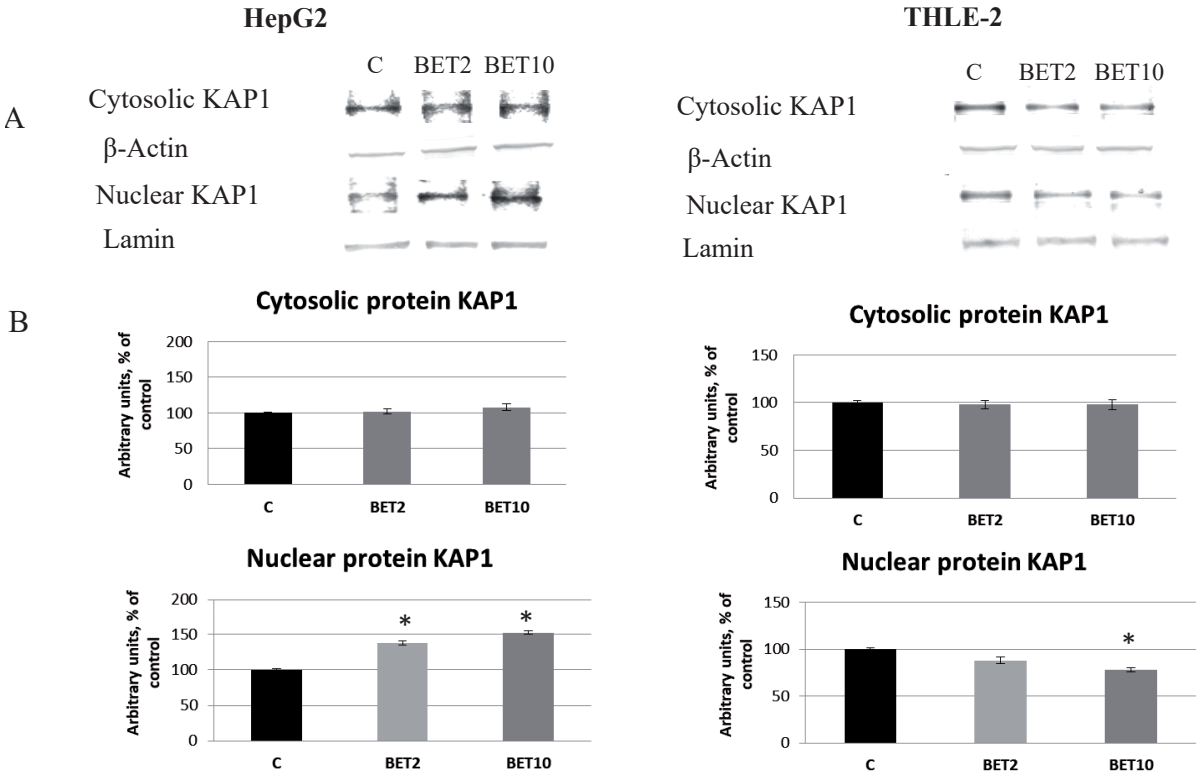

Figure 4. The effect of betanin on the level of the KAP1 protein in HepG2 and THLE-2 cells. A. Representative immunoblots showing the cytosolic and nuclear levels of KAP1 protein. B. Data (mean±SEM) from three separate experiments in comparison to the control cells set to 100%. Asterisk (*) above the bar indicates statistically significant differences from the control group, $p < 0.05$.

Discussion

Many studies have investigated the involvement of STAT3 in tumor development [17–19]. Recent evidence indicates that STAT5 plays a significant role in progression of several cancers, such as breast, colorectal, lung, prostate, and liver cancer [12].

In the case of liver cancer, STAT5 plays a dual role. First, STAT5 can act as an oncogene promoting tumor development and progression by enhancing cell proliferation of cancer stem cells (CSCs), chemoresistance, and epithelial–mesenchymal transition, a key mechanism that could lead to increased invasion and metastasis [20–22]. Fu et al. [20] demonstrated that in HCC increased STAT5 expression regulated by GRAM domain-containing 1A (GRAMD1A), a cholesterol transporter, could induce tumor growth, and chemoresistance and increased CSC side population and tumor cell survival by upregulating cyclin D1, Bcl-2, c-Myc, and c-Jun, as well as downregulating caspase 3 and poly (ADP-ribose) polymerase (PARP). Second, STAT5 can act as a tumor suppressor in the liver. Studies by Yu et al. [23] showed the upregulation of NADPH oxidase 4 (NOX4), an enzyme involved in the generation of reactive oxygen species, as well as the induction of pro-apoptotic proteins: p53-upregulated modulator of apoptosis (PUMA) and Bcl-2-interacting mediator of cell death (BIM).

These arguments convinced us to search for potential inhibitors of the abovementioned transcription factors. Betanin is one such compound, which is found in beetroot and exhibits pleiotropic effects [3, 24], including on transcription factors.

In this study, we examined the translocation of STAT3 and STAT5 from the cytosol to the nucleus. Our results showed that betanin decreased the nuclear levels of STAT3 and STAT5 in HepG2 hepatoma cells. The most significant inhibitory effect of betanin was observed at the higher dose (10 μM), at which the nuclear level of STAT3 was decreased in HepG2 cells. It should be emphasized that the inhibitory effect of betanin on STATs has not been explored in hepatoma cells. In normal hepatocytes, THLE-2, we did not observe any significant changes in the levels of STATs. A similar effect has been reported for other components of sugar beet, namely betavulgarin in breast CSCs [25]. In addition, studies have demonstrated STAT inhibition in HepG2 cells using other phytochemicals or active biological compounds. For instance, a study by Aggarwal and team [26] showed a reduction in the nuclear level of STAT3 in HepG2 cells after treatment with *Aegle marmelos* leaf extract, rutin, and quercetin. Soni et al. [27] observed a similar inhibitory effect of curcumin on the STAT3 protein level in the liver cancer line HepG2 and T-cell lymphoma line HuT78.

Some studies verified the possible involvement of Ser727 phosphorylation of STAT3 in cell survival and activities. For example, a study showed the reduction of phosphorylation of STAT3 at position Ser727 in the HepG2 cell line under the influence of resveratrol at a concentration of 25 μM [28]. Additionally, Liu et al. [29] examined the effect of sulforaphane, which is abundant in cruciferous vegetables, such as cauliflower and broccoli, on the levels of total and phosphorylated STAT3 in the HepG2 cell line. The authors observed a reduction in the level of both proteins after treatment with sulforaphane at a concentration range of 5–20 μM.

Understanding the role of STAT3 could be crucial to determine the potential application of STAT3 as a marker in liver cancer.

Studies conducted so far on the effect of phytochemicals on the JAK/STAT signaling pathway in an *in vitro* model mainly focused on the STAT3 protein, while very few concerned the STAT5 protein. Therefore, the effect of betanin on STAT5 may be of interest.

The present study confirmed the same direction of change for STAT5 also. At a dose of 10 μM, betanin significantly decreased the nuclear level of STAT5a and STAT5b proteins in HepG2 cells. Jung et al. [30] also reported the inhibition of STAT5 protein, but with other compounds and in different cell lines. The authors showed that oxymatrine, a major alkaloid found in radix *Sophorae flavescent* extract, inhibited the activation of STAT5 and its binding to DNA in the lung cancer cell line A549. Sulforaphane has also been shown to inhibit STAT5 in the human leukemia cell line K562 [31].

Recent studies suggest that the KAP1 protein may interfere with STATs. KAP1 is a universal corepressor protein for the transcriptional repressors belonging to the KRAB zinc finger protein superfamily [13,15]. Tsuruma et al. [13] observed that endogenous KAP1 was associated with endogenous STAT3 in an *in vivo* model. KAP1 is susceptible to several posttranslational

modifications, including phosphorylation, which directly alters its biological functions. This protein is responsible for differentiation, transcriptional regulation, gene silencing, and response to DNA damage. It also plays a role in the control of oxidative stress and carcinogenesis [32]. Hence, there has been increasing interest in using the KAP1 protein for diagnostic purposes or as a marker for cancer treatment.

In this study, we assessed the effect of betanin on the level of KAP1. Our densitometric analysis showed that betanin increased the nuclear level of this protein in hepatoma cells while it decreased its level in normal cells. This discrepancy may be due to the different mechanisms of action of betanin in cancer and normal cells or its interaction with other transcription factors. It should be emphasized that our study is the first to determine the effect of betanin on KAP1 levels in hepatoma cells.

Few studies have been conducted by others to determine the level of KAP1 protein. Wang et al. [16] reported the overexpression of KAP1 in a human hepatoma cell line. In addition, comparisons between cancer and non-cancerous tissues proved that the expression level of KAP1 was significantly higher in tumor tissues obtained from HCC patients. These data confirm that KAP1 plays an important role in the development of HCC and could be a valuable biomarker for tumor diagnosis and prognosis prediction, as well as a potential therapeutic target for the treatment of this disease.

Similarly, in other research models, the expression of KAP1 has been shown to higher in cancer cells of various tissues (e.g. lung cancer cells) compared to noncancerous cells [33].

In summary, natural compounds can be effective in the prevention and treatment of liver cancer. Our study highlighted the inhibitory effect of betanin on STAT3 and STAT5 in HepG2 cells, thus confirming its potential therapeutic effect. However, further *in vitro* and *in vivo* studies must be carried out to explain the mechanisms of chemopreventive and antitumor activity of phytochemicals, including betanin.

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Conflict of interest statement
The authors declare no conflict of interest.

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Author Contributions
Conceptualization: V. Krajka-Kuźniak and H. Szaefer; methodology: V. Krajka-Kuźniak, K. Hadryś, H. Gajewska, K. Migdałek and H. Szaefer; validation: V. Krajka-Kuźniak and H. Szaefer; formal analysis: V. Krajka-Kuźniak and H. Szaefer; investigation: V. Krajka-Kuźniak and H. Szaefer; writing original draft preparation: V. Krajka-Kuźniak and H. Szaefer.

References

1. He G, Karin M. NF-κB and STAT3 - key players in liver inflammation and cancer. Cell Res. 2011 Jan;21(1):159-168. doi:10.1038/cr.2010.18.
2. Kotecha R, Takami A, Espinosa JL. Dietary phytochemicals and cancer chemoprevention: a review of the clinical evidence. Oncotarget. 2016 Aug 9;7(32):52517-52529. doi: 10.18632/oncotarget.9593.
3. Hadipour E, Taleghani A, Tayarani-Najaran N, Tayarani-Najaran Z. Biological effects of red beetroot and betalains: A review. Phytother Res. 2020 Aug;34(8):1847-1867. doi: 10.1002/ptr.6653.
4. Santamaria P. Nitrate in vegetables: Toxicity, content, intake and EC regulation. J Sci Food Agric. 2006; 86: 10-17. doi: 10.1002/jsfa.2351.
5. Lee EJ, An D, Nguyen CTT, Patil BS, Kim J, Yoo KS. Betalain and betaine composition of greenhouse- or field-produced beetroot (Beta vulgaris L.) and inhibition of HepG2 cell proliferation. J Agric Food Chem. 2014 Feb 12;62(6):1324-31. doi: 10.1021/jf404648u.
6. Rakshanaa R, ShafreenM, Kumar N. Inhibition of Proliferation in Ovarian Cancer Cell Line (PA-1) by the Action of Green Compound "Betanin". Appl Biochem Biotechnol. 2022 Jan;194(1):71-83. doi: 10.1007/s12010-021-03744-0.
7. Zhang Q, Pan J, Wang Y, Lubet R, You M. Beetroot red (betanin) inhibits vinyl carbamate- and benzo(a)pyrene-induced lung tumorigenesis through apoptosis. Mol Carcinog. 2013 Sep;52(9):686-91. doi: 10.1002/mc.21907.
8. Salimi A, Bahiraei T, Ahdeno S, Vatanpour S, Pourahmad J. Evaluation of Cytotoxic Activity of Betanin Against U87MG Human Glioma Cells and Normal Human Lymphocytes and Its Anticancer Potential Through Mitochondrial Pathway. Nutr Cancer. 2021;73(3):450-459. doi: 10.1080/01635581.2020.1764068.
9. Zou X, Yu K, Chu X, Yang L. Betanin alleviates inflammation and ameliorates apoptosis on human oral squamous cancer cells SCC131 and SCC4 through the NF-κB/PI3K/Akt signaling pathway. J Biochem Mol Toxicol. 2022 Aug;36(8):e23094. doi: 10.1002/jbt.23094.
10. Krajka-Kuźniak V, Paluszczak J, Szaefer H, Baer-Dubowska W. Betanin, a beetroot component, induces nuclear factor erythroid-2-related factor 2-mediated expression of detoxifying/antioxidant enzymes in human liver cell lines. Br

J Nutr. 2013 Dec;110(12):2138-49. doi: 10.1017/S0007114513001645.
11. Svinka J, Mikulits W, Eferl R. STAT3 in hepatocellular carcinoma: new perspectives. Hepat Oncol. 2014 Jan;1(1):107-120. doi: 10.2217/hep.13.7.
12. Halim CE, Deng S, Ong MS, Yap CT. Involvement of STAT5 in Oncogenesis. Biomedicines. 2020 Aug 28;8(9):316. doi: 10.3390/biomedicines8090316.
13. Tsuruma R, Ohbayashi N, Kamitani S, Ikeda O, Sato N, Muromoto R, Sekine Y, Oritani K, Matsuda T. Physical and functional interactions between STAT3 and KAP1. Oncogene. 2008 May 8;27(21):3054-9. doi: 10.1038/sj.onc.1210952.
14. Kamitani S, Ohbayashi N, Ikeda O, Togi S, Muromoto R, Sekine Y, Ohta K, Ishiyama H, Matsuda T. KAP1 regulates type I interferon/STAT1-mediated IRF-1 gene expression. Biochem Biophys Res Commun. 2008 May 30;370(2):366-70. doi: 10.1016/j.bbrc.2008.03.104.
15. Kamitani S, Togi S, Ikeda O, Nakasuji M, Sakauchi A, Sekine Y, Muromoto R, Oritani K, Matsuda T. Krüppel-associated box-associated protein 1 negatively regulates TNF-α-induced NF-κB transcriptional activity by influencing the interactions among STAT3, p300, and NF-κB/p65, J Immunol. 2011 Sep 1;187(5):2476-83. doi: 10.4049/jimmunol.1003243.
16. Wang Y, Jiang J, Li Q, Ma H, Xu Z, Gao Y. KAP1 is overexpressed in hepatocellular carcinoma and its clinical significance. Int J Clin Oncol. 2016 Oct;21(5):927-933. doi: 10.1007/s10147-016-0979-8.
17. Bromberg J. Stat proteins and oncogenesis. Clin Invest. 2002 May;109(9):1139-42. doi: 10.1172/JCI15617.
18. Chai EZP, Shanmugam MK, Arfuso F, Dharmarajan A, Wang C, Kumar AP, Samy RP, Lim LHK, Wang L, Goh BC, Ahn KS, Hui KM, Sethi G. Targeting transcription factor STAT3 for cancer prevention and therapy. Pharmacol Ther. 2016 Jun;162:86-97. doi: 10.1016/j.pharmthera.2015.10.004.
19. Thilakasiri PS, Dmello RS, Nero TL, Parker MW, Ernst M, Chand AL. Repurposing of drugs as STAT3 inhibitors for cancer therapy. Semin Cancer Biol. 2021 Jan;68:31-46. doi: 10.1016/j.semcancer.2019.09.022.
20. Fu B, Meng W, Zhao H, Zhang B, Tang H, Zou Y, Yao J, Li H, Zhang T. GRAM domain-containing protein 1A (GRAMD1A) promotes the expansion of hepatocellular carcinoma stem cell and hepatocellular carcinoma growth through STAT5. Sci Rep. 2016 Sep 2;6:31963. doi: 10.1038/srep31963.
21. Zhao C, Wang Q, Wang B, Sun Q, He Z, Hong J, Kuehn F, Liu E, Zhang Z. IGF-1 induces the epithelial-mesenchymal transition via Stat5 in hepatocellular carcinoma. Oncotarget. 2017 Dec 5;8(67):111922-111930. doi: 10.18632/oncotarget.22952.
22. Lee TK, Man K, Poon R TP, Lo C M, A Yuen AP, Ng IO, Ng KT, Leonard W, Fan ST. Signal transducers and activators of transcription 5b activation enhances hepatocellular carcinoma aggressiveness through induction of epithelial-mesenchymal transition. Cancer Res. 2006 Oct 15;66(20):9948-56. doi:10.1158/0008-5472.CAN-06-1092.
23. Yu JH, Zhu BM, Riedlinger G, Kang K, Hennighausen L. The liver-specific tumor suppressor STAT5 controls expression of the reactive oxygen species-generating enzyme NOX4 and the proapoptotic proteins PUMA and BIM in mice. Hepatology. 2012 Dec;56(6):2375-86. doi: 10.1002/hep.25900.
24. Lechner JF, Stoner GD. Red Beetroot and Betalains as Cancer Chemopreventative Agents. Molecules. 2019 Apr 23;24(8):1602. doi: 10.3390/molecules24081602.
25. Liu R, Choi HS, Zhen X, Kim S-L, Kim J-H, Ko Y-Ch, Yun B-S, Lee DS. Betavulgarin Isolated from Sugar Beet (Beta vulgaris) Suppresses Breast Cancer Stem Cells through Stat3 Signaling. Molecules. 2020 Jul; 25(13): 2999. doi: 10.3390/molecules25132999.
26. Aggarwal H, Nair J, Sharma P, Sehgal R, Naeem U, Rajora P, Mathur R. Aegle marmelos differentially affects hepatic markers of glycolysis, insulin signalling pathway, hypoxia, and inflammation in HepG2 cells grown in fructose versus glucose-rich environment. Mol Cell Biochem. 2018 Jan;438(1-2):1-16. doi: 10.1007/s11010-017-3108-8.
27. Soni VK, Shukla D, Kumar A, Vishvakarma NK. Curcumin circumvent lactate-induced chemoresistance in hepatic cancer cells through modulation of hydroxycarboxylic acid receptor-1. Int J Biochem Cell Biol. 2020 Jun;123:105752. doi: 10.1016/j.biocel.2020.105752.
28. Li Y, Zhu W, Li J, Liu M, Wei M. Resveratrol suppresses the STAT3 signaling pathway and inhibits proliferation of high glucose-exposed HepG2 cells partly through SIRT1. Oncol Rep. 2013 Dec;30(6):2820-2828. doi: 10.3892/or.2013.2748.
29. Liu P, Atkinson SJ, Akbareian SE, Zhou Z, Munsterberg A, Robinson SD, Bao Y. Sulforaphane exerts anti-angiogenesis effects against hepatocellular carcinoma through inhibition of STAT3/HIF-1α/VEGF signalling. Sci Rep. 2017 Oct 4;7(1):12651. doi: 10.1038/s41598-017-12855-w.
30. Jung YY, Shanmugam MK, Narula AS, Kim Ch, Lee JH, Namjoshi OA, Blough BE, Sethi G, Ahn KS. Oxymatrine attenuates tumor growth and deactivates STAT5 signaling in a lung cancer xenograft model. Cancers (Basel). 2019 Jan 7;11(1):49. doi: 10.3390/cancers11010049.
31. Pinz S, Unser S, Rascle A. The natural chemopreventive agent sulforaphane inhibits STAT5 activity. PLoS One. 2014 Jun 9; 9(6): e99391: 1-14.doi: 10.1371/journal.pone.0099391.
32. Cheng CT, Kuo CY, Ann DK. KAPtain in charge of multiple missions: Emerging roles of KAP1. World J Biol Chem. 2014 Aug 26;5(3):308-20. doi: 10.4331/wjbc.v5.i3.308.
33. Wu GJ, Pen J, Huang Y, An S, Liu Y, Yang Y, Hao Q, Guo X-X, Xu T-R. KAP1 inhibits the Raf-MEK-ERK pathway to promote tumorigenesis in A549 lung cancer cells Mol Carcinog. 2018 Oct;57(10):1396-1407. doi: 10.1002/mc.22853.

ORIGINAL PAPER

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Spectrum of neuroendocrine neoplasms of GIT – a histomorphological study in a tertiary care centre

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ABSTRACT

Background. Neuroendocrine neoplasms are diverse in terms of sites of origin, functional status, and degrees of aggressiveness. Since neuroendocrine cells are ubiquitous in the human body, these neoplasms can arise in different organs, with gastrointestinal tract being most frequently involved. The past few years have seen a surge in the diagnosis of these neoplasms, which were earlier considered to be rare. Their nomenclature, classification, and diagnostic criteria are revamped frequently, as new knowledge emerges.

Aim. To study the histopathological spectrum of neuroendocrine neoplasms of gastrointestinal tract and assess the immunohistochemical expression of neuroendocrine markers in them.

Material and methods. Ours is a descriptive study of the distribution and pathologic characteristics of gastrointestinal neuroendocrine neoplasms in a tertiary care hospital in Kerala, over a three year period. Neoplasms were categorised based on 2019 updated WHO classification.

Results. Among the 59 cases, we observed a male predominance. (Male to female ratio – 1.8:1). Most patients were in 6th and 7th decades of life. Duodenum was most frequently involved followed by rectum and appendix. NET G2 and G1 constituted the predominant histologic grades (47% and 24% respectively). NEC and MiNEN were infrequent. All cases were positive for synaptophysin, with variable positivity for chromogranin. Ki67 helped establish the histologic grade. We also came across a rare case of neuroendocrine tumor with coexisting mucinous neoplasm in appendix.

Conclusions. With evolving knowledge and advanced imaging modalities, the incidence of these neoplasms is increasing with time. Histopathology is the mainstay of diagnosis and plays a decisive role in influencing management protocols and prognosis.

Introduction

Neuroendocrine neoplasms (NENs) are a diverse group of neoplasms composed of cells containing dense-core neuroendocrine secretory granules in their cytoplasm. Accumulating evidence over the years has paved way for newer and updated clas-

sification of these neoplasms. The consensus meeting held in Lyon, under the auspices of the WHO Classification of Tumors Group established a unitary classification system for NENs, that was published in the 5th edition of 2019 WHO classification of tumors of the digestive system. Presently, NENs are categorised as well differentiated neuroendocrine tumors (NET) and poorly differentiated neuroendocrine carcinomas (NEC).

Most of these neoplasms are neuroendocrine tumors and possess an indolent disease biology. 10–20% are neuroendocrine carcinomas, which are highly proliferative tumors characterized by rapid disease progression [1].

These tumors have a diverse spectrum of clinical presentation with variable etiologies, clinical features, morphologic and genomic findings, and degree of aggressiveness.

The incidence and prevalence of NETs has increased substantially over time, with greatest rates of increase in USA, Canada and Norway [2]. Availability of powerful functional imaging modalities such as endoscopy & ultrasound guided fine needle biopsies, immunohistochemistry for a definitive diagnosis, better awareness and health care utilisation have contributed to the global rise in incidence.

Approximately two-thirds of NETs are found in the gastrointestinal tract, 25% occur in the lungs, and the remaining cases arise in other endocrine tissues, such as the thyroid [3].

However, there are few concise reports which give the entire spectrum and prevalence of these tumors in the GI tract in our geographic area. In this study, we attempt to put forward our experience of gastrointestinal NENs.

Materials and methods

This is a two and half year retrospective and six months prospective study of all gastrointestinal neuroendocrine neoplasms reported in our hospital from July 2019-June 2022. Approval of institutional ethics committee was taken.

Clinical data, including gender, age at diagnosis, and anatomic locations, were obtained for all the cases from the hospital database. The cases were categorised based on 2019 WHO classification of tumors of digestive system (see **Table 1**).

Haematoxylin and eosin (H & E) stained sections were reviewed and histomorphological features were analysed.

Immunohistochemistry with synaptophysin (Rabbit polyclonal antibody), chromogranin (Mouse monoclonal antibody), CD56 and Ki 67 cell proliferation index (Mouse monoclonal antibody) were performed on fully automated immunostainer (Leica Bondmax) by using poly horse radish peroxide (HRP) technique. The Ki-67 index was assessed in areas with highest nuclear labelling (hot spot areas). 500–2000 cells were counted manually for assessment.

Socio demographic data and characteristics of tumors were expressed as number, percentage and mean value.

Results

A total of 59 cases including biopsies and resection specimens were studied. Mean age at diagnosis was 56.13 years, with an age range of 14 – 82 years. Individuals in 7th and 8th decades were

Table 1. WHO 2019 Classification and grading criteria for neuroendocrine neoplasms of the gastrointestinal tract [7].

Terminology	Differentiation	Grade	Mitotic rate (Mitoses/2 mm^2)	Ki 67 index (%)
NET G1	Well differentiated	Low	<2	<3
NET G2		Intermediate	2-20	3-20
NET G3		High	>20	>20
NEC (Small cell type)	Poorly differentiated	High	>20	>20
NEC (Large cell type)			>20	>20
MiNEN	Well or poorly differentiated	Variable	Variable	Variable

NET – Neuroendocrine tumor, G 1,2 & 3 – Grade 1,2 & 3, NEC – Neuroendocrine carcinoma, MiNEN – Mixed neuroendocrine-non-neuroendocrine neoplasm.

most commonly affected. We observed a male predominance with male: female ratio of 1.8:1. The gender distribution with respect to location is depicted in **Figure 1**. The diagnosis was made on endoscopic biopsies in 42 cases and resected surgical specimens were available in 17 cases.

Location

Most common site of involvement was duodenum (28.8%) followed by rectum (22%). Esophagus and GE junction were least frequently involved.

Grading

We came across 28 cases of NET G2, 14 cases of NET G1, 4 cases of NET G3, 5 cases of NEC and 5 cases of MiNEN. Three cases showed poorly differentiated adenocarcinoma with neuroendocrine differentiation. The distribution of NEN in different locations and their grades is shown in **Table 2**.

Of the 10 appendiceal NEN, most belonged to pathological stage pT1 (4 cases) followed by pT3 (3 cases). One case had coexisting low grade appendiceal mucinous neoplasm (LAMN, **Figure 2**). Of the 17 resected specimens, nodal metastasis were found in three. **Figures 2-5** show the different grades of NEN encountered.

Among the MiNEN, all cases had an admixture of adenocarcinomatous areas and NEC (**Figure 5**).

On IHC, all cases showed diffuse cytoplasmic positivity for synaptophysin. 81% and 14% cases showed diffuse and focal positivity for chromogranin respectively. 5% cases were negative for chromogranin, of which majority were high grade NET and NEC. CD 56 was done in 21 cases and was found to be positive.

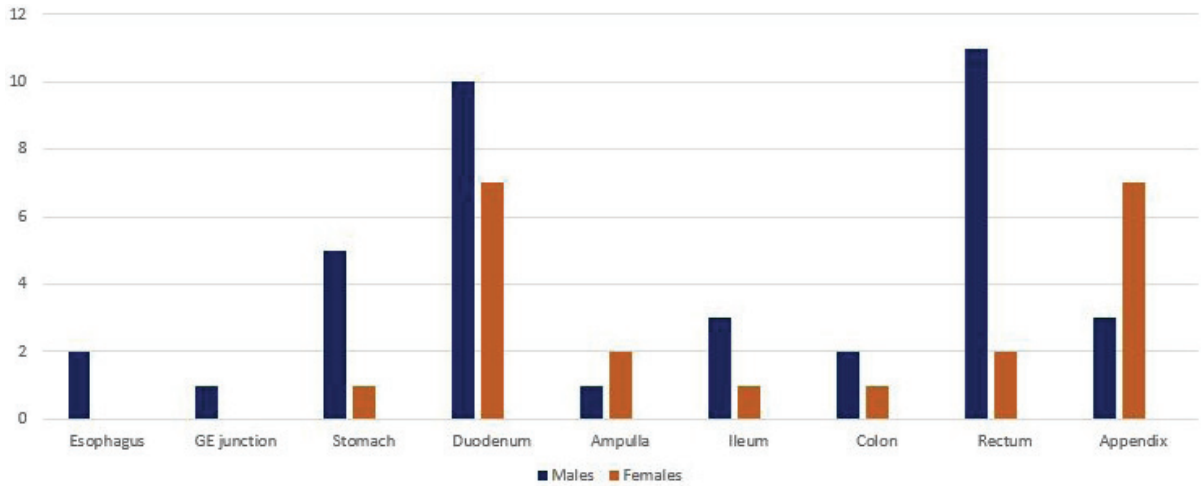

Figure 1. Gender distribution according to location.

Table 2. Distribution of neuroendocrine neoplasms by location and grade.

Site	No. of cases n (%)	G1	G2	G3	NEC	MiNEN	Neuroendocrine differentiation
Esophagus	2 (3.4)	0	0	0	2	0	
GE junction	1 (1.7)	0	0	0	0	0	1
Stomach	6 (10.2)	2	2	1	1	0	
Duodenum	17 (28.8)	4	12	1	0	0	
Ampulla	3 (5.1)	1	0	0	0	2	
Ileum	4 (6.8)	1	3	0	0	0	
Colon	3 (5.1)	0	0	0	0	3	
Rectum	13 (22)	1	7	2	2	0	1
Appendix	10 (16.9)	5	4	0	0	0	1

GE junction – Gastroesophageal junction, G1,2,3 – Neuroendocrine tumor grade 1, 2, 3, NEC – Neuroendocrine carcinoma, MiNEN – Mixed neuroendocrine-non-neuroendocrine neoplasm.

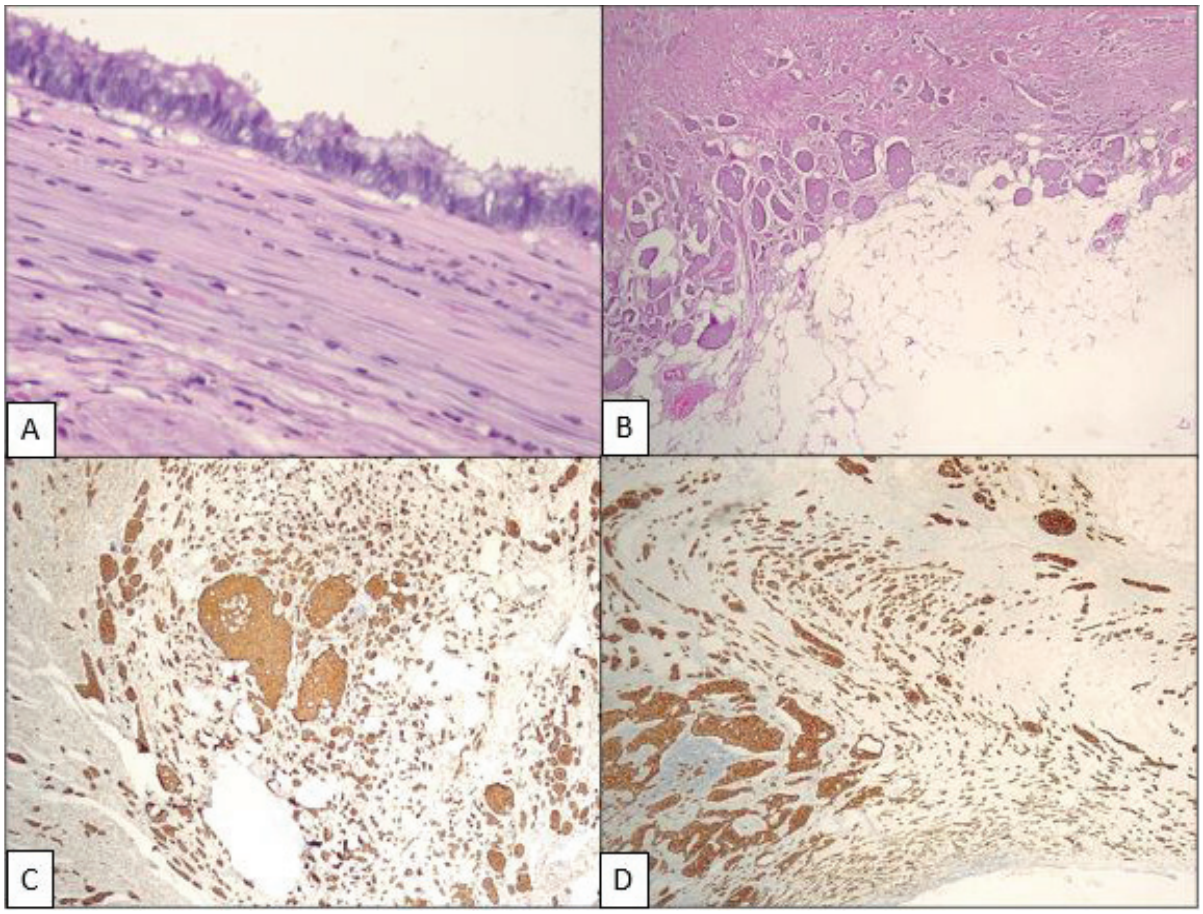

Figure 2. Low grade appendiceal mucinous neoplasm with neuroendocrine tumor grade 2. A – Appendix lined by mucinous cells with low grade nuclear atypia, atrophy of underlying lymphoid tissue and effaced muscularis mucosae, H&E, 100×. B – Neuroendocrine tumor extending into periappendiceal fat, H&E, 100×. C – Tumor cells showing diffuse positivity for synaptophysin, 100×. D – Tumor cells showing diffuse positivity for chromogranin, 100×.

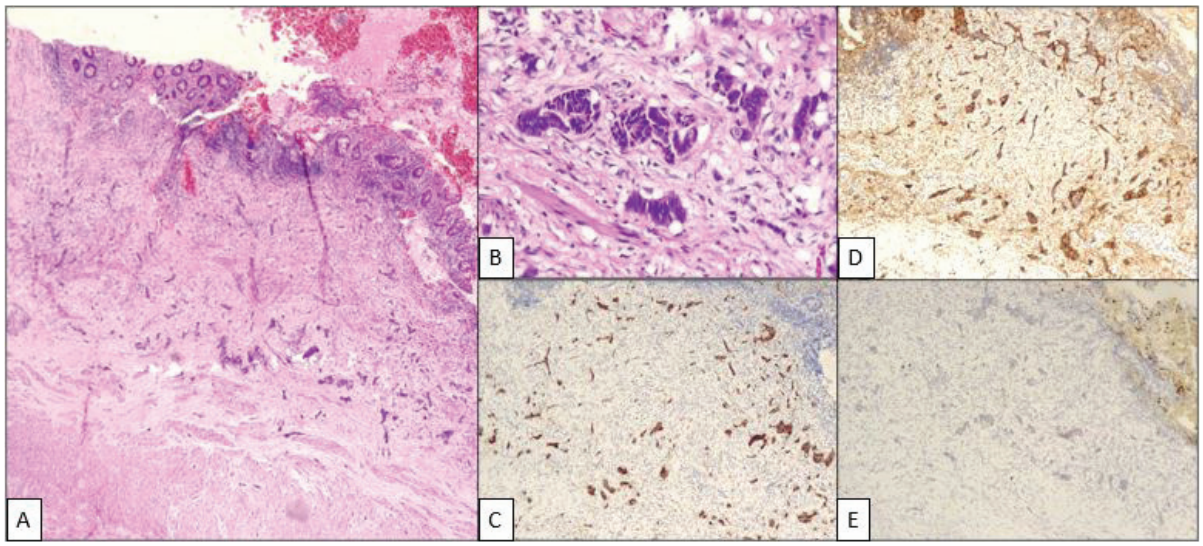

Figure 3. Neuroendocrine tumor grade 1, appendix. A – Appendix showing NET, H&E, 100×. B – Nests of neuroendocrine cells, H&E,400×. C – Synaptophysin – diffuse positivity, 100×. D – CD56 – diffuse positivity, 100×. E – Ki 67 – 1%, 100×.

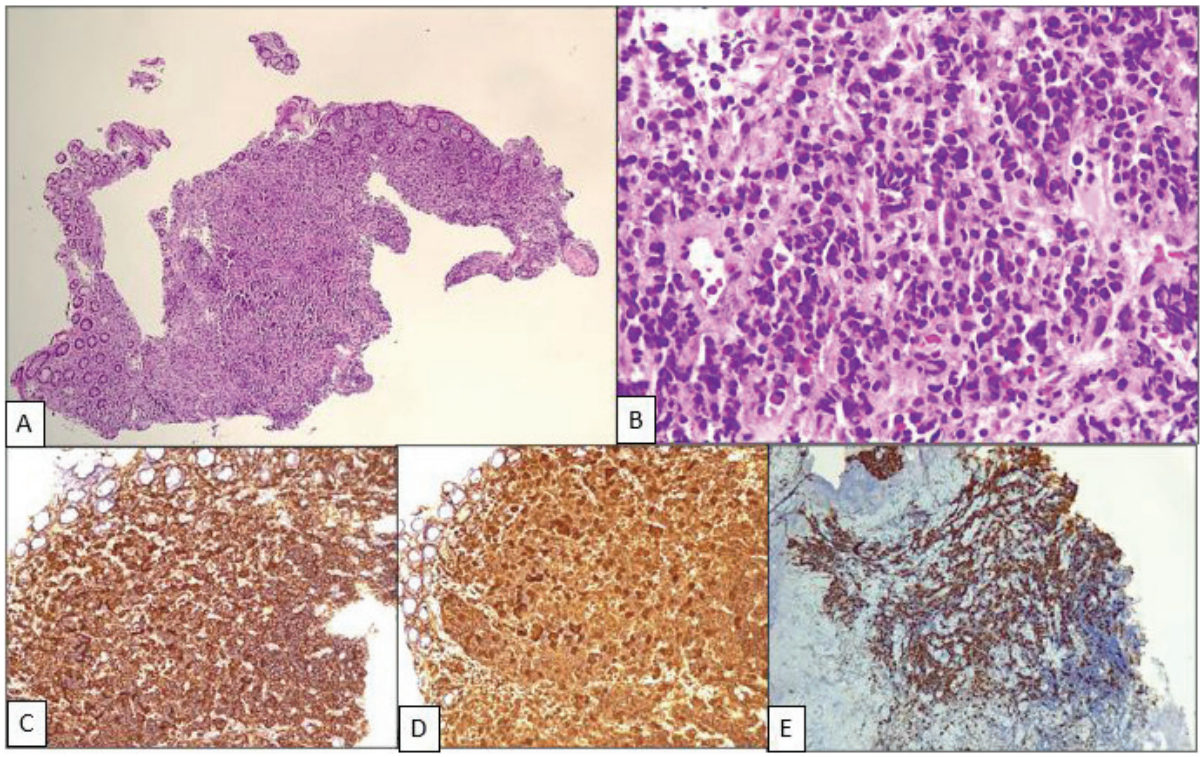

Figure 4. Neuroendocrine carcinoma, Rectum. A – Rectal mucosa showing infiltration by atypical cells, H&E, 40×. B – Small mildly pleomorphic atypical cells, H&E, 400×. C – Tumor cells show diffuse positivity for synaptophysin, 100×. D – Tumor cells show diffuse positivity for chromogranin, 100×. E – Ki 67 (86%), 100×.

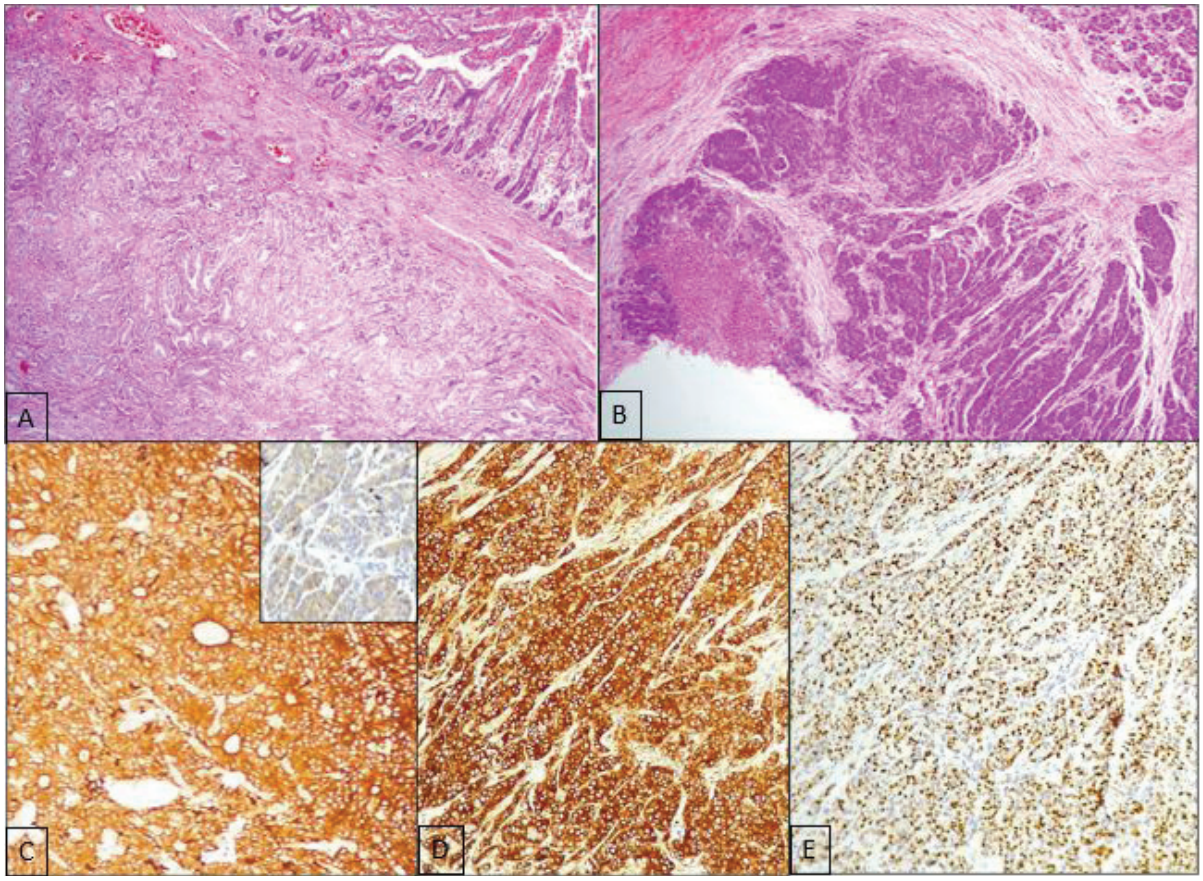

Figure 5. Mixed neuroendocrine non neuroendocrine neoplasm. A – Adenocarcinomatous component, H&E,40×. B – Solid nests of neuroendocrine cells, H&E,40×. C – IHC showing positivity for CK7 in glandular component, 400×. Inset shows CK7 negativity in neuroendocrine component,100×. D – IHC showing positivity for synaptophysin, 100×. E – Ki 67 (60%), 100×.

Discussion

The neuroendocrine cells of GIT are derived from the neural crest, neuroectoderm, and endoderm. Endocrine cells are interspersed within the mucosa of the GIT and comprise approximately 1% of all mucosal cells [4].

Pathologically, neuroendocrine differentiation is defined as architectural and cytological patterns reminiscent of non-neoplastic neuroendocrine cells (nesting or trabecular growth pattern and coarsely stippled chromatin) and production of characteristic neurosecretory proteins that can be detected by immunohistochemistry. These include synaptophysin and chromogranin A. Some authorities consider CD56 and neuron specific enolase (NSE) as adequate evidence of neuroendocrine differentiation [5].

The World Health Organisation, in 2018, proposed a uniform classification framework for all NEN, according to which NEN are categorised into NET and NEC. The two groups differ in clinical, epidemiological, genetic and prognostic factors. Tumors with a non neuroendocrine component in addition to NEN (each component should be >30%) are categorised as MiNEN. NET and NEC are subcategorised as shown in **Table 1**.

The new category "NET G3" was first introduced for pancreatic tumors in 2017, and later extend to all gastrointestinal NEN in 2019. In the earlier classifications, NET G3 was considered to be synonymous with poor differentiation, i.e NEC. However, recent evidence suggests that NET G3 show differences in morphology, genotype, clinical features, and treatment response, compared to NEC [6].

NECs have a less nested architectural pattern and abundant necrosis. They are said to arise from precursors that give rise to non neuroendocrine carcinomas of respective organs. Emerging genomic data on pancreatic NENs suggests differing mutation profiles in NET and NEC of pancreas. NETs more frequently harbour mutations in MEN1, ATRX, and DAXX; while TP53, Rb and SMAD4 are more commonly inactivated in NEC. Genomic comparisons in NEN of GIT are still emerging [7].

Clinically, prognosis of NET G3 seems closer to that of NET G2 rather than that of NEC, but with a worse overall survival. Localised tumors are treated by surgery. For advanced tumors with Ki67 index <55% alkylant based regimens are used, while platinum based chemotherapy is preferred for those with Ki67 index >55% [6].

Studies show that NET G-3 are more often found in the pancreas with a frequency ranging from 10% to 65%. Other main tumor sites are the colon/rectum and stomach, with frequencies ranging from 8% to 24% and 8% to 29%, respectively [6].

The most frequent primary sites of gastrointestinal NETs vary in different regions of the world. An epidemiological study by Satya Das et al. [2] found small intestinal and colorectal NETs to be most common in North America. In Europe, small intestine and pancreas were found to be most frequently involved. Rectal, gastric, and pancreatic NETs occurred in highest frequencies in Asia. The reason for these differences could be a combination of environmental factors and biological differences due to differing national demographics [2].

A multicentre longitudinal NET registry from India reported the most common primary sites of NET to be pancreas (42.9%), small intestine (22.1%), colorectum (9%), and appendix (2.7%) in diminishing order [8].

In our study, small intestine and rectum were most frequently affected, similar to other Indian studies [9,10,11]. Studies in Brazil and Turkey found gastric NENs to be most frequent.

NETs of the esophagus are rare, representing only 0.04 -1% of all the gastrointestinal NETs reported [7]. Studies conducted in different regions show this anatomical location to be rarely involved, with a higher frequency in males [3,9], as our study concurs.

The most common age group at presentation was 61-80 years, with a mean age of 56 years, comparable to Uppin et al and Zeng et al. [10,12]. Studies conducted in Brazil [3], Kashmir [9], Japan [12], and Turkey [13] found a female predominance. Our study and Uppin et al. [10] observed a male predominance, attributing no specific gender predilection.

Most NET were low grade tumors (G1 and G2), in concordance with studies across different regions [3,9,10]. NET G3 and NEC involved rectum predominantly. NECs are rare in the large bowel, representing approximately 0.6% of all carcinomas in this location. Nevertheless, they are more common in rectum than in any other part of the intestine [3] as seen in our study as well.

MiNEN represent a rare diagnosis of the GI tract. Compared with the previous definition of mixed adeno neuroendocrine carcinomas (MANEC), MiNEN better represents the spectrum of variability of differentiation and morphology of these neoplasms [7].

For a neoplasm to qualify as MiNEN, both neuroendocrine and non neuroendocrine component should be morphologically and immunohistochemically recognisable. Each component should constitute >30% of the neoplasm. In GI tract, both components are usually carcinomas. Rarely, the neuroendocrine component may be well differentiated [7]. These neoplasms may arise as a combination of two neoplastic clones or as the proliferation of one precursor cell with divergent differentiation. Molecular and genetic studies point towards a monoclonal origin of both components. The behaviour is thought to be driven by the neuroendocrine component [14,15].

Li et al analyzed the pathologic characteristics of mixed colorectal glandular-neuroendocrine tumors in 87 cases. Majority of the lesions were located in the right colon (56%) and the left colon (41%), while rectum was uninvolved. Another case series by Guerrera et al. [17] found colon to be most frequently affected. In our study as well, colon was most frequently involved, followed by ampulla. Three cases showed neuroendocrine differentiation, but did not fulfil the criteria for MiNEN, hence were categorised as adenocarcinomas with neuroendocrine differentiation.

Appendiceal collision tumors are extremely rare with very few cases reported in literature [18]. They result from proliferation of two different cellular lines. We came across one such case in a 40 year old male with a low grade appediceal mucinous neoplasm (LAMN) and well differentiated NET G2. Management options for such cases depend on nodal status, pathological stage and margin status.

Markers of neuroendocrine differentiation include synaptophysin (imparts a diffuse cytoplasmic staining), chromogranin A and CD56. Chromogranin A is an acidic glycoprotein of the granin family, being expressed in well to moderately differentiated NENs and tends to be only focally positive in PD-NECs/SC-NEC [19]. CD56 is sensitive, but not highly specific marker for NENs because it is often expressed in several non-NENs.

Conclusion

Our study focusses on NEN of the GIT, based on files of a single institution in Kerala. Mean age at presentation was 56.13 years with a male predominance. Duodenum was most commonly involved. Majority of the tumors were well differentiated NET grade 2. Tumors from colorectal region were mostly NEC. We also encountered a rare case of collision tumor in appendix. The present study represents a concise overview of spectrum of GI NEN, based on WHO 2019 criteria.

Histomorphology plays a pivotal role in tailoring treatment options in this diverse spectrum of gastrointestinal neoplasms.

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Conflict of interest statement
The authors declare no conflict of interest.

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References

1. Sorbye H, Strosberg J, Baudin E, Klimstra DS, Yao JC. Gastroenteropancreatic high-grade neuroendocrine carcinoma. Cancer. 2014 Sep 15;120(18):2814-23. doi: 10.1002/cncr.28721. Epub 2014 Apr 25. PMID: 24771552.
2. Das S, Dasari A. Epidemiology, Incidence, and Prevalence of Neuroendocrine Neoplasms: Are There Global Differences? Curr Oncol Rep. 2021 Mar 14;23(4):43. doi: 10.1007/s11912-021-01029-7. PMID: 33719003.
3. Estrozi B, Bacchi CE. Neuroendocrine tumors involving the gastroenteropancreatic tract: a clinicopathological evaluation of 773 cases. Clinics (Sao Paulo). 2011;66(10):1671-5. doi: 10.1590/s1807-59322011001000002. PMID: 22012036.
4. Schonhoff SE, Giel-Moloney M, Leiter AB. Minireview: Development and differentiation of gut endocrine cells. Endocrinology. 2004 Jun;145(6):2639-44. doi: 10.1210/en.2004-0051. Epub 2004 Mar 24. PMID: 15044355.
5. Klimstra DS, Beltran H, Lilenbaum R, Bergsland E. The spectrum of neuroendocrine tumors: histologic classification, unique features and areas of overlap. Am Soc Clin Oncol Educ Book. 2015:92-103. doi: 10.14694/EdBook_AM.2015.35.92. PMID: 25993147.
6. Pellat A, Cottereau AS, Palmieri LJ, Soyer P, Marchese U, Brezault C, Coriat R. Digestive Well-Differentiated Grade 3 Neuroendocrine Tumors: Current Management and Future Directions. Cancers (Basel). 2021 May 18;13(10):2448. doi: 10.3390/cancers13102448. PMID: 34070035.
7. WHO Classification of Tumors Editorial Board. WHO Classification of Tumors, 5th ed. Digestive System

Tumors. Lyon: International Agency for Research on Cancer,2019.
8. Palepu J, Shrikhande SV, Bhaduri D, Shah RC, Sirohi B, Chhabra V, Dhar P, Sastry R, Sikora S. Trends in diagnosis of gastroenteropancreatic neuroendocrine tumors (GEP-NETs) in India: A report of multicenter data from a web-based registry. Indian J Gastroenterol. 2017 Nov;36(6):445-451. doi: 10.1007/s12664-017-0808-7. Epub 2018 Feb 19. PMID: 29457213.
9. Abdullah H, Wani Z, Nabi Z, Shah P, Bhat S. Neuroendocrine tumors of gastrointestinal tract with special reference to immunohistochemistry markers at a tertiary care hospital. APIK Journal of Internal Medicine. 2022 Apr 1;10(2):78. doi: 10.4103/ajim.ajim_14_21
10. Uppin MS, Uppin SG, Sunil CS, Hui M, Paul TR, Bheerappa N. Clinicopathologic study of neuroendocrine tumors of gastroenteropancreatic tract: a single institutional experience. J Gastrointest Oncol. 2017 Feb;8(1):139-147. doi: 10.21037/jgo.2016.12.08. PMID: 28280618.
11. Amarapurkar DN, Juneja MP, Patel ND, Amarapurkar AD, Amarapurkar PD. A retrospective clinico-pathological analysis of neuroendocrine tumors of the gastrointestinal tract. Tropical Gastroenterology. 2010 Aug 12;31(2):101-4. PMID: 20862983.
12. Zeng YJ, Liu L, Wu H, Lai W, Cao JZ, Xu HY, Wang J, Chu ZH. Clinicopathological features and prognosis of gastroenteropancreatic neuroendocrine tumors: analysis from a single-institution. Asian Pac J Cancer Prev. 2013;14(10):5775-81. doi: 10.7314/apjcp.2013.14.10.5775. PMID: 24289577.
13. Yucel B, Babacan NA, Kacan T, Eren AA, Eren MF, Bahar S, Celasun MG, Seker MM, Hasbek Z. Survival analysis and prognostic factors for neuroendocrine tumors in Turkey. Asian Pac J Cancer Prev. 2014 Jan;14(11):6687-92. doi: 10.7314/apjcp.2013.14.11.6687. PMID: 24377589.
14. La Rosa S, Sessa F, Uccella S. Mixed Neuroendocrine-Nonneuroendocrine Neoplasms (MiNENs): Unifying the Concept of a Heterogeneous Group of Neoplasms. Endocr Pathol. 2016 Dec;27(4):284-311. doi: 10.1007/s12022-016-9432-9. PMID: 27169712.
15. Frizziero M, Chakrabarty B, Nagy B, Lamarca A, Hubner RA, Valle JW, McNamara MG. Mixed Neuroendocrine Non-Neuroendocrine Neoplasms: A Systematic Review of a Controversial and Underestimated Diagnosis. J Clin Med. 2020 Jan 19;9(1):273. doi: 10.3390/jcm9010273. PMID: 31963850.
16. Li Y, Yau A, Schaeffer D, Magliocco A, Gui X, Urbanski S. Colorectal glandular-neuroendocrine mixed tumor: Pathologic spectrum and clinical implications. Am J Surg Pathol 2011;35:413-25. doi: 10.1097/PAS.0b013e3182093657. PMID: 21317713.
17. Guerrera LP, Suarato G, Napolitano R, Perrone A, Caputo V, Ventriglia A, Martini G, Della Corte CM, Orditura M, Martinelli E, Ciardiello F, Montella M, Franco R, Troiani T, Napolitano S. Mixed Neuroendocrine Non-Neuroendocrine Neoplasms of the Gastrointestinal Tract: A Case Series. Healthcare (Basel). 2022 Apr 11;10(4):708. doi: 10.3390/healthcare10040708. PMID: 35455885.
18. Villa M, Sforza D, Siragusa L, Guida AM, Ciancio Manuelli M, Pirozzi BM, Pocci M, Palmieri G, Grande M. A Low-Grade Appendiceal Mucinous Neoplasia and Neuroendocrine Appendiceal Collision Tumor: A Case Report and Review of the Literature. Am J Case Rep. 2021 Feb 23;22:e927876. doi: 10.12659/AJCR.927876. PMID: 33619239.
19. Lin F, Liu H. Immunohistochemistry in undifferentiated neoplasm/tumor of uncertain origin. Arch Pathol Lab Med 2014;138:1583-610. doi: 10.5858/arpa.2014-0061-RA. PMID: 25427040.

ORIGINAL PAPER

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Assessment of chronic pain prevalence and impact on quality of life in the general population and visitors of a pain clinic in Makkah region, Saudi Arabia, 2022–2023

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ABSTRACT

Introduction. Chronic pain is a frequent, complicated, stressful health condition thatsignificantly affects society and people. Chronic pain also is believed to be one of themost important causes of suffering and infirmity worldwide. It can impact various aspects of the person and cause emotional, social, and functional capabilities. Assessing Health-related quality of life is a significant outcome in studies concerningchronic pain patients. It is a different way to evaluate patients' perceptions of their pain experience and its effect on their lives.

Aim. The aim of this study is to establish the prevalence of chronic pain among the generalpopulation in the Makkah region, Saudi Arabia.

Material and methods. An online cross-sectional study design using a structured self-administered questionnaire was distributed electronically in Arabic through social media.

Results. A total of 610 participants completed the survey. Females represented 63.3%of participants, while male participants represented 36.4%. Most participants were Saudi (98.9%), and 72.3% had bachelor's degrees or Diplomas. Single participants were almost equal to married ones (53.1 % and 46.2%, respectively), and most were nonsmokers.

Conclusions. chronic pain from patients' perspectives has physical, psychological, and social functioning and well-being effects.

Introduction

Chronic pain is a multidimensional health problem defined by the International Association for the Study of Pain (IASP) as pain persisting for more than six months,although being much more related to peripheral and central nervous system sensitization than to whole duration time [1].

Chronic pain is a frequent, complicated, stressful health condition affecting society and persons [2]. The frequent cause of chronic pain is the presence of an injury or a particular disease condition; however, chronic pain should be considered a distinct condition, not only an associated symptom of other disorders. Thus, Chronic pain hasits particular categorization and characterization [3].

Chronic pain might be an intense, persistent, and incapacitating disorder, considerably reducing individuals' healthy lifestyles and decreasing their quality of life [4]. This condition is believed to be one of the most important causes of misery and infirmity worldwide [5]. Chronic pain can impact different facets of the individual and cause emotional, behavioral, and functional infirmities. Previous studies indicate that undesirable outcomes like depressive disorders [6], anxiety, and feelings of confusionare correlated with prolonged periods of chronic pain, such as movement incapacity, job incapacity [7], heightened health care expenses [8], death, and suicide [9]. In Europe, of five people having chronic pain conditions, one employee loses his career because of this sustained health condition, and one-third of the persons experienced chronic pain conditions and their consequences during their work hours, either partially or totally [10]. Therefore, pain is considered a defense mechanism. The JointCommission on Accreditation of Healthcare Organizations describes the pain as a familiar experience with unfavorable physical and emotional consequences when it cannot be managed [11]. Hence, pain experience involves mental, social, emotional, and physical characteristics; on the other hand, quality and life have a broad perspective that covers all these aspects [12].

Health-related quality of life describes the effect of well-being on individuals' capability to achieve and contribute to meaningful actions inside the family, work, and society [13]. Assessing the quality of life is an essential effect in investigations ofpatients suffering from the problem of chronic pain. It is a different approach to evaluating patients' points of view on their pain experience and its effect on their lives [14].

There are available data about the consequence of chronic pain on the quality of life in the Arab world; thus, this study aims to assess the incidence of chronic pain amongthe general population in Makkah region, Saudi Arabia, and investigate the intensity of chronic pain and evaluate the quality of life for those patients who are suffering from the chronic pain.

Methods

Study design

A cross-sectional study in which we enrolled participants through the year 2022 from the Makkah region, Saudi Arabia.

Settings

Well designed, structured self-administered questionnaire for assessing the quality of life was used. Data was collected through an online google form and distributed electronically in Arabic through WhatsApp and telegram.

Inclusion and exclusion criteria

The study included participants of both sexes, participants were asking if they are 18 years and above, and patients with chronic diseases who agreed to participate in this study. Exclusion criteria included individuals younger than 18 and those who refused to participate. In first section, participants were asked how long they had been experiencing pain (if more than 3 months it considered as chronic pain). Numerical Rating Scale (NRS) were used for measuring pain intensity and is well validated. It is scored from 0–10 (0 meaning no pain and 10 meaning the worst pain imaginable).

Data collection and sample size

After excluding incomplete data, a total of 610 participants were included in the study; By using the Raosoft sample size calculator website [15], a total of 377 was calculated as the minimum sample size sufficient to detect the prevalence of chronicpain in Makkah at 95% confidence level and 5% estimation error.

Materials

PROMIS® Scale v1.2–Global Health and RAND 36-Item Health Survey 1.0 questionnaire items were used [16]. The questionnaire had two sections. The first oneinvestigated the demographic characteristics of participants (age, education, smokingconditions, and marital status). The second section investigated chronic medical illness and chronic pain status. Responses were recorded using a bimodal approach.

Outcome Assessment

We used a structured questionnaire to assess the quality of life in two steps. First, preceded numeric values are recorded. Then, each item is scored so that a high score describes a more favorable health state. In addition, each item is scored on a 0 to 100 range so that the most minor and greatest possible scores are 0 and 100, respectively. In step two, items in the same scale are averaged together to create the eight-scale score. Items left blank (missing data) should be considered when calculating the scalescores.

Data analysis

Statistical Package for Social Sciences (SPSS) software version 22.0 for data entry and analysis was used. Descriptive statistics were described for categorical variablesas frequencies and percentages. In contrast, continuous variables were expressed as Mean (Standard deviation) for normally distributed variables and Median (Interquartile range) for non-normal variables. In addition, crude and adjusted Odds ratios and 95% Confidence Intervals were reported, and p-values of < 0.05 were considered statistically significant.

Ethical approval

This study was approved by King Abdullah medical city Institutional Research Board (IRB number: 22-1013). Survey responses were collected anonymously. No identifying information was collected from participants, no private information, and all responses were confidentiality maintained; at the beginning of the survey, participants consent was obtained after explaining the study's objective.

Results

This section presented the data of the participant sample with its statistical analysis results of 610 participants, with 63.3% females and 36.4% males completed the questionnaire. The prevalence of chronic pain is 47.5% (.475), with a 95% level of confidence and confidence interval [.436,.515].

This study included four sections: socio-demographic factors and their relationship with chronic pain, outcomes of RAND 36-item survey of self-reported chronic pain and its frequency, results of RAND 36-item survey of severe pain, and accessibility tochronic pain doctors or chronic pain clinics over the past six months.

The socio-demographic was investigated through gender, nationality, education, marital status, and smoker and nonsmoker factors. From a gender perspective, more than half of female responder participants were found to be with chronic pain (n = 210,54.1%), while male positive cases were only one-third (n = 80, 36%); this result was statistically significant around (p = <.001). 50% of the participants responded having chronic pain from both categories, Saudi and nonsaudi. From the education level point of view, two-thirds of doctors experience chronic pain while only half of master's and bachelor's holders suffer from chronic pain; unlike doctorate, two-thirds of high school and lower educated people have no chronic pain. Although there is no statistical significance (p = .313), Almost half of the married and single people reported chronic pain (n = 140,147, 49.6%,45.4%), respectively. Smokers and non-smokers were almost equally complaining of chronic pain (n = 40,245, 48.2%,47.2%), respectively (**Table 1**).

The first part of this work asked the participant whether they have chronic pain and are categorized based on socio-demographic factors, as explained above. In the second and third parts of this study, the RAND 36-item survey was assessed. Initially, the survey was conducted for individuals with self-reported chronic pain. After that, the outcomes of severe chronic pain were

Table 1. Distribution of socio-demographic factors and their relationship with chronic pain among the population in Saudi Arabia, 2022.

Variable		Chronic pain			
		Yes	No		
		N (%)		Chi-square	*p*-value
Gender				18.524	<.001*
Female	388 (63.3)	210(54.1)	178(45.9)		
Male	222 (36.4)	80(36)	142(64)		
Nationality				.262	.609
Saudi	603 (98.9)	286(47.4)	317(52.6)		
Non-Saudi	7 (1.1)	4(57.1)	3(42.9)		
Education				6.709	.082
High Schoolor lower	124 (20.3)	49(39.5)	75(60.5)		
Bachelor/Diploma	441 (72.3)	214(48.5)	227(51.5)		
Master	27 (4.4)	15(55.6)	12(44.4)		
Doctorate	18 (3)	12(66.7)	6(33.3)		
Marital Status				2.322	.313
Married	282 (46.2)	140(49.6)	142(50.4)		
Single	324 (53.1)	147(45.4)	177(54.6)		
Widow	4 (0.7)	3(75)	1(25)		
Smoker				.755	.685
Yes	83 (13.6)	40(48.2)	43(51.8)		
No	519 (85.1)	245(47.2)	274(52.8)		

The *p*-value was obtained from the chi-square test.
* $p < .05$

analyzed distinctly. The results of binary logistic regression of survey data are summarized in **table 2** and **table 3**, respectively.

The answers to 36 questions are scaled out of 100, and a higher score indicates betterhealth. Then the questions are divided into six factors: the weight of each element is calculated by the mean of its questions score. Finally, the coefficient of the logistic regression model is calculated for each factor. The relationship between energy/fatigue factor and chronic pain is inverse based on the odds ratio (OR). In other words, more energetic behavior indicates less chronic pain — like the relationship between social functioning and general health — however, physical functioning and role limitation due to emotional problems are directly related to chronic pain.

For the severe chronic pain group, social functioning, physical functioning, and general health factors have an inverse relationship concerning reported chronic pain, while energy/fatigue and role limitation due to emotional problems are directly related to reported chronic pain, as indicated by OR value in **table 3**.

The accessibility to chronic pain doctors or chronic pain clinics over the past six months is

Table 2. The results of binary logistic regression analysis of self-reported chronic painwith scores of different outcome factors from the RAND 36-Item Health survey.

Variable	Coefficient	Standard error	Statistics (*p*-value)	OR [95%CI]
Energy/fatigue	-.023	.006	13.584 (<.001)*	.978 [.966-.989]
Social Functioning	-.022	.005	21.616 (<.001)*	.979 [.970-.988]
Physical Functioning	.006	.003	4.199 (.040)*	1.006 [1.000-1.012]
General Health	-.033	.008	19.284 (<.001)*	.968 [.953-.982]
Role limitations due toemotional problems	.003	.003	.350 (.554)	1.002 [.997-1.007]

* $p < .05$

Table 3. Results of binary logistic regression analysis of severe pain with scores of different outcome factors from RAND 36-Item Health survey.

Variable	Coefficient	Standard error	Statistics (*p*-value)	OR [95%CI]
Energy/fatigue	.004	.016	.051 (.821)	1.004 [.973-1.036]
Social Functioning	.064	.016	16.087 (<.001)*	.938 [.909-.968]
Physical Functioning	-.007	.009	.711 (.399)	.993 [.976-1.010]
General Health	-.043	.021	4.380 (.036)*	.957 [.919-.997]
Role limitations due to emotional problems	.010	.007	1.692 (.193)	1.010 [.995-1.024]

* $p < .05$

reported by 290 participants. Slightly over 37% of the participants discussed their chronic pain with their doctors and sought help. Thirty-eight point three percent have visited chronic pain specialists. However, only 26.9 have taken treatment for chronic pain, like neuroleptics and depression medications, in the last six months. Around 10% have conducted direct therapy like botox or anesthesia injection to relieve pain.

Discussion

Our study involved 610 participants with chronic pain; females represented 63.3% of participants with more affection by chronic pain than males; this was by a previous study done in Brazil [1], in which (56.6%) of participants were female and reported having pain or being under the pharmacological treatment of pain. Also, Pain crises frequency and duration were significantly higher among females, who reported further interference of pain in self-care, work, sexual life, and sleep interruption.

Another study [17] was done to associate factors of chronic pain in nurses, which showed that 67% of participants with chronic pain were females. Also, a recent study [18] in England showed that 67.1% of participants were females.

Most of our participants had Bachelor's degrees or diplomas; this was to the previous study done in Saudi Arabia [19], which found that 77.89% of participants were university students. Also, a study done in the USA [20] showed that the most affected group with chronic pain graduated from college or higher. The mental and physical stresses may explain this in this group.

In contrast to our study, the study done on Iranian nurses [17] found that participants with a bachelor's degree were the minor group affected by chronic pain; another study done in Canada [21] showed that the high-education group of participants was the least affected by chronic pain.

In our study, marital status did not affect chronic pain status. In contrast, in the USA,63.5% of females with chronic pain were married [20], and the same was reported in Iran [17], 66.5% of chronic pain participants were married.

Our study reported That the odds of reporting chronic pain were significantly lower with higher scores in energy, social functioning, and general health factors. The odds of reporting chronic pain were significantly higher, with a lower score at the physical functioning factor.

This was by a study done in Croatia [22], which stated that the participants with sharper pain had significant emotional limitations, lower energy, poorer psychological health and social functioning, and poorer general health.

The social life of chronic pain patients was affected, as they had to give up their social lives and became socially isolated either because of the restricted physical activity [18] associated with chronic pain or due to depression resulting from the pain.

Chronic pain may affect the patient's sleep, which causes tiredness in the daytime and low energy. Constant feelings of fatigue had an unfavorable impact on the general health status.

Conclusion

We assessed the burden of chronic pain from patients' perspectives in multiple physical, psy-

chological, and social functioning and well-being domains. Chronic pain significantly affects social functioning and general health factors. Chronic pain also seemed to impact negatively patients' energy, although this did not reach statistical significance. In addition, chronic pain affects females and highly educated individuals more.

Limitations

This study has many limitations as it was an online survey, the response rate could be low,and there was a lack of communication between the participants and researchers which may limit the ability to explain some questions.

Also, the study was done in the Makkah region; the results cannot be generalized to the whole kingdom of Saudi Arabia.

Recommendations

More studies about chronic pain and its effects on quality of life should be done in other regions of Saudi Arabia and other Arab countries, as there is a lack of information and studies available on this issue; this will be necessary to manipulate this problem and improve the quality of life.

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Conflict of interest statement
The authors declare no conflict of interest.

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References

1. de Souza JB, Grossmann E, Perissinotti DMN, de Oliveira Junior JO, da Fonseca PRB, Posso IP. Prevalence of Chronic Pain, Treatments, Perception, and Interference on Life Activities: Brazilian Population-Based Survey. Pain Res Manag. 2017;2017:4643830. doi:10.1155/2017/4643830.
2. Fayaz A, Croft P, Langford RM, Donaldson LJ, Jones GT. Prevalence of chronic pain in the UK: a systematic review and meta-analysis of population studies. BMJ Open. 2016;6(6):e010364. Published 2016 Jun 20. doi:10.1136/bmjopen-2015-010364.
3. Tracey I, Bushnell MC. How neuroimaging studies have challenged us to rethink: is chronic pain a disease?. J Pain. 2009;10(11):1113-1120. doi:10.1016/j.jpain.2009.09.001.
4. Boonstra AM, Reneman MF, Stewart RE, Post MW, Schiphorst Preuper HR. Life satisfaction in patients with chronic musculoskeletal pain and its predictors. Qual Life Res. 2013;22(1):93-101. doi:10.1007/s11136-012-0132-8.
5. Babadi ME, Nazari F, Safari R, Abdoli S. The effect of reflexology on pain perception aspects in nurses with chronic low back pain in Isfahan. Iran J Nurs Midwifery Res. 2016;21(5):487-492. doi:10.4103/1735-9066.193395.
6. Nicholas MK. Depression in people with pain: There is still work to do Commentary on 'Understanding the link between depression and pain'. Scand J Pain. 2011;2(2):45-46. Published 2011 Apr 1. doi:10.1016/j.sjpain.2011.02.003.
7. Meints SM, Edwards RR. Evaluating psychosocial contributions to chronic pain outcomes. Prog Neuropsychopharmacol Biol Psychiatry. 2018;87(Pt B):168-182. doi:10.1016/j.pnpbp.2018.01.017.
8. Smith D, Wilkie R, Uthman O, Jordan JL, McBeth J. Chronic pain and mortality: a systematic review. PLoS One. 2014;9(6):e99048. Published 2014 Jun 5. doi:10.1371/journal.pone.0099048.
9. Hassett AL, Aquino JK, Ilgen MA. The risk of suicide mortality in chronic pain patients. Curr Pain Headache Rep. 2014;18(8):436. doi:10.1007/s11916- 014-0436-1.
10. Langley PC, Van Litsenburg C, Cappelleri JC, Carroll D. The burden associated with neuropathic pain in Western Europe. J Med Econ. 2013;16(1):85-95. doi:10.3111/13696998.2012.729548.
11. Soyuer F, Kepenek-Varol B. Quality of life and pain. Int J Fam Commun Med. 2019;3(3):110-114. DOI: 10.15406/ijfcm.2019.03.00140.
12. Marchand S. The physiology of pain mechanisms: from the periphery to the brain. Rheum Dis Clin North Am. 2008;34(2):285-309. doi:10.1016/j.rdc.2008.04.003.
13. Jonsdottir T, Aspelund T, Jonsdottir H, Gunnarsdottir S. The relationship between chronic pain pattern, interference with life and health-related quality of life in a nationwide community sample. Pain Manag Nurs. 2014;15(3):641- 651. doi:10.1016/j.pmn.2013.07.005.
14. Angst F, Verra ML, Lehmann S, Aeschlimann A. Responsiveness of five condition-specific and generic outcome assessment instruments for chronic pain. BMC Med Res Methodol. 2008;8:26. Published 2008 Apr 25. doi:10.1186/1471-2288-8-26.
15. Sample size calculator. Raosoft, Inc. makes high quality web survey software. (2004). http://www.raosoft.com/samplesize.html.
16. PROMIS® Scale v1.2-Global Health and RAND 36-Item Health Survey 1.0 questionnaire items (accessed on June 2022) https://www.rand.org/health-care/surveys_tools/mos/36-item-short-form.html.
17. Kheiry F, Rakhshan M, Shaygan M. The prevalence and associated factors of chronic pain in nurses Iran. Revista Latinoamericana de Hipertension. 2019;14(1):21-5.

18. Hadi MA, McHugh GA, Closs SJ. Impact of Chronic Pain on Patients' Quality of Life: A Comparative Mixed-Methods Study. J Patient Exp. 2019;6(2):133-141. doi:10.1177/2374373518786013.
19. El-Metwally A, Shaikh Q, Aldiab A, et al. The prevalence of chronic pain and its associated factors among Saudi Al-Kharj population; a cross sectional study. BMC Musculoskelet Disord. 2019;20(1):177. Published 2019 Apr 25. doi:10.1186/s12891-019-2555-7.
20. Kawai K, Kawai AT, Wollan P, Yawn BP. Adverse impacts of chronic pain on health-related quality of life, work productivity, depression and anxiety in a community-based study. Fam Pract. 2017;34(6):656-661. doi:10.1093/fampra/cmx034.
21. Toth C, Lander J, Wiebe S. The prevalence and impact of chronic pain with neuropathic pain symptoms in the general population. Pain Med. 2009;10(5):918-929. doi:10.1111/j.1526-4637.2009.00655.x.
22. Hnatešen D, Pavić R, Radoš I, et al. Quality of Life and Mental Distress in Patients with Chronic Low Back Pain: A Cross-Sectional Study. Int J Environ Res Public Health. 2022;19(17):10657. Published 2022 Aug 26. doi:10.3390/ijerph191710657.

ORIGINAL PAPER

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Phyllanthus amarus protects against potassium-dichromate pituitary toxicity via the oxidative pathway and improves the gonadotropins in male Wistar rats

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ABSTRACT

Background. *Phyllanthus amarus* is an antioxidant plant with numerous beneficial biological activities, but scarce information on its neuroprotective role against potassium dichromate (PDC)-induced neurotoxicity. This research investigated the antioxidant effect of aqueous *Phyllanthus amarus* leaf extract (APALE) on PDC-induced rats.

Materials and methods. Fifty male Wistar rats (120-130g) were randomized into five groups (A-E, n=10). Group A: (Control) distilled water; B: 300mg/kg APALE; C: 17mg/kg PDC; D: 17mg/kg PDC + 400mg/kg APALE; E: 17mg/kg PDC + 200mg/kg APALE. Administrations were once daily via an orogastric cannula for 28 consecutive days. At the end of the experiment, blood samples were obtained for hormonal assay (FSH and LH). The animals were euthanized, and pituitary glands were harvested and homogenized for Superoxide Dismutase (SOD) and Catalase (CAT), Glutathione Reductase (GSH) by x-ray crystallography, Malondialdehyde (MDA) by thiobarbituric acid reacting substances (TBARS) and paraffin embedding sections, for histological and histochemical evaluations.

Results. Morphometric analysis revealed that PDC caused a reduction in body and brain weights, volume, and weight of the pituitary gland. Masson trichrome demonstrates excessive accumulation of collagen fibers on PDC-treated tissues resolved by APALE. There was a significant increase in MDA in the PDC group and a decrease in the APALE groups compared to the control. In APALE groups, the SOD, CAT, GSH, and T-Protein levels significantly increased compared to the control group. PDC significantly decreased LH and FSH levels compared to the control. However, APALE restored these changes.

Conclusions. APALE demonstrated potent protective activity against PDC-induced pituitary toxicity.

Introduction

Neurotoxicity is the direct or indirect effect of chemicals that disrupt the nervous system of humans or animals [1]. It is a form of toxicity when a biological, chemical, or physical agent introduced into the body adversely affects the anatomy and physiology of the central and peripheral nervous systems. Neurotoxins are synthetic or natural substances that can damage or impair the nervous system's proper functioning. Neurotoxicity is caused by exposure to neurotoxins like chemotherapy drugs, radiation, heavy metals, industrial solvents, insecticides, and pesticides.

Potassium is a silvery white metal that reacts quickly with atmospheric oxygen and is found naturally in foods. It maintains the normal level of fluids in the body and conducts electrical impulses throughout the body. Overdose of potassium has been linked to fatigue, paralysis, nausea, vomiting, and diarrhea [2]. The potassium salt of dichromic acid (potassium Dichromate) is an inorganic chemical with oxidizing, allergen, and sensitizer attributes. Potassium dichromate is mainly used in producing potassium dichromate alum in the leather tanning industry. It is also used as a raw material for chromic acid and cement production [3]. It is chronically harmful to human health and the environment. It affects vision by causing blurred vision, redness, pain, and severe tissue burns. Severe exposure to potassium dichromate can result in blindness, ulceration, and skin ulceration. It has been demonstrated to induce toxicity associated with oxidative stress in animals and humans [4].

Phyllanthus amarus (Bhumya *amalaka*, *Phyllanthus amarus* Schumis) of the family of Euphorbiaceae, is an herbal leaf plant primarily found in tropical and subtropical regions of Americas, China, South East Asia and Africa. It contains different classes of organic compounds of medicinal importance, including alkaloids, flavonoids, hydrolyzable tannins (Ellagitannins), major lignans, polyphenols, triterpenes, sterols, and volatile oil. *Phyllanthus amarus* contains several pharmacologically important biomolecules whose efficacy has been well-established by several biochemical and pharmacological studies [5,6]. It is most commonly used in the Indian Ayurvedic system of medicine in conditions of the stomach, genitourinary system, liver, spleen, and kidney problems [7-9] also for a variety of ailments including dropsy, diabetes, jaundice, asthma and bronchial infections [10]. *Phyllanthus amarus* has been proven to have antioxidant properties and devoid of genotoxicity and pro-oxidant property [5,8]. This study aims to elucidate the neuroprotective effect of *Phyllanthus amarus* on oxidative stress, hormonal imbalance, and histology of potassium dichromate induced-neurotoxicity in the pituitary gland of male Wistar rats

Materials and methods

Research design

Fifty adult male Wistar rats weighing an average of 120-130 g divided into five groups (n = 10), as shown in **Table 1**.

Extraction of Aqueous Extract of *Phyllanthus amarus*

Fresh leaves of *Phyllanthus amarus* were harvested in bulk from the environs of the University of Medical Sciences, Ondo, Ondo State. The leaves were immediately taken for identification at the Plant Biology Department, Adeyemi College of Education, with Batch Number CAE/BIO/22/010. The fresh leaves were air-dried for seven days, pulverized, weighed, and kept in an air-tight con-

Table 1. Treatment regimen across experimental groups.

Groups	Treatment
1 – Control	Received saline water
2 – APALE only	Received 300 mg/kg of aqueous *Phyllanthus amarus* leaf extract
3 – PDC only	Received 17 mg/kg of Potassium Dichromate
4 – High APALE + PDC	Received 400 mg/kg of aqueous *Phyllanthus amarus* leaf extract+ 17 mg/kg of Potassium Dichromate
5 – Low APALE + PDC	Received 200 mg/kg of aqueous *Phyllanthus amarus* leaf extract+ 17 mg/kg of Potassium Dichromate

tainer. Afterward, (600 g) was macerated in 6 liters of distilled water for 48 hours at 4˚C in the refrigerator. The bottle with its container was sealed, kept at room temperature, and allowed to stand for seven days with irregular shaking. The extract was sieved, and the mixture was filtered on Whatman (No. 1) filter paper. The filtrate was evaporated in an air-circulating oven at 42˚C until dried. The crude extract was placed in small glass dishes and incubated at 28˚C. Afterward, the extract was dissolved in an appropriate volume of distilled water to make different doses of 200 mg/kg, 300 mg/kg, and 400 mg/kg to be administered orally to experimental animals.

Chemicals and drugs

Normal saline; Potassium Dichromate (500 g; molecular weight 294.18; UN Number 3288) purchased from Pyrex Scientific Company, Benin; Donepezil from Uche Care Pharmacy LTD, Ondo.

Ethical approval and care of animals

The study was submitted for review, and approval was granted by the Research Ethical Committee of the University of Medical Sciences, with the ethical approval number NHREC/TR/UNIMED-HREC-Ondo St/22/06/21. The experiment was conducted at the Department of Anatomy, Faculty of Basic Medical Sciences, University of Medical Sciences. The animals were acquired from the Laboratory Animal Centre of the University of Medical Sciences, Ondo. They were fed with standard rodent pellet food and water ad libitum throughout the experimental period. They were acclimatized for one week before the commencement of the experiments, after that; each animal was randomly distributed according to the experimental design. Experimental animals used in this research were cared for and maintained in the animal facility regulations, guidelines, and policies governing the use of animal research as described in public health service policy on human care and use of laboratory animals, as approved by the Institute of Laboratory Animal Resource, National Research Council [11].

Determination of body weight, brain weight, and brain volume

The experimental animals were weighed weekly before the commencement of the experiment and during the experiment using an electronic digital weighing scale (Ohaus). On completion of the experiment, the rats were sacrificed by cervical dislocation. The brains were dissected and weighed using a light-sensitive weighing balance. Brain volume was accessed by displacement of liquid.

Determination of Histological Demonstration

After 28 days of administration with a 24 (twenty-four) hours window of the last administration, the skulls were opened using bone forceps to expose the rat's brain, and the whole brain was fixed in 10% formal saline. The brain tissues were excised and dissected, and the pituitary glands were obtained stereotaxically. The pituitary gland was processed for paraffin embedding. Sections were stained for histological and histochemical evaluations using Hematoxylin and eosin and Masson trichrome staining techniques respectively.

Determination of hormonal level

Blood samples collected through the rats' retro-orbital vein for analysis of the Follicle Stimulating Hormone and Luteinizing hormones were centrifuged for 15 minutes using a centrifuge model 800B. The serum extracted was frozen until the time of the analysis. The hormones were analyzed through immunoenzymometry method using a Microplate Reader model: MSLER08 manufactured by Medsinglong Global Group Co., Ltd, China.

Determination of concentration of biochemical activities

The homogenate of the pituitary glands was obtained and then centrifuged at 12,000 rpm to obtain the supernatant containing tissue lysates. The supernatants obtained were stored at low temperatures and assayed to analyze the concentration of Superoxide dismutase (SOD), catalase (CAT), Malondialdehyde (MDA), and Glutathione Reductase (GSH) activities in the tissue lysates using appropriate enzyme lysate immunosorbent assay kits.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism statistical package (version 8).

Results generated from the studies were presented as Mean ± S.E.M. One–way ANOVA and Two-way ANOVA were used to determine any significant difference among the groups with a confidence limit of 95% (0.05).

Results

Physical observations

The rats treated with PDC had a reduced feeding habit and an increased mortality rate. Some clinical signs observed include swollen testes, eye discharge, gum injuries, and cannibalism. Rats administered with only APALE for 28 days did not exhibit the above signs.

Effect of treatment on body weight, brain weight, pituitary gland weight, and brain volume

Figure 1 shows a Morphometric analysis of body weight, pituitary gland weight, brain weight, and volume across all the experimental groups. A significant decrease ($p < 0.05$) was observed in the final body weight of rats treated with PDC only when compared to the control. Also, there was a significant ($p < 0.05$) increase in the final body weight of rats pretreated with 200 mg/kg, 300 mg/kg, and 400 mg/kg body weight of *Phyllanthus amarus* when compared to the PDC-only group (chart A). Morphometric analysis of the brain weights revealed a substantial reduction in the brain weight of the PDC-only treated group compared to the control group (chart B). Brain volume showed no significant difference between the groups, although a slight reduction in brain volume was noticed in the PDC group compared to the control group (chart C). A significant difference was observed between the control and PDC-only-treated groups in pituitary gland weight. Results showed that PDC-treated rats substantially reduced the pituitary gland's weight compared with the control (chart D).

Effect of treatment on histology of pituitary gland

Hematoxylin and Eosin (H&E)

Figure 2 shows the histology of the sections of the pituitary gland showing the distinct blue-stained nuclei of pituitary neurons in the groups (yellow arrows). In plates A and B (control & APALE groups), there is no distinct or observable alteration in tissue morphology compared to C (PDC group). Plate C (PDC group) shows several distorted neurons at different stages of degeneration, nuclear dissolution and fragmentation, and

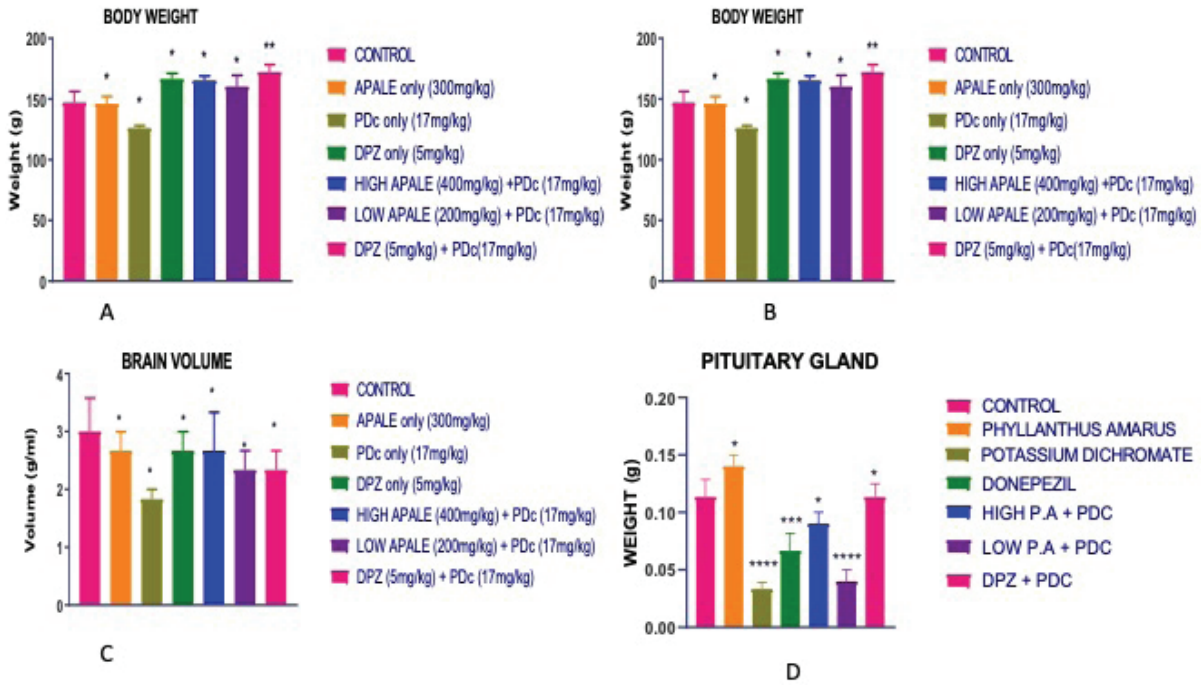

Figure 1. Morphometric analysis of body weight, pituitary gland weight, brain weight, and volume across all the experimental groups.

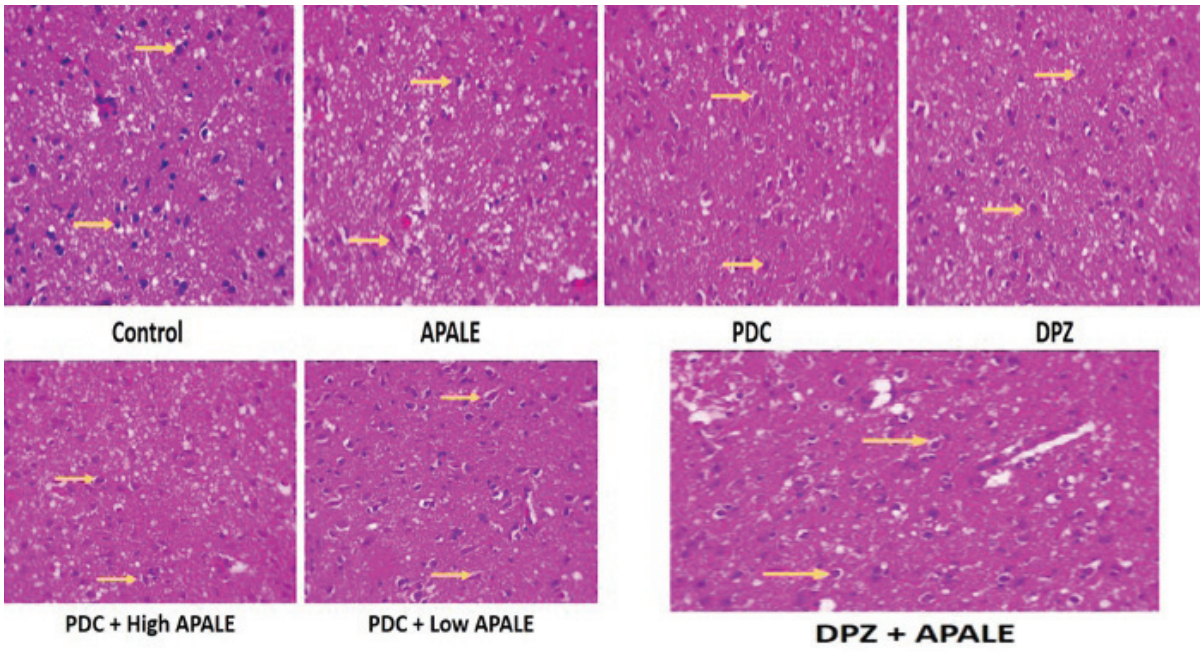

Figure 2. Histology of the sections of the pituitary gland showing the distinct blue-stained nuclei of pituitary neurons in the groups (yellow arrows).

vacuolation compared to the other groups. Plates D & E (low & high APALE groups) show signs of recovery compared to the control group.

Masson trichrome staining

Figure 3 shows the histology of the sections of the pituitary gland tissue with red-stained distinct neurons on a red background in all tissues. However, the administration of PDC (plate C) resulted in the accumulation of collagen fibers compared to the other groups (plate A, B, D, and E). the treatment with APALE at low and high doses significantly restored tissue appearance compared to control.

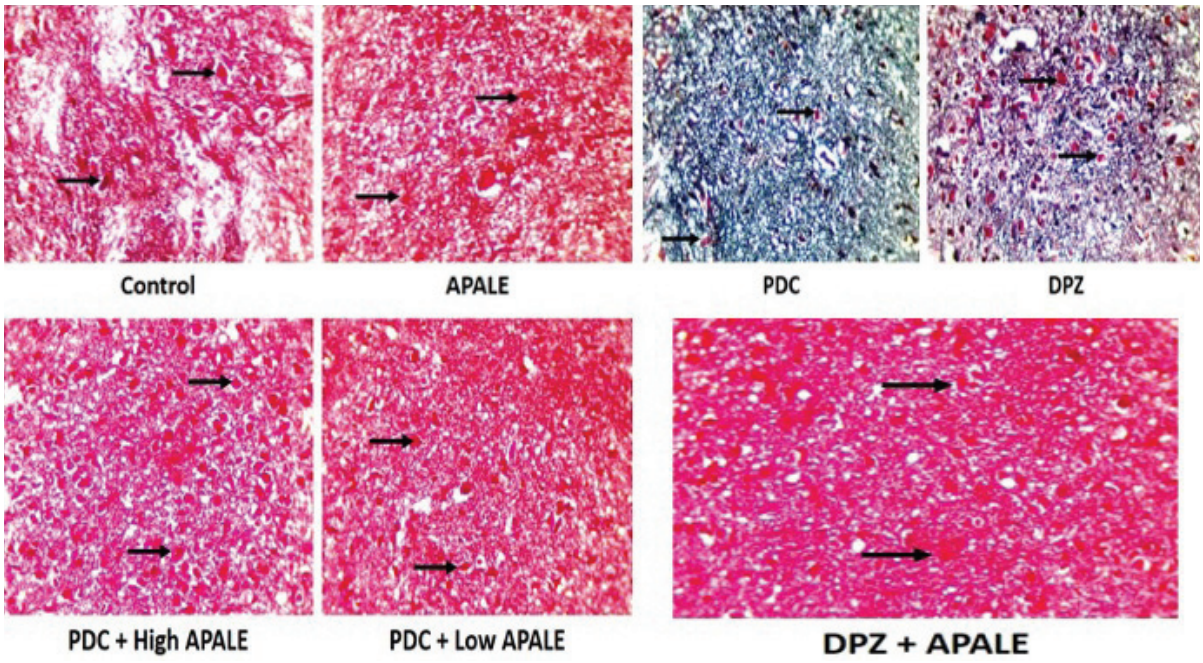

Figure 3. Histology of the sections of the pituitary gland tissue with red-stained distinct neurons on a red background.

Effect of treatment on the biochemical activity of the pituitary gland

Figure 4 shows the charts for the oxidative stress markers. A significant increase ($p < 0.05$) was observed in the SOD level of rats treated with 200 mg/kg of APALE compared to the control and other groups. There was no significant difference in T-protein levels among all the groups (chart B). A significant increase ($p < 0.05$) was observed in Catalase-level rats treated with 200 and 400 mg/kg of APALE compared to the control and PDC groups (chart C). A significant increase ($p < 0.05$) was observed in the GR level of rats treated with 300 mg/kg of APALE compared to the control. No significant difference was observed in the other treatment groups compared to the control group (chart D). A significant increase ($p < 0.05$) was observed in the MDA level of rats treated with PDC compared to the control, while a significant decrease ($p < 0.05$) in the level of MDA of rats treated with 200 mg/kg and 400 mg/kg of APALE compared to the PDC only group (chart E).

Effect of treatment on the hormonal level

Figure 5 shows the charts for the hormonal assay. No significant difference was observed in the LH and FSH levels of rats treated with PDC compared to the control. However, there was a significant ($p < 0.05$) increase in the level of LH and FSH of rats treated with 200 mg/kg, 300 mg/kg, and

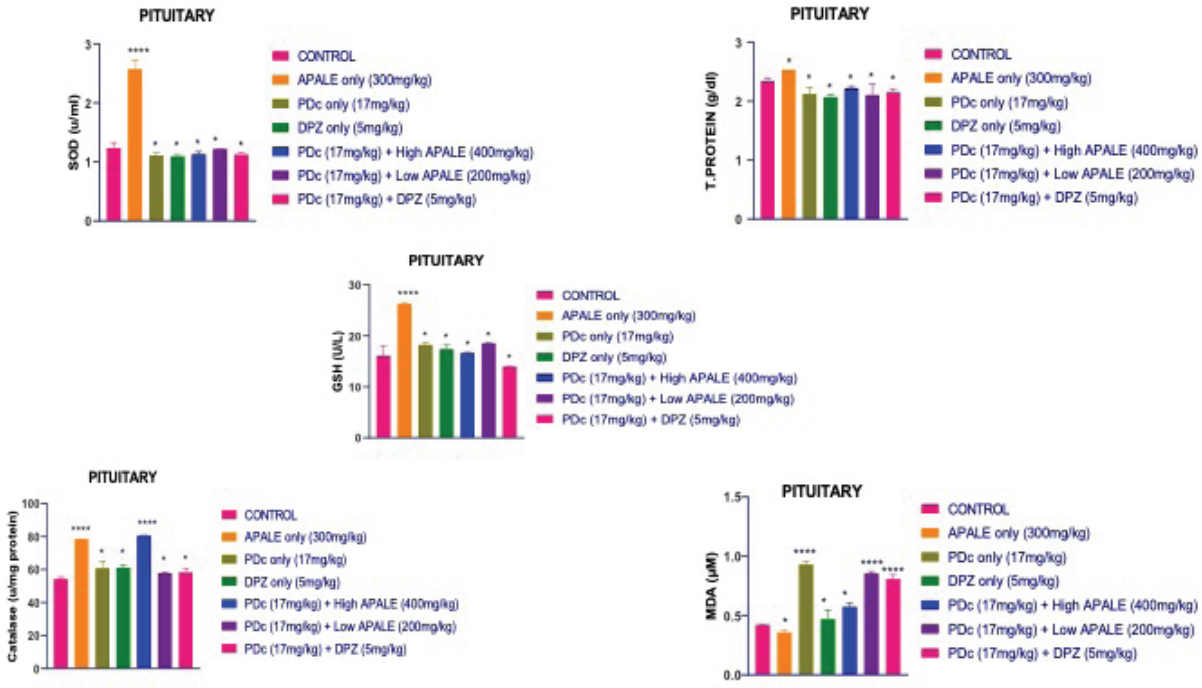

Figure 4. Charts for the oxidative stress markers.

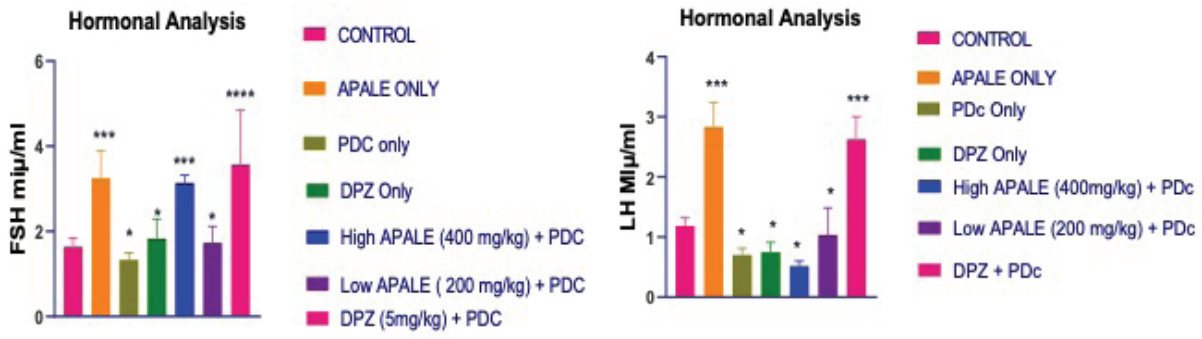

Figure 5. Charts for the hormonal assay.

400 mg/kg of Phyllanthus amarus when compared to the PDC-only group.

Discussion

This study sought to develop a novel natural-based neuroprotective agent against chromium-induced pituitary damage and consequent infertility following exposure to potassium dichromate. *Phyllanthus amarus* (APALE) was chosen for the study because of its protective properties [12-14]. In this study, the overall body weight of all experimental animals across the groups was measured and analyzed at the end of the administration period. It was noticed that oral administration of potassium dichromate to Wistar rats led to a significant decrease in body, brain, and pituitary weight. However, different treatments with APALE resulted in a significant weight gain in the body, brain, and pituitary gland. Weight loss is considered an indication of an underlying condition. Evidence indicates that metal toxicity in rats is associated with body and brain weight loss and morphological damage if not treated appropriately [15]. According to Momo [4], animals treated with potassium dichromate registered a significant decrease in body weight in a dose-dependent manner compared to animals that received only distilled water. In addition, Snejana and colleagues reported a significant decrease in daily feed consumption in animals exposed to potassium dichromate and, consequently, a reduction in body weight compared to animals treated with water [16], corroborating the findings of the above studies, our results elaborate on the body, brain, and pituitary weight loss due to reduced feeding in the rats exposed to PDC. Besides, it has been proven that the toxicity of heavy metals is a significant threat, and several health risks are associated with it; chronic exposure to heavy metals was associated with weight loss and brain damage [17]. Significantly, APALE proved its neuro-protective role against PDC-induced neurotoxicity by protecting against body, brain, and pituitary gland weight loss.

Collagen accumulation (fibrosis) was observed and stained with Masson trichrome in tissues of animals treated with oral administration of potassium dichromate. In addition, PDC caused nuclear distortion and fragmentation, as shown in our H & E photomicrographs. However, different treatment doses of APALE resulted in significant restoration of the tissue appearances and distortion. Fibrosis is the formation of fibrous connective tissue in response to an injury. It is characterized by collagen accumulation at the injury site; continued activation is highly detrimental and a final pathway of numerous diseases [18]. Besides, Bucher [19] has observed focal ulceration, hyperplasia, and metaplasia in rats and mice consuming sodium dichromate-treated water. Furthermore, it has been previously reported in some studies that chromium accumulates in both the pituitary and hypothalamus, thereby resulting in apoptotic damage to neuronal nuclei (nuclear fragmentation) and caspase 3 upregulation [19,20] this is consistent with our findings from this study.

The mechanism of PDC action is associated with mitochondrial and lysosomal injury by biologic chromium (VI) reactive intermediates and reactive oxygen species (ROS) [21]. Hence, biochemical activities were measured and analyzed at the end of the administration period by assaying the level of concentration of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Reductase (GSH), Total protein, and Malondialdehyde (MDA). Superoxide Dismutase (SOD) is an enzyme that constitutes a crucial antioxidant defense against oxidative stress. Its mechanism of action involves acting as a good therapeutic agent against reactive oxygen species-mediated diseases [22]. Our study observed that rats treated with APALE only had the highest level of SOD compared to the other groups pretreated with PDC before APALE.

In contrast, those treated with PDC only showed reduced levels of SOD compared to the control group. This finding was also evident in the report of Momo et al., which indicated that rats treated with PDC registered a significant decrease in the level of SOD [15]. In addition, Ogunmoyole and colleagues noted that treatment with APALE restored the activity of SOD to a level comparable with the negative control [23]. It was further noticed that APALE in the treatment group could not restore the significant decrease in T-protein observed in the rats treated with PDC compared to the control group.

Catalase is a crucial antioxidant enzyme that reduces oxidative stress by destroying cellular

hydrogen peroxide to produce water and oxygen [24]. Rats treated with PDC had significantly lower catalase levels than the other groups. However, treatment with various doses of APALE significantly increased the level of catalase in rats treated with PDC. This result contradicts that of Momo [4], who posited that PDC administration decreased catalase activity levels compared to control animals. However, our result agrees with that of Ogunmoyole and colleagues, stating that treatment with APALE restored catalase activity in serum and tissue homogenates [23]. Besides, an increase in catalase suggests a response toward increasing ROS generation [25], which is important in the oxidative pathway.

Glutathione Reductase maintains the supply of reduced glutathione [26]. Protecting cells from toxicity and oxidative damage and maintaining redox balance are critical [27]. It was observed that rats solely treated with APALE had the highest level of GSH, while those pretreated with PDC before APALE had reduced GR but were not significant compared to the control. This finding contrasts with that of Momo and his colleagues, which suggests that PDC patients significantly had decreased GSH (also known as GR) [4]. However, it partly corroborates that Ogunmoyole and colleagues observed that treatment with APALE markedly raised intracellular GSH (GR) concentration [23].

Malondialdehyde (MDA), a lipid peroxidation product, is a biomarker for oxidative stress. It was observed that PDC administration resulted in an increase in the levels of MDA in rats. At the same time, those treated with various doses of APALE had significantly reduced levels of MDA. Momo et al. stated that those treated with PDC registered an increased level of MDA relative to the control [4]. Also, it is reported that APALE restored antioxidant capacity by inhibiting lipid peroxidation [23]. Significantly treatment with APALE restored and increased superoxide dismutase, glutathione reductase, total protein, and catalase activities while reducing malondialdehyde.

The gonadotropic hormones consist of LH and FSH, secreted by the adenohypophysis and only essential for reproduction. They are called gonadotropin because they stimulate the gonads (the testes in males and ovaries in females) [28]. LH is answerable for gonadal release of sex steroids. In the testes, LH binds to receptors on Leydig cells causing synthesis and secretion of testosterone. In the ovary, theca cells are activated in response to LH and secrete testosterone, this is converted to estrogen through the help of adjacent granulosa cells [28]. FSH is responsible for the stimulation and maturation of ovarian follicle in females and for sperm production in males. FSH supports Sertoli cells functions thereby aiding many facets of sperm cell maturation. The actuator of the secretion of gonadotropic hormone is GnRH, which is the chief controller of the HPG axis [28].

In this research, it was noticed that oral administration of potassium dichromate to Wistar rats led to a significant decrease in FSH and LH. However, different treatments with APALE resulted in a significant increase in FSH and LH levels. This protective effect could be occasioned by its antioxidant function, increasing the antioxidant protective mechanism of cells and tissues, thereby scavenging ROS and restraining lipid peroxidation of tissues. Evidence indicates that exposure to heavy metals like potassium dichromate adversely affects FSH and LH levels, including the hypothalamic-pituitary-gonadal axis, which plays a crucial role in the normal functioning of the reproductive system. According to Momo [4], animals treated with potassium dichromate registered abnormal levels of sex hormones. In addition, a significant decrease in levels of FSH and LH was recorded in potassium dichromate-treated as compared with control [4], corroborating the results of the above studies. The reduced secretion of LH could be an indication of increased Glucocorticoids secretion and decreased sensitivity of pituitary gonadotroph to GnRH, [29]. The gonads are directly affected by stress hormones which reduce the rate of Leydig cells response to LH or reduces testicular receptors to this hormone [29], with concurrent change in sex steroid production [30]. However, Glucocorticoids reduces sexual hormones receptors concentration by creating resistance in target tissues of gonadal steroids [29]. As a result, abatement in testosterone and reducing sexual incitement and fertility [30,31].

It has been proven that PDC (VI) toxicity can be a potential risk to the reproductive system [32]. Significantly, APALE proved its neuroprotective role against PDC-induced neurotoxicity by protecting against the decreased level of FSH and LH.

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Conflict of interest statement
The authors declare no conflict of interest.

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Authors' contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by all authors. The first draft of the manuscript was written by Iteire Afoke Kingsley Ph.D., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Availability of data and materials
All data are available on request from the corresponding Author.

References

1. Lein PJ, Spencer PS. Neurotoxicity In: Wexler, P. (ed.), Encyclopedia of Toxicology, Elsevier Inc., Academic Press, 2014;3(3):489-500. ISBN: 9780123864543.
2. Oria M, Harrison M, Stallings VA (eds.). Potassium: Dietary Reference Intakes for Toxicity - Dietary Reference Intakes for Toxicity - Dietary Reference Intakes for Sodium and Potassium - NCBI Bookshelf. 2019, March 5. https://doi.org/10.17226/25353. Accessed 20/12/2022.
3. Turito. Dichromate: Characteristics, Uses, Types. Retrieved December 23, 2022, from https://www.turito.com/blog/chemistry/dichromate.
4. Mary Momo CM, Ferdinand N, Omer Bebe NK, Alexane Marquise MN, Augustave K, Bertin Narcisse V, Herve T, Joseph T. Oxidative Effects of Potassium Dichromate on Biochemical, Hematological Characteristics, and Hormonal Levels in Rabbit Doe (Oryctolagus cuniculus). Vet Sci. 2019 Mar 18;6(1):30. doi: 10.3390/vetsci6010030.
5. Verma S, Sharma H, Garg M. Phyllanthus Amarus: A Review. Journal of Pharmacognosy and Phytochemistry. 2015;3(2):18-22.
6. Sharma SK, Sheela MA. Pharmacognostic evaluation of leaves of certain Phyllanthus species used as a botanical source of Bhumyamalaki in Ayurveda. Ayu. 2011 Apr;32(2):250-3. doi: 10.4103/0974-8520.92552.
7. Patel JR, Tripathi P, Sharma V, Chauhan NS, Dixit VK. Phyllanthus amarus: ethnomedicinal uses, phytochemistry and pharmacology: a review. J Ethnopharmacol. 2011 Nov 18;138(2):286-313. doi: 10.1016/j.jep.2011.09.040.
8. Karuna R, Reddy SS, Baskar R, Saralakumari D. Antioxidant potential of aqueous extract of Phyllanthus amarus in rats. Indian J Pharmacol. 2009 Apr;41(2):64-7. doi: 10.4103/0253-7613.51342.
9. Jayaram S, Thyagarajan SP, Sumathi S, Manjula S, Malathi S, Madanagopalan N. The efficiency of Phyllanthus amarus treatment in acute viral hepatitis A.B and non-A and non-B: an open clinical trial. Indian Journal of Virology. 1997;13:59-64.
10. Foo LY, Wong H. Phyllanthusiin D an unusual hydrolyzable tannin from Phyllanthus amarus. Phytochemistry. 1992;31:711-713.
11. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th edition. Washington (DC): National Academies Press (US); 2011. Available from: https://www.ncbi.nlm.nih.gov/books/NBK54050/ doi: 10.17226/12910.
12. Alagan A, Jantan I, Kumolosasi E, Ogawa S, Abdullah MA, Azmi N. Protective Effects of Phyllanthus amarus Against Lipopolysaccharide-Induced Neuroinflammation and Cognitive Impairment in Rats. Front Pharmacol. 2019 Jun 4;10:632. doi: 10.3389/fphar.2019.00632.
13. Joseph B, Raj SJ. An Overview: Pharmacognostic Properties of Phyllanthus amarus Linn. International Journal of Pharmacology. 2011;7:40-45. doi: 10.3923/ijp.2011.40.45.
14. Karuna R, Bharathi VG, Reddy SS, Ramesh B, Saralakumari D. Protective effects of Phyllanthus amarus aqueous extract against renal oxidative stress in Streptozotocin -induced diabetic rats. Indian J Pharmacol. 2011 Jul;43(4):414-8. doi: 10.4103/0253-7613.83112.
15. Fiati Kenston SS, Su H, Li Z, Kong L, Wang Y, Song X, Gu Y, Barber T, Aldinger J, Hua Q, Li Z, Ding M, Zhao J, Lin X. The systemic toxicity of heavy metal mixtures in rats. Toxicol Res (Camb). 2018 Jan 29;7(3):396-407. doi: 10.1039/c7tx00260b.
16. Petrovici S, Trif A, Petrovici M. Dumitrescu E, Olariu L, Tulcan C, Ghise A. Effect of potassium dichromate intake on feed intake and body weight in female rats, Rattus norvegicus (exposure on three generations). Human & Veterinary Medicine International Journal of the Bioflux Society. 2010;2(1);31-35.
17. Jaishankar M, Tseten T, Anbalagan N, Mathew BB, Beeregowda KN. Toxicity, mechanism and health effects of some heavy metals. Interdiscip Toxicol. 2014 Jun;7(2):60-72. doi: 10.2478/intox-2014-0009.
18. Murtha LA, Schuliga MJ, Mabotuwana NS, Hardy SA, Waters DW, Burgess JK, Knight DA, Boyle AJ. The Processes and Mechanisms of Cardiac and Pulmonary Fibrosis. Front Physiol. 2017 Oct 12;8:777. doi: 10.3389/fphys.2017.00777.
19. Bucher JR. NTP toxicity studies of sodium dichromate dihydrate (CAS No. 7789-12-0) administered in drinking water to male and female F344/N rats and B6C3F1 mice and male BALB/c and am3-C57BL/6 mice. Toxic Rep Ser. 2007 Jan;(72):1-G4. PMID: 17342194.
20. Nudler SI, Quinteros FA, Miler EA, Cabilla JP, Ronchetti SA, Duvilanski BH. Chromium VI administration induces oxidative stress in hypothalamus

and anterior pituitary gland from male rats. Toxicol Lett. 2009 Mar 28;185(3):187-92. doi: 10.1016/j.toxlet.2009.01.003.
21. Patlolla AK, Barnes C, Hackett D, Tchounwou PB. Potassium dichromate induced cytotoxicity, genotoxicity and oxidative stress in human liver carcinoma (HepG2) cells. Int J Environ Res Public Health. 2009 Feb;6(2):643-53. doi: 10.3390/ijerph6020643.
22. Younus H. Therapeutic potentials of superoxide dismutase. Int J Health Sci (Qassim). 2018 May-Jun;12(3):88-93. PMID: 29896077; PMCID: PMC5969776.
23. Ogunmoyole T, Awodooju M, Idowu S, Daramola O. Phyllanthus amarus extract restored deranged biochemical parameters in rat model of hepatotoxicity and nephrotoxicity. Heliyon. 2020 Dec 18;6(12):e05670. doi: 10.1016/j.heliyon.2020.e05670.
24. Nandi A, Yan LJ, Jana CK, Das N. Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. Oxid Med Cell Longev. 2019 Nov 11;2019:9613090. doi: 10.1155/2019/9613090.
25. Amin KA, Hassan MS, Awad el-ST, Hashem KS. The protective effects of cerium oxide nanoparticles against hepatic oxidative damage induced by monocrotaline. Int J Nanomedicine. 2011 Jan 17;6:143-9. doi: 10.2147/IJN.S15308.
26. Couto N, Wood J, Barber J. The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. Free Radic Biol Med. 2016 Jun;95:27-42. doi: 10.1016/j.freeradbiomed.2016.02.028.
27. Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. Mol Aspects Med. 2009 Feb-Apr;30(1-2):1-12. doi: 10.1016/j.mam.2008.08.006.
28. VIVO pathophysiology, 2018. Available at http: www.vivo.colostate.edu/hbooks/pathphys/endocrine/hypopit/lhfsh.html. Accessed 20/12/2022.
29. Retana-Márquez S, Juárez-Rojas L, Casillas F. Interaction of Adrenal and Gonadal Axes during Stress in Males. Research & Reviews: Journal of Zoological Sciences. 2016;4(1):13-17
30. Hardy MP, Gao HB, Dong Q, Ge R, Wang Q, Chai WR, Feng X, Sottas C. Stress hormone and male reproductive function. Cell Tissue Res. 2005 Oct;322(1):147-53. doi: 10.1007/s00441-005-0006-2.
31. Retana-Márquez S, Vigueras-Villaseñor RM, Juárez-Rojas L, Aragón-Martínez A, Torres GR. Sexual behavior attenuates the effects of chronic stress in body weight, testes, sexual accessory glands, and plasma testosterone in male rats. Horm Behav. 2014 Nov;66(5):766-78. doi: 10.1016/j.yhbeh.2014.09.002.
32. Banu SK, Samuel JB, Arosh JA, Burghardt RC, Aruldhas MM. Lactational exposure to hexavalent chromium delays puberty by impairing ovarian development, steroidogenesis and pituitary hormone synthesis in developing Wistar rats. Toxicol Appl Pharmacol. 2008 Oct 15;232(2):180-9. doi: 10.1016/j.taap.2008.06.002.

ORIGINAL PAPER

JMS Journal of Medical Science

Comparison of effectiveness between two different doses of intravenous dexmedetomidine as adjuvant to subarachnoid block for sub umbilical surgeries

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ABSTRACT

Background. Spinal anesthesia was a commonly used technique in anesthetic practice for lower abdominal and lower limb surgeries. To prolong the duration of bupivacaine spinal anesthesia adjuvants like α2 agonists and opioids have been used intrathecally. Clonidine and dexmedetomidine have also been found to prolong the duration of spinal anesthesia when given intravenous. Dexmedetomidine was more suitable adjuvant to spinal anesthesia compared to clonidine as it has more sedative and analgesic effects due to more selective α2A receptor agonist activity. Dexmedetomidine has been shown to prolong the duration of analgesia of spinal anaesthesia in various studies. Here we compare the two doses of Dexmedetomidine in prolonging the duration of analgesia.

Material and methods. 60 American Society of Anaesthesiologists(ASA) physical status I/II patients scheduled for elective lower abdominal and lower limb surgeries under spinal anesthesia were randomized into two groups of 30 each. Immediately after subarachnoid block with 3.5ml of 0.5% hyperbaric bupivacaine, Group A patients received a loading dose of 0.5µg/kg of dexmedetomidine intravenously in 100ml NS over 10 mins whereas Group B received 1.0µg/kg of dexmedetomidine intravenously in 100ml NS over 10 mins.

Results. Time for rescue analgesic were higher in Group B compared to Group A which was statistically significant but clinically the extra duration was insignificant. Time for two segment regression and duration of motor blockade was significantly prolonged in Group B. Requirement of Mephentermine was comparable in both the groups. There was no excessive sedation in both the groups.

Conclusions. Dexmedetomidine administered as isolated loading dose of 0.5 µg/kg IV immediately after spinal anaesthesia was clinically equi-efficacious in prolonging the duration of analgesia of spinal anaesthesia compared to a larger dose of 1.0 µg/kg. The side effect profile, hemodynamic stability, sedation levels, need for vasopressors and atropine were comparable in both groups.

Introduction

Spinal anesthesia was a commonly used technique in anesthetic practice for lower abdominal and lower limb surgeries. To prolong the duration of bupivacaine spinal anesthesia adjuvants like α2 agonists and opioids have been used intrathecally [1]. Clonidine and dexmedetomidine have also been found to prolong the duration of spinal anesthesia when given intravenously [2]. Dexmedetomidine was initially launched for sedation in humans and most commonly used in ICUs globally for short term sedation [3]. It was a highly selective alpha 2 receptor agonist(alpha2: alpha 1 of 1600:1) when compared to Clonidine(200:1). The analgesic action of dexmedetomidine was by its action on presynaptic membrane, inhibiting the release of norepinephrine, which in turn induces hyperpolarization and inhibits the pain signals to the brain [4]. The usage of intravenous alpha agonists in prolonging the duration and quality of spinal anaesthesia was not very popular. Intrathecal usage of alpha agonists are more commonly employed though it was an off-label use of the drug. There are only limited studies describing the most effective doses of Dexmedetomidine as an intravenous adjunct to subarachnoid block.

In this study we try to compare the two commonly employed doses(1.0 µg/kg and 0.5 µg/kg over 10 minutes) of intravenous dexmedetomidine as an adjunct to spinal anaesthesia. We evaluate difference between the two doses with respect to total duration of analgesia(time to rescue analgesia), two segment regression of sensory blockade and motor blockade. Secondarily we also observe the differences if any, with regards to the hemodynamic stability(Heart rate, Blood pressure), side effect profile, sedation levels both intra-operative and in post-operative period.

Materials and methods

After approval of Institutional Ethical committee clearance (IEC/NRIIMS/A/05/2019), sixty patients scheduled for surgeries under Subarachnoid block in NRI INSTITUTE OF MEDICAL SCIENCES, Visakhapatnam, India were enrolled into the study. The study was conducted during the period of October 2019 to November 2020. Sample size was calculated as 30 for each group, estimated based on study by Madhavi Unmesh Santpur et al [5]. Patients of age between 18 to 60 years, ASA (American Society of Anaesthesiologists) grade: I – II, Patients undergoing infra umbilical surgeries were included in the study. Patients in whom there was a contraindication for spinal anaesthesia, ASA grade III – V, Systolic blood pressure <90 mm Hg, Heart rate less than 50/min, patients on Calcium channel blockers, ACE inhibitors, clonidine, patients on opioids, patients on antidepressants a week prior to surgery and patients undergoing lower segment Caesarean sections were excluded from the study. Sixty patients were divided by computer generated random number table into Group A and Group B with 30 subjects in each group. **Group A** patients received a loading dose of 0.5 µg/kg of dexmedetomidine intravenously in 100 ml NS over 10 mins whereas **Group B** received 1.0 µg/kg of dexmedetomidine intravenously in 100 ml NS over 10 mins immediately after administration of Spinal anaesthesia.

During pre-anaesthetic evaluation an informed and written consent was taken from patients who were included in the study and patients were explained on the methods of sensory and motor assessments. Patients were also educated on the usage of Visual analogue scale in the post operative period. All patients in study groups were kept nil by mouth from midnight before day of surgery. On the day of surgery before commencement of anaesthesia, intravenous line was secured with 18-gauge cannula. Preloading was done with 15 ml/kg Ringer Lactate 30 min prior to procedure. Pulse oximeter, noninvasive blood pressure (NIBP), and electrocardiography monitors were connected to all patients on arrival to operating room and baseline parameters were noted. The patient and anesthesiologist were blinded to the study groups, and all the recordings were noted by an anesthesiologist, who was blinded to randomization schedule. Under strict aseptic precautions, lumbar puncture was done at the level of L3-L4 intervertebral space through midline approach by using a 25-gauge Quincke spinal needle. After confirmation of free flow of cerebrospinal fluid 17.5 mg of 0.5% hyperbaric bupivacaine was given intrathecally. Group A: Intravenous dexmedetomidine 0.5 µg/kg in 100 ml NS loading dose was administered in the first 10 min immediately after spinal anesthesia. Group B: Intravenous dexme-

detomidine 1 µg/kg in 100 ml NS loading dose was administered in the first 10 min immediately after spinal anesthesia. Heart rate, non-invasive blood pressure, and saturation of oxygen was recorded before the subarachnoid block, every five-minute interval in the initial 30 mins of surgery, later for every 15 mins throughout surgery, and after 30 minutes in postoperative period.

Sensory blockade was checked with pin prick in mid axillary line from caudal to cephalad direction. Onset of analgesia was checked by loss of sensation to pinprick at T10 dermatome. The highest level of analgesia after 10 min was assessed. Time for two segment regression from highest level of sensory block (duration) was noted. Time from onset of subarachnoid block to the time of administration of rescue analgesia was considered as duration of analgesia. Motor blockade was assessed by Modified Bromage Scale. Time taken for motor blockade to reach Modified Bromage Scale 3 was taken as onset of motor blockade and regression to Modified Bromage Scale 0 was taken as duration of motor blockade. The sedation level was evaluated using Ramsay Level of Sedation Scale The level of sedation was assessed intraoperatively for every 5 for initial 30 min and for every 15 mins till the end of the surgery and for every 30 min till 12 h. in postoperative period in PACU. Excessive sedation was considered as score greater than 4/6. Hypotension, defined as decrease in systolic blood pressure of more than 20% from baseline and was treated with an intravenous bolus dose of 6 mg mephenteramine. The total number of bolus doses required throughout the intraoperative period was noted. Heart rate <50, defined as bradycardia, was treated by a bolus dose of 0.6 mg atropine. The total number of doses of atropine required was noted. Pain score was measured using visual analogue scale in postoperative period for every 30 min for 2h, thereafter for every 1hr. Rescue analgesic was given when VAS score was greater than 3. Time for rescue analgesic was noted. Patients were given 100 mg of Tramadol as slow IV as rescue analgesic.

Descriptive statistical analysis was done in present study. Results of continuous measurements are represented as Mean ± SD and results of categorial measurements are represented in Number (%). Chi-square test was used for calculation of significance of study parameters on categorial scale between two or more groups. Fishers exact test was used for calculation of significance of the study parameters on categorial scale (frequency tables). Paired t test was used for calculation of significance of the study parameters on continuous scale within group. Student independent t test was used for calculation of significance of the study parameters on continuous scale between groups. P value <0.05 was considered as statistically significant. Jamovi software [6] was used for analysis of the data and Microsoft word and excel were used to generate graphs, tables.

Results

This study was carried out on 60 patients operated under spinal anesthesia. Demographic data, intraoperative and postoperative hemodynamics, oxygen saturation, Ramsay sedation scores, postoperative analgesia and side effects were compared between Group A and Group B.

Demographic data: The mean age of Group A was 42.03 ± 10.85 yrs. compared to 41.27 ± 8.20 yrs. in the Group B and difference was statistically not significant (P value 0.759). BMI: The mean BMI in Group A was 24.91 ± 4.94 kg/m^2, compared to 23.23 ± 3.02 kg/m^2 in Group B and difference was statistically insignificant (P value-0.118). Weight distribution in both the groups as summarized (**Table 1**). ASA grading of patients from both the groups was not statistically significant (P value-0.417). The mean duration of surgery of Group A was 104.83 ± 17.83 minutes, compared to 106.83 ± 22.07 minutes in Group B and the difference was not statistically significant (P value – 0.701) (**Table 1**). Gender distribution in both groups was also not statistically significant (P value – 0.592) (**Table 2**).

There was no significant difference in time for onset of sensory and motor blockade as shown in (**Table 3**). Preoperative, intraoperative and postoperative heart rate in both the groups are shown in (**Figure 1**). The average systolic blood pressure was lower in Group B (Dexmedetomidine 1 µg/kg) (116.24 ± 9.77), compared to Group A (Dexmedetomidine 0.5 µg/kg) (120.25 ± 13.44). The average postoperative SBP was lower in Group B (112.00 ± 12.96 mmHg) as compared to Group A (117.27 ± 17.02 mmHg) (**Figure 2**). Intraoperative and postoperative DBP in both groups was

Table 1. Baseline Characteristics of the groups.

	Group A	Group B	(p-value)
Age	42.03 ± 10.85	41.27 ± 8.2	0.759
BMI	24.91 ± 4.94	23.23 ± 3.02	0.118
Surgery duration	104.83 ± 17.83	106.83 ± 22.07	0.701

Table 2. Gender distribution.

Study groups	Gender	Frequency	%	P value
Group A	Male	18	60	0.592
	Female	12	40	
	Total	30	100	
Group B	Male	20	66.7	
	Female	10	33.3	
	Total	30	100	

summarized in **Figure 3**. There was no statistically significant difference in SPO2 levels between both groups during surgery and in postoperative period. VAS scores are summarized in **Figure 4**. Intraoperative Ramsay sedation scores were high in Group B (3.93 ± 0.25) compared to Group A (3.20 ± 0.66) (P value <0.05). Ramsay sedation scores are summarized in **Figure 5**. Requirement of Mephentermine and Atropine doses in both groups was also comparable.

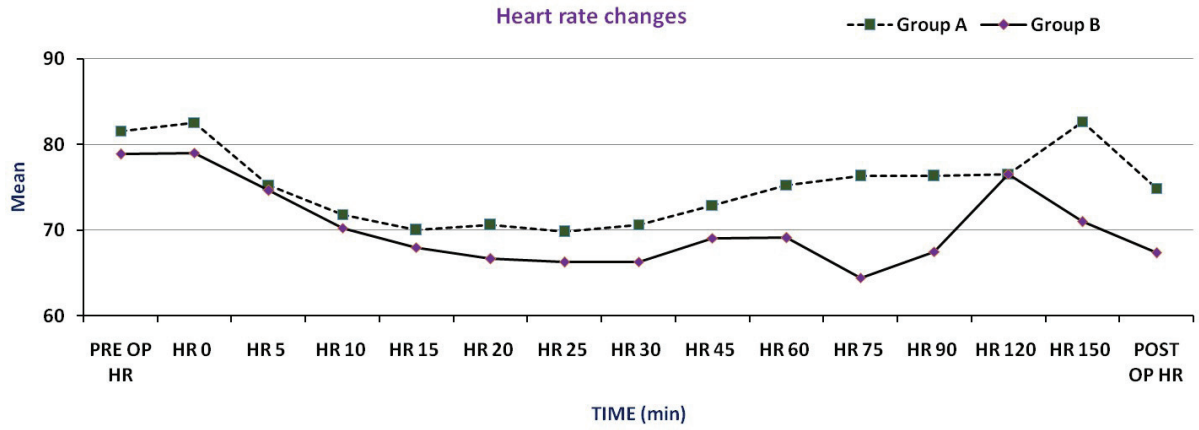

Figure 1. Heart rate changes.

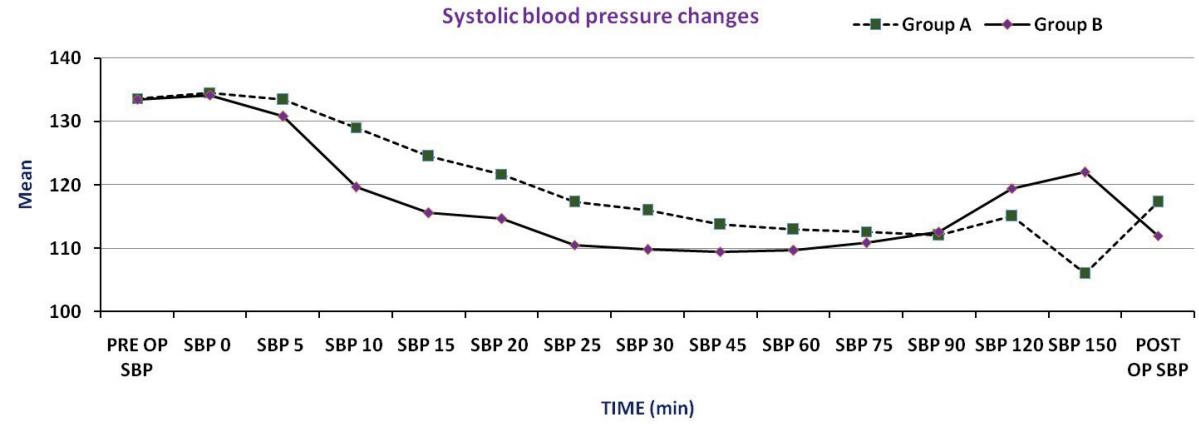

Figure 2. Systolic Blood Pressure changes.

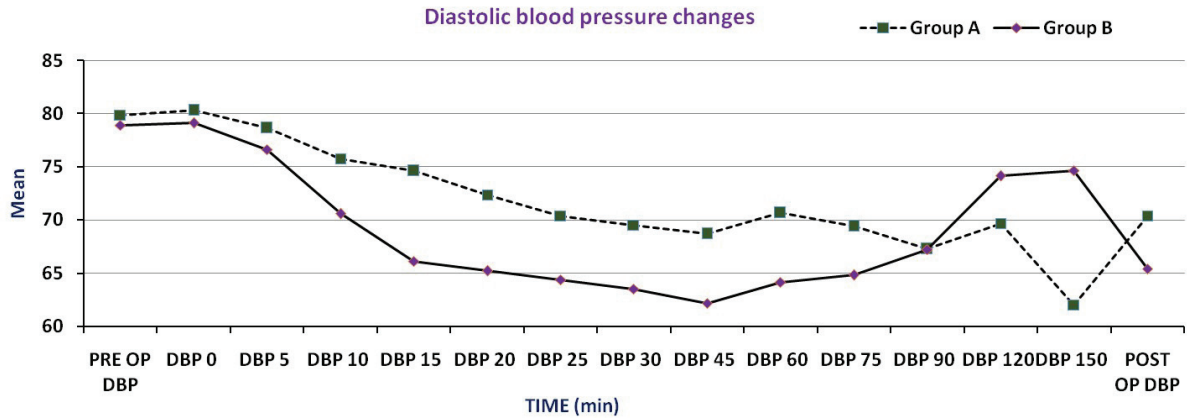

Figure 3. Diastolic BP.

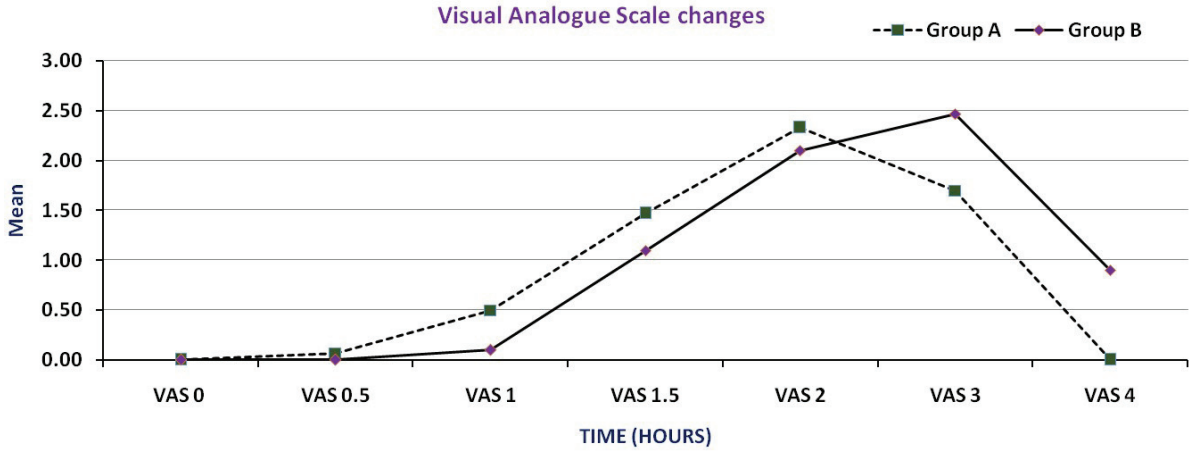

Figure 4. VAS scores.

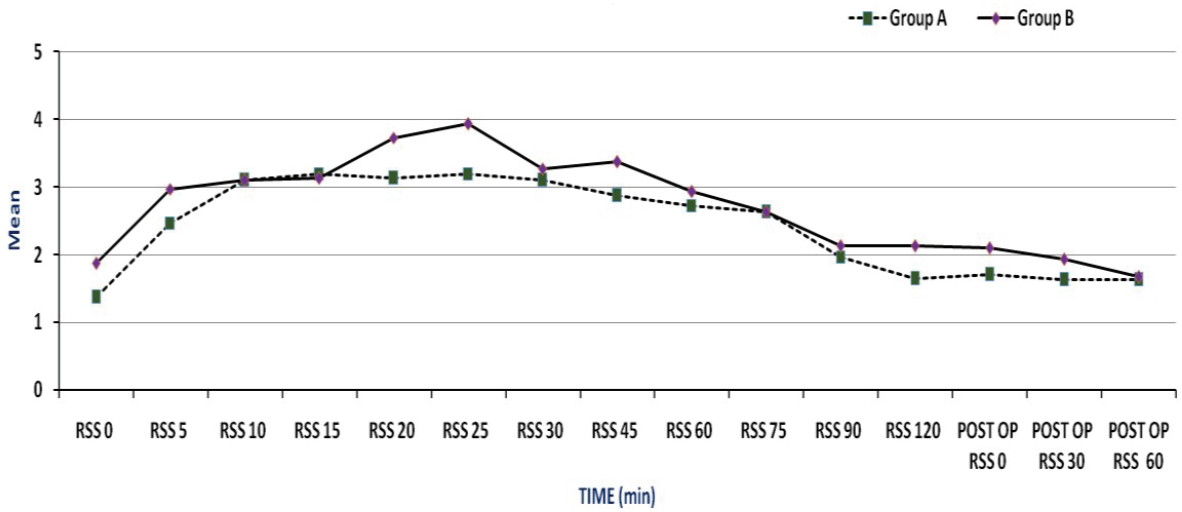

Figure 5. Ramsay sedation scores.

Table 3. Sensory and motor blockade in both groups.

	Group A	Group B	(p-value)
Onset sensory (min)	2.96 ± 0.52	2.78 ± 0.54	0.184
Onset motor (min)	5.19 ± 1.01	4.99 ± 0.99	0.448
Duration of motor	265 ± 19.61	276.83 ± 20.53	0.026
Time to two segment regression	Group A	116.83 ± 11.33	0.025
	Group B	123 ± 9.34	

The duration for two segment regression of sensory blockade and duration of motor block i.e, regression to Modified Bromage Scale 0 was significantly prolonged in Group B(123 ± 9.34) as compared to Group A(116.83 ± 11.33) (P value < 0.05) (**Table 3**). Time for first request of analgesic was longer in Group B (276.00 ± 13.80) compared to Group A) p value 0.07 as statistically significant (**Table 3**).

Discussion

Adjuvants to local anaesthetics such as epinephrine, magnesium sulphate, sodium bicarbonate, opioids and α2 agonists such as clonidine and dexmedetomidine have been used to extend the duration of spinal anaesthesia [7]. Clonidine, an α2 agonist, was commonly used as adjuvant to prolong spinal anaesthesia via oral, intrathecal, and intravenous methods [8]. Both intrathecal and intravenous dexmedetomidine have been demonstrated to prolong spinal anaesthesia in recent studies. Due to its more selective α2 receptor agonist activity, dexmedetomidine was a better adjuvant to spinal anaesthesia than clonidine because it provides more sedative and analgesic effects [9]. Dexmedetomidine acts at the spinal level, lamina VII and VIII of the ventral horns, to cause analgesia when injected intravenously or intrathecally. Sedation and analgesia are also produced by the drug, which acts on the locus coeruleus and dorsal raphe nucleus [4]. Prolongation of spinal anaesthesia following intravenous dexmedetomidine was due to this supra spinal effect [9]. Present study was designed to compare the effect of two different doses of intravenous dexmedetomidine on bupivacaine spinal anaesthesia. Comparing both the groups with respect to Age, Sex, BMI, ASA physical status of the patients that were enrolled into the groups we found that both groups are evenly matched without any statistically significant mismatch. In our study, we observed that the onset of sensory block had a mean duration of 2.96 ± 0.52 minutes in Group A (Dexmedetomidine 0.5 µg/kg) whereas it was 2.78 ± 0.54 minutes in Group B (Dexmedetomidine 1 µg/kg). Student unpaired T test was used to compare the above data and the resultant P value was 0.184. There was no significant difference in the mean duration of onset of sensory block between both groups. This correlates with the study conducted by Mi Hyeon Lee et al [11], which showed both 0.5 and 1 µg/kg of dexmedetomidine as isolated boluses without maintenance infusions showed no significant difference in duration of onset of sensory block. Similarly, Upadhya R Kavya et al [12] in their study also showed time of onset of sensory blockade was not significantly altered by use of dexmedetomidine. In our study, we observed that two segment regression had mean duration of 116.83 ± 11.33 minutes in Group A (Dexmedetomidine 0.5 µg/kg) whereas it was 123 ± 9.34 minutes in Group B (Dexmedetomidine 1 µg/kg). Student unpaired T test was used to compare the above data and the resultant P value was 0.025. Mean duration of two segment regression was slightly higher in Group B compared to Group A, which was statistically significant. In contrast to our study Mi Hyeon Lee et al [11], showed that there were no statistically significant differences in time for two segment regression of sensory blockade between 0.5 and 1 µg/kg dexmedetomidine. The highest level of sensory block was comparable in both groups, 3 patients (47.9%) in Group A achieved T4 sensory level compared to 4 patients (57.1%) in Group B. P value was 0.3 by using fishers exact test. There was no difference in highest level of sensory block achieved in both the groups, which was thus not statistically significant. In our study, we observed that onset of motor blockade was 5.19 ± 1.01 minutes in Group A, whereas it was 4.99 ± 0.99 minutes in Group

B. Student unpaired T test was used to compare the above data and the calculated P value was 0.448. There was no difference in mean duration of onset of motor blockade between both the groups, which was statistically not significant. Similar to our study, Upadhya R kavya et al [12], showed that there was no difference in onset of motor block between Control group, Dexmedetomidine 1mcg/kg bolus Group and Dexmedetomidine 0.5 µg/kg bolus plus 0.5 µg/kg/min infusion Group. Dinesh CN et al [10] also showed that using dexmedetomidine doesn't change onset of motor block.

In our study, we observed that duration of motor block (regression to Bromage 0) was 265 ± 19.61 minutes in Group A compared to 276.83 ± 20.53 minutes in Group B. Student unpaired T test was used to compare the above data and calculated P value was 0.026, which was statistically significant. Mean duration of motor block was slightly higher in Group B compared to Group A, which was statistically significant. Upadhya R Kavya et al [12] also showed duration of motor block was 321.6 ± 35.7 minutes in Dexmedetomidine 1 µg/kg bolus Group, 302.4 ± 18.2 minutes in Dexmedetomidine 0.5 µg/kg bolus plus 0.5mcg/kg/min maintenance Group, and 233.4 ± 34.1 minutes in control group, P value <0.001. In contrast to our study Mi Hyeon Lee et al [11], showed that there was no statistically significant difference in time of regression of motor blockade to Bromage 0 between Dexmedetomidine 0.5 µg/kg bolus Group and Dexmedetomidine 1 µg/kg bolus Group.

In our study the time for first request of analgesic in Group A was 265.5 ± 15.11 minutes, where as it was 276 ± 13.8 minutes in Group B, student unpaired T test was used to compare the above data and calculated P value was 0.007. The mean time for rescue analgesic was slightly higher in Group B compared to Group A, which was statistically significant but clinically the increased duration was not of much significance. Similar to our study Jyotsna Kubre et al [13] in their study showed that first request for postoperative analgesic was significantly prolonged 234.67 ± 7.649 minutes in dexmedetomidine 0.5 µg/kg loading dose group compared to control group 164.17 ± 6.170 minutes. Hong J et al [14], in their study also showed that mean time to first request for postoperative analgesia was longer with dexmedetomidine 1 µg/kg loading dose group 6.6h compared to control group 2.1h. In our study Intraoperative Ramsay Sedation Scores in series were slightly high in Group B compared to Group A. Maximum mean score in Group B was 3.93 ± 0.25, whereas it was 3.20 ± 0.66, P value < 0.05 by using Student unpaired T test. There was no excessive sedation (RSS > 4) in either of the groups. Upadhya R kavya et al [12], showed that patients receiving dexmedetomidine in their study had higher sedation scores (score 3 or 4) with minimal respiratory depression, they were easily arousable. In our study in postoperative period the maximum mean score of sedation in Group B was 2.10 ± 0.61, whereas it was 1.70 ± 0.61 in Group A, P value was 0.006, which was statistically significant. Though it was statistically significant, it was clinically insignificant, which can be attributed to the shorter duration of action of dexmedetomidine. Upadhya R kavya et al [12], Dinesh CN et al [10], failed to detect any difference in postoperative sedation. The average systolic blood pressure was lower in Group B 116.24 ± 9.77, compared to Group A 120.25 ± 13.44. The average postoperative SBP was lower in Group B 112.00 ± 12.96, compared to Group A 117.27 ± 17.02. In our study 2 patients in Group A and 2 patients in Group B had hypotension. The difference was not statistically significant in the incidence of hypotension between 2 groups. Hypotension was easily treatable with IV fluids and Mephenteramine. In similar to our study, Mohammad K AI Nobani et al [15] reported that there was no statistically significant difference in incidence of hypotension between IV dexmedetomidine and control groups. Intraoperative lowest mean heart rate was lower in Group B 64.47 ± 7.60 compared to Group A 69.80 ± 9.09. In our study 2 patients in Group B and none of them in Group A had bradycardia. Incidence of bradycardia was noted in dexmedetomidine 1 µg/kg group and was treated with Atropine 0.6 mg IV. Similar to our study Mohammad K AI Nobani et al [15] reported that higher doses of dexmedetomidine are associated with higher incidence of bradycardia. There was no oxygen desaturation in both groups, though there was increased sedation.

Conclusions

We conclude that Dexmedetomidine prolongs the duration of analgesia/sensory blockade when administered intravenously before administration of spinal anaesthesia. Dexmedomidine in a dose of 1.0 µg/kg has a slightly longer duration of analgesia as well as longer duration of motor blockade compared to a dose of 0.5 µg/kg (10-20 minutes longer approximately) but this duration is not significant in a clinical setting.

We didn't find any major variations between the two doses with regards to the Motor blockade duration, side effects profile, hemodynamic profile, or intra and post-op sedation levels. Hence we recommend that 0.5 µg/kg intravenously should be the preferred dosing as a co-analgesic with spinal anaesthesia.

Limitations

The findings of this study were based on the data having relatively small sample size from single centre.

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Conflict of interest statement
The authors declare no conflict of interest.

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References

1. Adriaensen Khan ZP, Ferguson CN, Jones RM. alpha-2 and imidazoline receptor agonists. Their pharmacology and therapeutic role. Anaesthesia. 1999 Feb;54(2):146-65. doi: 10.1046/j.1365-2044.1999.00659.x.
2. Gabriel JS, Gordin V. Alpha 2 agonists in regional anesthesia and analgesia. Curr Opin Anaesthesiol. 2001 Dec;14(6):751-3. doi: 10.1097/00001503-200112000-00024.
3. Keating GM. Dexmedetomidine: A Review of Its Use for Sedation in the Intensive Care Setting. Drugs. 2015 Jul;75(10):1119-30. doi: 10.1007/s40265-015-0419-5.
4. Tang C, Xia Z. Dexmedetomidine in perioperative acute pain management: a non-opioid adjuvant analgesic. J Pain Res. 2017 Aug 11;10:1899-1904. doi: 10.2147/JPR.S139387.
5. Santpur MU, Kahalekar GM, Saraf N, Losari A. Effect of intravenous dexmedetomidine on spinal anaesthesia with 0.5% hyperbaric bupivacaine in lower abdominal surgeries: A prospective randomized control study.Anesth Essays Res. 2016, 10:497-501. 10.4103/0259-1162.179319.
6. The jamovi project (2022). jamovi. (Version 2.3) [Computer Software]. Retrieved from: https://www.jamovi.org.
7. Krishna Prasad GV, Khanna S, Jaishree SV. Review of adjuvants to local anesthetics in peripheral nerve blocks: Current and future trends. Saudi J Anaesth. 2020, 14:77-84. 10.4103/sja.SJA_423_19.
8. Allen TK, Mishriky BM, Klinger RY, Habib AS. The impact of neuraxial clonidine on postoperative analgesia and perioperative adverse effects in women having elective Caesarean section-a systematic review and meta-analysis. Br J Anaesth. 2018, 120:228-240. 10.1016/j.bja.2017.11.085.
9. Gertler R, Brown HC, Mitchell DH, Silvius EN. Dexmedetomidine: a novel sedative-analgesic agent. Proc(Bayl Univ Med Cent. 2001, 14:13-21. 10.1080/08998280.2001.11927725.
10. Dinesh CN, Sai Tej NA, Yatish B, Pujari VS, Mohan Kumar RM, Mohan CV. Effects of intravenous dexmedetomidine on hyperbaric bupivacaine spinal anesthesia: A randomized study. Saudi J Anaesth. 2014, 2:202-8. 10.4103/1658-354X.130719.
11. Lee MH, Ko JH, Kim EM, Cheung MH, Choi YR, Choi EM. The effects of intravenous dexmedetomidine on spinal anesthesia: comparision of different dose of dexmedetomidine. Korean J Anesthesiol. 2014, 4:252-7. 10.4097/kjae.2014.67.4.252.
12. Kavya UR, Laxmi S, Ramkumar V. Effect of intravenous dexmedetomidine administered as bolus or as bolus plus-infusion on subarachnoid anesthesia with hyperbaric bupivacaine. J Anaesthesiol Clin Pharmacol.2018, 34:46-50. 10.4103/joacp.JOACP_132_16.
13. Kubre J, Sethi A, Mahobia M, Bindal D, Narang N, Saxena A. Single dose intravenous dexmedetomidine prolongs spinal anesthesia with hyperbaric bupivacaine. Anesth Essays Res. 2016, 2:273-7. 10.4103/02591162.174465.
14. Hong JY, Kim WO, Yoon Y, Choi Y, Kim SH, Kil HK. Effects of intravenous dexmedetomidine on low-dose bupivacaine spinal anaesthesia in elderly patients. Acta Anaesthesiol Scand. 2012, 56:382-7. 10.1111/j.13996576.2011.02614.x.
15. Al Nobani MK, Ayasa MA, Tageldin TA, Alhammoud A, Lance MD. The Effect of Different Doses of Intravenous Dexmedetomidine on the Properties of Subarachnoid Blockade: A Systematic Review and Meta-Analysis. Local Reg Anesth. 2020, 15:207-215. 10.2147/LRA.S288726.

REVIEW PAPER

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Iron Deficiency in Pregnancy: A Brief Review

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ABSTRACT

This paper highlights iron deficiency anaemia in pregnancy; its prevalence, causes, screening, and management. Iron deficiency is a spectrum that ranges from iron depletion to iron deficiency anaemia. Iron deficiency is the most common and leading cause of anaemia in pregnancy both in developed and underdeveloped countries. The incidence of iron deficiency anaemia varies worldwide depending on differences in race, socioeconomic factors, nutritional status, health condition, and the frequency of parasitic illnesses. Untreated Iron deficiency has significant adverse foetal and maternal consequences. The management of iron deficiency anaemia includes education regarding dietary modification, specifically ways to enhance iron absorption and iron supplementation. Although oral supplementation is typically the mainstay of treatment, more severe cases of iron deficiency anaemia may require intravenous supplementation.

Introduction

Anaemia is a worldwide public health problem, affecting more than 50% of pregnant women globally and about 90% in low-resourced countries [1]. Iron deficiency (ID) is the depletion of total-body iron, especially of macrophage and hepatocyte iron stores [2]. Because the largest amount of iron is consumed for haemoglobin (Hb) synthesis to produce 200 billion erythrocytes daily, anaemia is the more evident sign of iron deficiency, and iron deficiency anaemia (IDA) is often considered synonymous with iron deficiency [3]. However, iron deficiency is a broader condition that often precedes the onset of anaemia or indicates a deficiency in organs/tissues other than those involved in erythropoiesis, such as skeletal muscles and the heart. The heart is highly iron-dependent for myoglobin and energy production to sustain mechanical contraction [4]. ID was reported as the most prevalent nutrient disorder in both low-and-middle incomes as well as developed countries [5]. Due to regular menstrual flow, and increased iron requirement during pregnancy, women of reproductive age are at a higher risk of ID [6]. Because of the high iron demand to support the placenta and foetus during pregnancy, ID stands to be one of the problems associated with low PCV and decrease blood volume in pregnant women [7]. Fatigue, fever, pagophagia,

and restless legs syndrome are considered the symptoms of ID in pregnancy. Pregnant women with ID are at a higher risk of preterm delivery [5]. Children whose mothers had ID in pregnancy are more likely to be born with cognitive impairment in addition to decreased language motor function and learning [8]. Anaemia is a haematological disorder that is common among pregnant women in many developing/low-income countries. It is a public health problem contributing to high maternal/perinatal morbidity and mortality in most low-income countries [9]. It is a condition of low circulating haemoglobin in which concentration has fallen below a threshold lying at two standard deviations below the median of a healthy population of the same age, sex, and stage of pregnancy, causing decreased oxygen-carrying capacity in a pregnant woman [7]. This reduced oxygen-carrying capacity may affect the oxygen supply to the mother, especially in severe anaemia and resulting in shortness of breath, dizziness and fatigue [4]. In developing countries, it is of serious concern as it has adverse effects on the mother and the foetus and contributes to maternal mortality [10]. It increases the risk of preterm delivery and postpartum maternal infections [6]. Iron deficiency anaemia is a shortage in iron stores, transport, and functional iron causing low haemoglobin (Hb), low serum ferritin, low transferrin saturation, and high erythrocyte protoporphyrin concentration [11]. The World Health Organization (WHO) defines anaemia as haemoglobin value <13g/dL and <12 g/dL in men and women who are not pregnant respectively. A serum iron and ferritin below 7.1µg/L and 30ng/l respectively or a transferrin saturation less than 15% is considered as iron deficiency [5,12–13]. Anaemia in pregnancy is defined as a haemoglobin level of less than 11 g/dL. However, a serum ferritin of <30ng/mL or transferrin saturation <20% is associated with a 98% sensitivity and 92% specificity for absent marrow hemosiderin. [5,14], a haemoglobin value of less than 10 g/dL is considered in most developing countries because previous studies report no significant harm to the foetus until the haemoglobin concentration drops below 10 g/dL [15].

Anaemia has many causes. Direct causes can be largely categorized as deficiency, or abnormal production of red blood cells; excessive destruction of red blood cells; and excessive loss of red blood cells [16]. Contributing causes include insufficient dietary intake, diet quality, hygiene, health performances, adverse environmental situations, deficiency of health facilities, socio-economic status of the family, traditional dietary habits of the area, and irregular eating habit [17]. This paper provides a brief review on IDA in pregnancy, its prevalence, causes, and management.

Methodology

Articles were sought from Google scholar, PubMed, Science direct and Web of Science databases using the following keywords; iron deficiency, Anaemia, iron deficiency anaemia, iron deficiency in pregnancy, anaemia in pregnancy, effects anaemia in pregnancy, causes of anaemia, causes of anaemia in pregnancy, prevalence of anaemia, anaemia screening and diagnosis, treatment of anaemia, management of anaemia, oral and parenteral iron supplementation etc. The articles on anaemia /iron deficiency, iron supplementation/therapy, bioavailability of iron supplements, risk factors of anaemia, diagnosis and management of anaemia, and effects of iron supplementation were selected for the review.

Iron Deficiency in Pregnancy

Haemoglobin concentration decreases during the first trimester. It is important to note that variations exist within the definition of normal haemoglobin levels during pregnancy; for example, normal haemoglobin levels may differ depending on altitude [5]. During a singleton pregnancy, maternal plasma volume increases by approximately 50% and is accompanied by a modest increase in red blood cell (RBC) mass [18]. These changes are responsible for producing the physiologic anaemia that occurs during pregnancy [2]. Iron requirements peak in the second and third trimesters to support the expansion of maternal blood volume and the development of the foetus and placenta [19]. Individuals at greatest risk for developing IDA during pregnancy include women with pre-conception heavy uterine bleeding, women with shorter inter-pregnancy intervals, women who had insufficient iron stores prior to conception, and women with poor dietary intakes

of iron (common in developing countries) [19, 20]. Untreated maternal iron deficiency can negatively impact foetal development, particularly in terms of brain development where iron is required to synthesize the myelin sheath [21].

Prevalence of Iron Deficiency Anaemia in Pregnant Women

The prevalence of IDA varies among countries but is a major public health problem in the developing world, reflecting differences in race, socioeconomic factors, nutritional habits, medical care, and the frequency of parasitic illnesses [22]. A previous study reported that anaemia in pregnancy has a prevalence rate of 38% in over 100 countries [9], of whom about 75% were manifested with ID [23]. The prevalence of IDA, the major type of anaemia, appears to vary across regions, from 3% in Europe [17] to over 50% in Africa [24]. **Table 1** highlight the prevalence and risk factors associated with IDA in pregnancy in under-developed and developing countries with more emphasis on Africa and Nigeria.

Causes of Iron Deficiency Anaemia in Pregnancy

The most common cause of IDA in pregnancy is blood loss and/or iron transfer to foetus. Other common contributing factors include nutritional deficiency (when the body is not getting enough iron) and low initial iron stores that cannot adequately support the increasing demand for iron [41]. Menstrual blood loss in excess of iron intake, gastrointestinal blood loss (e.g., intestinal parasites, chronic gastrointestinal diseases), and hereditary haemorrhagic telangiectasia could leads to blood loss over a long time with a resultant depletion of the body's iron stores [41].

Impact of Anaemia in Pregnancy

Anaemia in pregnancy has negative effects on both the woman and the foetus [42; 43]. The clinical presentation and the complications depend on the severity and duration of anaemia. If severe, it is associated with a significant maternal morbidity and mortality [44]. The presence of other risk factors or surgical procedures may disproportionality increase the adverse outcomes [45]. Anaemia has been associated with a neonate's low birth weight and prematurity [1]. Considering high prevalence, significant clinical impact, and many available preventive and treatment options, early recognition, and appropriate classification of anaemia in a pregnant woman is imperative. **Table 2** summarizes the consequences of IDA in pregnancy.

Iron Deficiency Anaemia Screening during Pregnancy

Considering the high prevalence of anaemia in pregnancy and its negative impact on maternal and foetal/neonatal morbidity and mortality, screening for anaemia, particularly IDA is recommended by many agencies [45]. The specific guidelines may vary in different countries, but Hb measurement together with serum ferritin and transferrin saturation once or twice during pregnancy is generally recommended [41]. According to the American College of Obstetrics and Gynaecologists, Centres for Disease Control and Prevention, and United Kingdom guidelines, every pregnant woman should receive a complete blood count (CBC), serum ferritin and transferrin saturation at the initial antenatal visit [41]. Depending on the general population and the prevalence of IDA and other anaemia's, some countries recommend additional screening strategies for other haemoglobin disorders (thalassemia, sickle cell trait/disease) and a possible trial of oral iron supplementation in cases of an unexplained anaemia [48].

Management of Iron Deficiency Anaemia

The goal of IDA treatment is to correct the anaemia and reduce the adverse outcomes of both the iron deficiency and anaemia [49]. The management of IDA includes education regarding dietary modification, specifically ways to enhance iron absorption and iron supplementation [50]. Although oral supplementation is typically the mainstay of treatment, in cases where

Table 1. Studies on the prevalence and risk factors associated with iron deficiency anaemia in pregnancy.

Country/Methodology	Sample size	Result/Conclusion	Reference
Nigeria/ Cross-sectional study	70	Anaemia and iron deficiency anaemia were found to be significantly higher among pregnant women (20.0%, 15.7%) when compared to non-pregnant women. The mean haemoglobin, haematocrit, serum iron, ferritin, and transferrin levels were significantly reduced in pregnant compared to non-pregnant women. Pregnant women in their third trimesters and multigravida had the highest prevalence of iron deficiency and iron deficiency anaemia.	[25]
Nigeria/ Cross-sectional study	2702	Lower prevalence of anaemia at the tertiary hospital may be attributed to the higher socioeconomic status of the clientele. Short-term early antenatal management of anaemia and long-term economic/educational empowerment is advocated.	[26]
Nigeria/ Cross-sectional study	202	The prevalence of IDA was 12.3%. IDA is still a fairly common condition among parturient in Lagos and it's mostly associated with maternal peripartum morbidities.	[27]
Nigeria/ Cross-sectional study	88	The mean values of the haematology and anaemia–related parameters among the pregnant subjects were; haemoglobin (10.14 ± 1.45 g/dL), PCV (30.567± 4.492%), SI (153.55 ± 66.061µg/dl), TIBC (4.33.18 ± 97.248 µg/dl), Serum Ferritin (32.9 ±14.2 ng/mL) and TS (7.69 ± 28.84%). The prevalence of IDA was significantly higher among women in the 3rd trimester of pregnancy compared to the 2nd trimester.	[28]
Nigeria/ Cross-sectional study	90	Serum ferritin among the pregnant and non-pregnant subjects were 26.0 µg/L and 70.3 µg/L, respectively. Even though iron deficiency was observed in 68/90 (75.6%) of pregnant women, it was latent in 61/68(89.7%) of the women while it was frank in 7/68 (10.3%).	[29]
Nigeria/ Cross-sectional study	307	Parity, low educational level, and economic status as factors responsible for IDA. Both serum iron level and haemoglobin concentration can be used to diagnose anaemia.	[30]
Nigeria/ Cross-sectional study	200	A 24.5 % prevalence of anaemia in pregnancy. A significant decrease ($p< 0.05$) in packed cell volume (PCV) of pregnant women [34.94 ± 4.98%] compared to non-pregnant women (38.11 ± 6.47%). Progressive increase in PCV from the first to the third trimester, while it decreases with advancing maternal age and parity.	[31]
Congo/Cross-sectional study	128	Anaemia is common in pregnant women living in low-income settings compare to pregnant women living in high-income settings. Malaria, large family size, and <18 years of age were associated with anaemia in pregnant women.	[32]
South Africa/ Cross-sectional study	2,000	The prevalence of anaemia in HIV-positive pregnant women was significantly higher relative to HIV-negative pregnant women (71% vs. 28.7%, $p<0.0001$).	[1]
Russia/ Cross-sectional study	390	The prevalence of anaemia was significantly higher in macro- and micro-somatotype compared to meso-somatotype.	[33]
Ethiopia/ Cross-sectional study	206	Anaemia in pregnant women was significantly ($p<0.05$) associated with rural dwelling [AOR= 9.17, 95%CI= 2.15–40, $p<0.001$] and intestinal parasite infection (AOR=55.09, 95%CI=6.88–441.19, $p<0.001$).	[34]
India/ Community-based study	446	Anaemia was reported as one of the main complication in pregnancy with a frequency of 62%. Other complications such as postpartum haemorrhage, preeclampsia, abortion and still birth ranging from 1.6% to 3.5%.	[35]
East Africa/ Cross-sectional study	8,583	The prevalence of anaemia in pregnancy was estimated at 41.8% in East Africa (95%CI= 40.78, 42.87) with over 23% in Rwanda and about 57% in Tanzania. An increased incidence of anaemia was observed in pregnant women with bad toilet facility (aPR=1.17, 95%CI=1.06, 1.27), women from countries with high illiteracy level (aPR=1.12, 95%CI=1.07, 1.18), and teenage women (aPR=1.22, 95%CI=1.02, 1.40).	[36]
Latin America, Africa, Western Pacific and Southeast Asia/ Cross-sectional study	312,281	Severe Anaemia was associated with maternal death among pregnant women (AOR=1.86, 95%CI=1.39–2.49).	[37]
USA/Population-based cohort study	20,690	Increased odds of postpartum haemorrhage and delivering a small neonate was observed in anaemic pregnant women. Successful treatment of anaemia in pregnant women lead to a significant decrease in odds for preterm birth (5.1% vs. 8.3%, AOR=0.59, 95%CI= 0.47–0.72) and preeclampsia (5.9% vs. 8.3%, AOR=0.75, 95%CI= 0.61–0.91). However, untreated anaemia was associated with increased odds for preterm birth (AOR=1.44, 95%CI= 1.16–1.76) and preeclampsia (AOR=1.54, 95%CI= 1.24–1.89).	[38]
Ghana/Cross-sectional study	400	The incidence of anaemia increased with the trimester of pregnancy. Women in the third trimester were 4 times more susceptible to anaemia relative to those in the first trimester (AOR=3.57, 95%CI= 1.91–6.67). Women's knowledge of anaemia and pregnancy trimester at interview time was associated with their anaemia status.	[39]
China/Retrospective cohort study	18,948,443	Moderate and severe anaemia during pregnancy was associated with increased risk of maternal death, still birth, restricted foetal growth, and maternal shock compared to no anaemia. Mild anaemia was associated with a decreased risk of maternal death, maternal shock, foetal growth restriction and still birth compared to moderate and severe anaemia after adjusting for demography and pregnancy complications.	[40]

Table 2. Consequences of IDA in pregnancy. Source: [49, 50].

Maternal complications	Foetal outcomes
Preeclampsia	Low birth weight
Intrauterine death	Congenital anomaly
High risk of preterm delivery	Low cognitive development
Antepartum and postpartum haemorrhage	High risk of schizophrenia
Postpartum depression	Neonatal anaemia
Premature membrane rupture	Still birth

oral supplementation is intolerance and/or ineffective, IDA patients may require intravenous (IV) supplementation.

Dietary Advice: Dietary iron occurs in two forms: haem and non-heme. Haem iron has a higher bioavailability than non-heme iron and is only found in meat, poultry, and fish products [51]. Dietary iron absorption is dependent on bioavailability, physiologic requirements, and the presence of absorption promoters (ascorbic acid) or inhibitors such as tannins, calcium, or phytates [52]. Ascorbic acid enhances iron absorption by reducing ferric iron to ferrous iron, allowing for iron uptake by the mucosal cells and forming a chelate with iron to allow for iron absorption in the duodenum [53].

The negative effects of iron inhibitors can be negated with the use of ascorbic acid [53]. The dietary reference intake of dietary iron for pregnant women is 27 mg. Approximately 1 to 2 mg of iron is lost per day due to mucosal shedding in the GI tract [54]. Because pregnant women have increased iron demands, merely increasing their intake of dietary iron may be insufficient to correct their IDA [55]. However, increased dietary iron would be beneficial for pregnant women with iron depletion, rather than IDA, as typically only 10% to 25% of dietary iron is absorbed from the GI tract [54]. This is due to a hormone called hepcidin, which is essential for iron homeostasis by controlling intestinal iron absorption [55].

Oral Iron Supplementation: Oral iron supplements are cheap, convenient, and readily available in different forms, these include ferrous sulphate (20% elemental iron/mg), ferrous gluconate (11% elemental iron/mg), and ferrous fumarate (33% elemental iron/mg) [51]. For pregnant women with IDA, the recommended elemental iron dose is 120 mg per day throughout the period of pregnancy and for at least, 3 months postpartum [56]. However, the bioavailability of oral iron is low especially in healthy individuals with 5% and 5.6% absorption rate in male and females respectively [57,58]. In severe iron deficiency, the absorption may reach 20% [59]. While some previous studies reported a significant decrease in iron absorption following daily administration, other studies report no significant changes [50–62]. Because of the decrease in iron absorption that is associated with daily administration, an alternate day iron supplementation is recommended. The iron that remain in the intestine may cause a change in gut microbiota and promote pathogenic specie growth leading to inflammation and mucosal injury [63]. Hence, parenteral iron supplementation is a better alternative.

Parenteral Iron: Supplemental iron is also available through intravenous (IV). Parenteral iron is a promising method of iron supplementation because it does not affect the intestinal mucosa and can be used to effectively treat severe iron deficiency [5]. Intramuscular iron injection is associated with increased pain, reduced efficacy, and a higher risk for permanent skin staining. Hence, it is not recommended for use [64]. The major benefit for IV iron is that it more effectively corrects IDA. However, infusion reactions and anaphylaxis may occur following IV iron supplementation [65]. Hence, iron formulations with low infusion reaction are required for IV iron [66]. The common formulations with high tolerability include ferric carboxymaltose (FCM), ferric derisomaltose (FDI), and ferrumoxytol (FMX) [67]. In Europe and Asia, FDI is recommended in cases of severe iron deficiency that requires rapid correction while in America and Africa FCM is indicated for patients with chronic kidney disease [68].

Conclusion

In both poor resource and developed countries, the most prevalent and pervasive dietary deficit is ID. Due to recurrent menstrual losses as well

as the higher iron demands of pregnancy and lactation, women of reproductive potential are most at risk of ID. The need for the extra iron required to maintain expansion of blood volume/red cell mass and growth of the foetus and placenta during pregnancy increases the risk for ID and IDA. IDA in pregnancy is readily manageable yet an unmet health demand. The management strategy is dependent upon the period of gestation and severity of anaemia. Organization of patient group meetings and the use of social media can spread awareness of this public health issue.

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Conflict of interest statement
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References

1. Tunkyi K, Moodley J. Anemia and pregnancy outcomes: a longitudinal study. J Matern Fetal Neonatal Med. 2018;31:2594-2598.
2. Lopez A, Cacoub P, Macdougall IC, et al. Iron deficiency anaemia. Lancet. 2016;387:907-916.
3. Bhavi SB, Jaju PB. Intravenous iron sucrose v/s oral ferrous fumarate for treatment of anemia in pregnancy: A randomized controlled trial. BMC Pregnancy Childbirth. 2017;17.
4. Lewkowitz AK, Gupta A, Simon L, et al. Intravenous compared with oral iron for the treatment of iron-deficiency anemia in pregnancy: a systematic review and meta-analysis. J Perinatol. 2019;1-14.
5. World Health Organisation. Iron deficiency anaemia: Assessment,prevention and control. World Health Organisation, Geneva 2001.
6. Steer PJ. Maternal hemoglobin concentration and birth weight. Am Journal of Clin Nutri. 2000;7:1285S-1287S.
7. Beutler EWJ. The definition of anaemia: What is the lower limit of normal of the blood hemoglobin concentration? Blood. 2006;107:1747-1750.
8. Shams S, Ahmad Z, Wadood A. Prevalence of Iron Deficiency Anemia in Pregnant Women of District Mardan, Pakistan. J Pregnancy Child Health. 2007;4.
9. Stevens G, Finucane MM, De-Regil LM, et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for. 1995-2011: a systematic analysis of population representative data. Lancet Glob Health. 2013;1:1016-1025.
10. Khaskheli M, Baloch S, Sheeba S, et al. Iron deficiency anaemia is still a major killer of pregnant women. Pak J Med Sci. 2016;630-634.
11. Pavord S, Myers B, Robinson S, et al. UK guidelines on the management of iron deficiency in pregnancy. Br J Haematol. 2012;156:588-600.
12. Loy SL, Lim LM, Chan S-Y, et al. Iron status and risk factors of iron deficiency among pregnant women in Singapore: a cross-sectional study. BMC Public Health. 2019;19:397.
13. Johnson-Wimbley TD, Graham DY. Diagnosis and management of iron deficiency anemia in the 21st century. Ther Adv Gastroenterol. 2011;4:177-184.
14. Mast Q, Syafruddin D, Keijmel S, et al. Increased serum hepcidin and alterations in blood iron parameters associated with asymptomatic P. falciparum and P. vivax malaria. Haematologica. 2020;95:1068-1074.
15. Ugwu NI, Uneke CJ. Iron deficiency anemiain pregnancy in Nigeria—A systematic review. Niger J Clin Pract. 2020;23:889-896.
16. Ahamed NH, Kotb SAM, Hassanen RH. Knowledge and Attitude of Pregnant Women about Iron Deficiency Anemia in Assist University Women Health Hospital, Egypt. J of Nur Health Sci. 2018;3:49-58.
17. Balci Y, Karabulut A, Gurses D, et al. Prevalence and risk factors of anemia among adolescents in Denizli, Turkey. Iran J Pediatr. 2013:22:77-81.
18. Gabee SG, Niebyl JR, Simpson JL. Obstetrics: Normal and Problem Pregnancies. 4th Eds. New York, Churchill Livingstone, 1996, pp. 1348.
19. Khalafallah, AA, Dennis AE. Iron deficiency anaemia in pregnancy and postpartum:pathophysiology and effect of oral versus intravenous iron therapy. J Pregnancy. 2012;2012:1-10.
20. Milman N, Taylor CL, Merkel J, et al. Iron status in pregnant women and women of reproductive age in Europe. Am J Clin Nutr. 2017, 106:1655S-1662S.
21. Lozoff B. Early iron deficiency has brain and behavior effects consistent with dopaminergic dysfunction. J Nutr. 2011:141:740S-746S.
22. Breymann C, Bian, XM, Blanco-Capito LR, et al. Expert recommendations for the diagnosis and treatment of iron-deficiency anemia during pregnancy and the postpartum period in the Asia-Pacific region. J Perinat Med. 2011;39:112-121.
23. Tan J, Qi Y, Yang H, et al. Prevalence of anemia and iron deficiency anemia in Chinese pregnant women (IRON WOMEN): a national cross-sectional survey. BMC Pregnancy Childbirth. 2020;20.
24. Rezk M, Marawan H, Dawood R. Prevalence and risk factors of iron-deficiency anaemia among pregnant women in rural districts of Menoufia governorate, Egypt. J Obstet Gynecol. 2015;35:663-666.
25. Okafor IM, Asemota EA, Antai AB, et al. Prevalence of Iron Deficiency Anaemia among Pregnant Women in Calabar, Cross River State, Nigeria. IOSR J Pharm Biol Sci. 2013;7:60-64.
26. Olubukola A, Odunayo A, Adesina O. Anemia in pregnancy at two levels of health care in Ibadan, south west Nigeria. Ann Afr Med. 2011;10:272-277.
27. Ajepe AA, Okunade KS, Sekumade AI, et al. Prevalence and foetomaternal effects of iron deficiency anaemia among pregnant womenin Lagos, Nigeria. PLoS ONE 2020;15: e0227965.

28. Erhabor O, Isaac IZ, Isah A, et al. Iron deficiency among antenatal women in Sokoto, Nigeria. British J of Med Health Sci. 2013;1:47-57.
29. Hassan AMAI, Adaji SMB, Kene S. Anemia and Iron Deficiency in Pregnant Women in Zaria, Nigeria. Sub-Saharan Afr J Med. 2014;1:36-39.
30. Obasi IO, Nwachukwu N. Comparative iron related anaemia at pregnancy in Ebonyi State, South-east Nigeria. J Med Sci. 2013;13:425-431.
31. Gwarzo MY, Ugwa EA. The pattern of anaemia in northern Nigerian pregnant women. J Med Med Sci. 2013;4:319-323.
32. Joseph CM, Albert MT, Herman TK, et al. Prevalence and associated factors of anemia during pregnancy in Lubumbashi, in the south of Democratic Republic of Congo: situation in. 2020. PAMJ Clinic Med. 2021;7(19):1-9.
33. Tomayeva KG, Gaydukov SN, Komissarova EN, et al. Prognosis of iron deficiency anemia in pregnant women with different somatotypes. Journal of Obstetrics and Women's Diseases. 2021;70(2): 83-89.
34. Asrie F. Prevalence of anemia and its associated factors among pregnant women receiving antenatal care at Aymiba Health Center, northwest Ethiopia. J Blood Med. 2017;8:35-40
35. Suryanarayana R, Chandrappa M, Santhuram AN, et al. Prospective study on prevalence of anemia of pregnant women and its outcome: A community based study. J Family Med Prim Care. 2017;6:739-743.
36. Liyew AM, Tesema GA, Alamneh TS, et al. Prevalence and determinants of anemia among pregnant women in East Africa; A multi-level analysis of recent Demographic and Health Surveys. PLoS ONE. 2021;16(4): e0250560.
37. Daru J, Zamora J, Fernández-Félix BM, et al. Risk of maternal mortality in women with severe anaemia during pregnancy and post partum: a multilevel analysis. Lancet Glob Health. 2018;6:e548-e554.
38. Detlefs SE, Jochum MD, Salmanian B, et al. The impact of response to iron therapy on maternal and neonatal outcomes among pregnant women with anemia. Am J Obstet Gynecol MFM. 2022;4:100569.
39. Wemakor A, Prevalence and determinants of anaemia in pregnant women receiving antenatal care at a tertiary referral hospital in Northern Ghana. BMC Pregnancy and Childbirth. 2019;19:495
40. Shi H, Chen L, Wang Y, et al. Severity of Anemia During Pregnancy and Adverse Maternal and Fetal Outcomes. JAMA Network Open. 2022;5(2):e2147046
41. Agarwal AM, Rets A. Laboratory approach to investigation of anemia in pregnancy. Int J Lab Hematol. 2021;43:65-70.
42. Margwe JA, Lupindu AM. Knowledge and attitude of pregnant women in rural Tanzania on prevention of anaemia. Afr J Reprod Health. 2018;22:71-79.
43. Serbesa ML, Iffa MT. Knowledge, attitude and practice on prevention of iron deficiency anemia among pregnant women attending ante natal care unit at public hospitals of Harar Town, Eastern Ethiopia: institutional based cross-sectional study. Int J Pregnancy Child Birth. 2019;5:48-55.
44. Daru J, Zamora J, Fernandez-Felix BM. Risk of maternal mortality in women with severe anaemia during pregnancy and post partum: a multilevel analysis. Lancet Glob Health. 2018;6:e548-e554.
45. Sobhy S, Arroyo-Manzano D, Murugesu N. Maternal and perinatal mortality and complications associated with caesarean section in low-income review and meta-analysis. Lancet. 2019;10184:1973-1982.
46. Pasricha SR, Flecknoe-Brown SC, Allen KJ. Diagnosis and management of iron deficiency anaemia: a clinical update. Med J Aust. 2010;193:525-532.
47. Tandon R, Jain A, Malhortra P. Management of Iron Deficiency Anemia in Pregnancy in India. Indian J Hematol Blood Transfus. 2018;34:204-215.
48. Zaman B, Rasool S, Jasim S, et al. Hepcidin as a diagnostic biomarker of iron deficiency anemia during pregnancy. J Matern Fetal Neonatal Med. 2019;1-10.
49. Vanamala VG, Rachel A, Pakyanadhan SS. Incidence and outcome of anemia in pregnant women: a study in a tertiary care centre. Int J Reprod Contracept Obstet Gynecol. 2018;7:462-466.
50. Zolotor AJ, Carlough MC. Update on prenatal care. Am Fam Physician. 2014;3:199-208.
51. Panigragi A, Sahoo B. Nutritional Anemia and its Epidemiological Correlates among Women of Reproductive Age in Urban Slum of Bhubaneswar, Orissa. Indian J Public Health. 2011;55.
52. Khuu G, Dika C. Iron deficiency anemia in pregnant women. Nurse Pract. 2017;42:42-47.
53. Hurrell R, Egli I. Iron bioavailability and dietary reference values. Am J Clin Nutr. 2010;91:1461S-1467S.
54. Abbaspour N, Hurrell R, Kelishadi R. Review on iron and its importance for human health. J Res Med Sci. 2014;19:164-174.
55. Raut K, Kumar B, Shretha J, et al. Prevalence of iron deficiency anemia among pregnant women before iron supplementation in Kathmandu University Hospital/Dhulikhel Hospital. J Obstet Gynecol. 2014;2:54-58.
56. Goot K, Hazeldine S, Bentley P, et al. Elevated serum ferritin: what should GPs know? Aus Fam Physician. 2012;41:945-949.
57. Jimenez K, Kulnigg-Dabsch S, Gasche C. Management of Iron Deficiency Anemia. Gastroenterol Hepatol. 2015, 11:241-250.
58. Stoffel NU, von Siebenthal HK, Moretti D, et al. Oral iron supplementation in iron-deficient women: How much and how often? Molecular Aspects of Medicine. 2020;75:100865.
59. Schwartz AJ, Das NK, Ramakrishnan SK, et al. Hepatic hepcidin/intestinal HIF-2alpha axis maintains iron absorption during iron deficiency and overload. J Clin Invest. 2019;129:336-348.
60. Viteri FE, Xunian L, Tolomei K, et al. True absorption and retention of supplemental iron is more efficient when iron is administered every three days rather than daily to iron-normal and iron-deficient rats. J Nutr. 1995;125:82-91.

61. Cook JD, Reddy MB. Efficacy of weekly compared with daily iron supplementation. Am J Clin Nutr. 1995;62:117-120.
62. Olivares M, Pizarro F, Walter T, et al. Bioavailability of iron supplements consumed daily is not different from that of iron supplements consumed weekly. Nutr Res. 1999;19:179-190.
63. Bloor SR, Schutte R, Hobson AR. Oral Iron Supplementation-Gastrointestinal Side Effects and the Impact on the Gut Microbiota. Microbiol Res. 2021; 12:491-502.
64. Christoph P, Schuller C, Studer H, et al. Intravenous iron treatment in pregnancy: comparison of high-dose ferric carboxymaltose vs. iron sucrose. J Perinat Med. 2012;40:469-474.
65. Simons FE, Ebisawa M, Sanchez-Borges M, Thong BY, Worm M, Tanno LK, Lockey RF, El-Gamal YM, Brown SG, Park HS, Sheikh A. 2015 update of the evidence base: World Allergy Organization anaphylaxis guidelines. World Allergy Organ J. 2015 Oct 28;8(1):32.
66. Rampton D, Folkersen J, Fishbane S, et al. Hypersensitivity reactions to intravenous iron: guidance for risk minimization and management. Haematologica. 2014;99:1671-1676.
67. Schaefer B, Meindl E, Wagner S, et al. Intravenous iron supplementation therapy. Molecular Aspects of Medicine. 2020;75:100862.
68. Peyrin-Biroulet L, Williet N, Cacoub P. Guidelines on the diagnosis and treatment of iron deficiency across indications: a systematic review. Am J Clin Nutr. 2015;102:1585-1594.

REVIEW PAPER

JMS Journal of Medical Science

An overview of medical applications of montmorillonite clay

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ABSTRACT

Clays are among the most important material available in nature. Montmorillonite MMT is an important type of clay mineral whose physical structure is typically perceptible as layers and sheets. Each layer is made up of one octahedral and two tetrahedral structural sheets. Due to its distinctive properties, such as swelling and adsorption, MMT has been used in a variety of industrial and therapeutic applications. The high adsorption capacity of MMT contributes to increasing drug intercalation and then its sustained release. By strongly adhering to the drug, MMT typically maintains drug release in many formulations and speeds up the solubility and bioavailability of hydrophobic drugs. MMT has also been used to develop composite delivery systems that combine it with other polymer-based materials. MMT could therefore be used to develop a variety of drug delivery systems to regulate and enhance a drug's pharmacological qualities, such as solubility, dissolution rate, and absorption. An important note to mention is that clays in general are traditionally considered bio-inert or even biocompatible. In this review, the distinguished applications of MMT clay as an agent in the medical field were discussed. Among those applications is its use as an antibacterial agent, detoxification agent, preventive obesity agent, drug carrier agent, and in the treatment of cancer, diarrhea, wounds, and bones.

Introduction

MMT is one of the three-layer clay minerals. The structure of each layer consists of an octahedral sheet sandwiched between two tetrahedral sheets. The silicon-oxygen tetrahedra that make up the tetrahedral sheet are connected by sharing three corners, creating a hexagonal network, while the fourth oxygen atom points downward to the adjacent octahedral sheet. In the aluminum- or magnesium-oxygen-hydroxyl sheet, aluminum or magnesium atoms are octahedrally coordinated to six oxygen or hydroxyl groups, which lie around the metal atom on the six corners of a regular octahedron. The oxygen atoms

are shared by neighboring octahedrons, and the hydroxyl groups and oxygen atoms form a hexagonal close packing in two parallel planes, with the metal atoms occupying a central plane (see **Figure 1**).

The unique property of MMT is the ability of water and other polar molecules to enter the unit layers, expanding the basal spacing. The basal spacing varies from 9.6 Å, when there are no polar molecules present in the interlayers, to almost complete separation in some cases [1-5].

MMT clay can be treated with some chemical compounds to improve its surface area and thus increase its adsorption capacity. Among these compounds used are acids such as sulfuric acid [6,7], hydrochloric acid [8], phosphoric acid [9], and bases such as sodium hydroxide [10]. Other organic and inorganic species can be used for this purpose, such as polysaccharides [11], dodecyltrimethylammonium bromide [12], hexadecyl dimethyl ammonium) chloride [13], octyltrimethylammonium bromide, dodecyltrimethylammonium bromide, cetyltrimethylammonium bromide, and stearyltrimethylammonium bromide [14], zirconium oxide [15]. The adsorption capacity may also be improved with the assistance of microwave and ultrasound [16]. The review aims to summerize the importent application of MMT in the medical feild, focusing on the last ten years.

The subsequent spread of antimicrobial resistance has brought the overuse of antibiotics throughout the years to attention. Certain kinds of microbes can have their growth inhibited or even eliminated by antimicrobial drugs. Several researchers are now particularly interested in finding new products with strong antibacterial action [17-19].

In recent years, varieties of approaches between clay minerals and antibacterial agents have been used to develop clay mineral-based antibacterial complexes. Due to their harmless and eco-friendly features and ease of manufacture through intercalation with organic antibacterial modifiers, clay minerals are being thoroughly investigated [20].

Studies have shown that the antibacterial action of organoclay is due to contact with cells. The activity is further enhanced by the intercalation of polymers with positive charges present on the clay as they nullify the translocation of biocidal cationic surfactant [21]. An antibacterial investigation showed that Co-MMT has

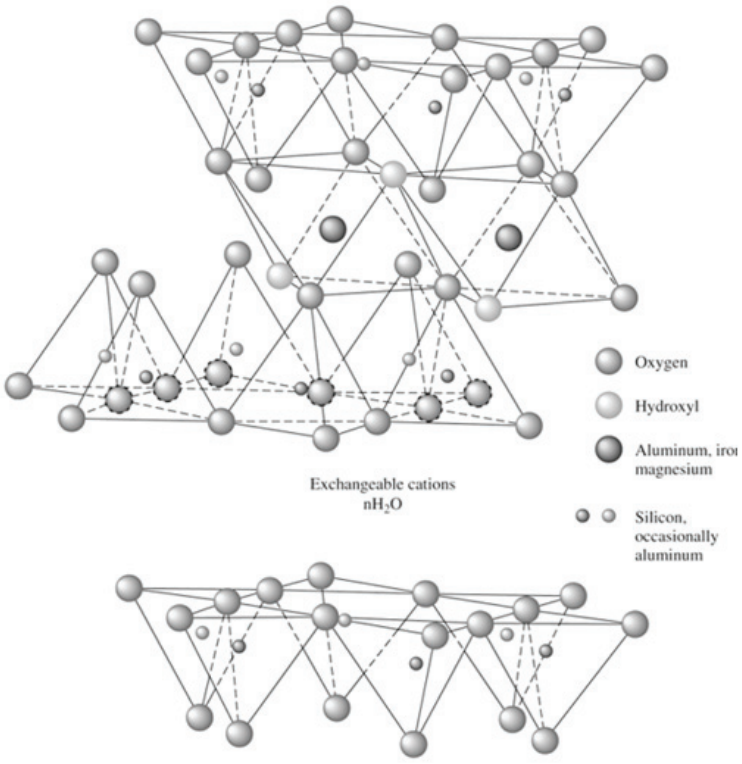

Figure 1. Schematic presentation of MMT antimicrobial application.

good antibacterial activity against *S. aureus* and *E. coli*. These results showed that Co-MMT could be a novel antibacterial agent for tissue engineering due to its superior biocompatibility and antibacterial activity [22]. A study was carried out to evaluate the mechanical characteristics, bonding capabilities, and antimicrobial activity of a new composite cement that contained MMT modified with cetylpyridinium chloride. The composite successfully maintained mechanical strength and bonding performance while achieving continuous anti-biofilm activity [23]. Cetylpyridinium chloride loaded MMT with a long-term antibacterial efficacy to prevent caries was developed as a long-term anti-bacterial agent [24]. A new antimicrobial tissue conditioner including cetylpyridinium chloride and MMT was tested for its mechanical characteristics, antibacterial efficacy, and biocompatibility. Within the limitations of this *in vitro* and *in vivo* study, the findings point to the fact that the newly developed tissue conditioner not only possesses excellent antimicrobial properties but also shares the same mechanical and biocompatibility characteristics as tissue conditioners that are currently on the market [25]. Acanthamoeba keratitis is a painful, potentially dangerous infection of the eye. It frequently tends to be associated with wearing contact lenses. There is a need to develop efficient disinfectants because several lines of evidence point to insufficient contact lens solutions, particularly against the cyst forms of pathogenic acanthamoeba. Cetylpyridinium chloride-MMT was used against keratitis-causing *A. castellanii* belonging to the T4 genotype was investigated. The results indicated that cetylpyridinium chloride-MMT complex has a power antiamoebic effects [26]. A study compares the production of Ag and Cu nanoparticles over MMT nanosheets produced using various reduction media and examines their antibacterial, antifungal, and toxicological activity. The hybrid materials were found to be prospective candidates for next-generation, highly effective antibacterial compounds with lower toxicity [27]. It was observed that a nanocomposite made of bacterial cellulose and silver-modified MMT was effective against Gram-positive *Staphylococcus aureus* and Gram-negative *Pseudomonas aeruginosa* [28]. High-density polyethylene nanocomposite monofilaments based on three different types of metal nanoparticles coated MMT have been the subject of comparative research on their antibacterial properties. In moist environment applications like ropes, sacks, agricultural products, and geotextiles where microbial growth is a concern, the antimicrobial filaments were found to be suitable candidates to replace neat high-density polyethylene counterparts [29]. The effect of MMT and carvacrol (as an antimicrobial agent) on the wettability, mechanical, gas barrier, thermal, and color characteristics of films made from nanoparticles based on methyl cellulose was studied. The mechanical properties of the film material were improved and the melting point of the methyl cellulose film was raised by the addition of MMT to the film matrix [30]. In a study to evaluate the effect of MMT filler on the antibacterial properties of polymer composites with a biodegradable polylactide matrix, the properties of the obtained composites suggest that the MMT may be potentially used as filler for polymer films in the packaging industry [31]. In an investigation on how a vinyl-modified MMT affected the physical and antibacterial qualities of a superabsorbent made of chitosan, graft, and polyacrylic acid, the results obtained encourage the use of the synthesized copolymer nanocomposites in several sectors as better physical antibacterial superabsorbents [32]. To deliver the necessary antibiotic dosages to fight post-implantation infection, a study developed chitosan-MMT nanoclay composites loaded with vancomycin and gentamicin. The results indicated that the prepared composite nanospheres can be a viable choice for preventing bone infections during the post-implantation period [33]. To improve the optical, mechanical, and antibacterial properties of chitosan, MMT-copper oxide nanocomposites were developed using an eco-friendly process. The nanocomposite exhibited strong antibacterial activity against pathogenic *S. aureus* and *B. cereus* and was more effective against these bacteria than it was against *E. coli* and *P. Aeruginosa* [34]. In a study, chitosan-poly(vinylalcohol)-MMT nanocomposites were prepared. The nanocomposites showed poweful antimicrobial properties [35]. A study concerns the potential use of MMT as a carrier and focuses on the intercalation of the clay with the aminoglycoside antibiotic, gentamicin showed the greatest capacity for killing *E. coli* bacteria in an *in vitro* test [36]. MMT clay modified with cetyltrimethyl ammonium bromide was used as a modified layered silicate. The results

revealed that the strong nanocomposite antimicrobial activity is due to the interaction between quaternized chitosan and MMT and the fine dispersion of the substance [37]. The antimicrobial activity of organically modified MMT was tested against two Gram-positive bacteria such as *Listeria monocytogenes* and *Staphylococcus aureus* and two Gram-negative ones such as *E. coli* and *Salmonella typhimurium*. The results indicated that the nano MMT caused cell membrane rupture and inactivation of the bacteria [38]. Silver nanoparticles-MMT were synthesized and found to be powerful against a group of Gram-positive and Gram-negative bacteria [39]. Chlorhexidine diacetate and MMT were used in varying amounts to develop organo-MMT. The antibacterial activity against *Escherichia coli* was evaluated and showed promising results [40]. By using electrostatic interaction to enclose a bio-synthesized peptide aptamer with MMT, an antifungal agent was produced. A powerful antifungal activity is shown by the nanocomposite against *Colletotrichum gloeosporioides* [41]. The combination of essential oils with MMT clay produces new materials for various applications including active packaging materials, insecticidal/repellent, antibacterial, and antifungal substances [42]. Silver halides (AgX, X=Cl, Br, I) in MMT were prepared by dispersion method, using silver nitrate as a silver precursor. The product exhibited good antibacterial effects [43].

Detoxification application

MMT clay with layered structures that have large surface areas, which enhances their capacity to bind different compounds on active interlayer surfaces and in pores, can be used as a powerful adsorbent and detoxifier to reduce the bioavailability of the toxic drugs [44].

The potential of MMT clay as an adsorbent for the organochlorine pesticide dieldrin was evaluated. It was found that MMT could be consumed as enterosorbents in the diet to reduce toxin toxicity and bioavailability [45]. Ca-MMT was found to have a high capacity, affinity, and a low therapeutic dose toward polychlorinated biphenyls, the environmental contaminants in food, water, and biota [46]. Ca-MMT was also found to be able to adsorb mixtures of glyphosate and aminomethylphosphonic acid that may reach humans through exposure to contaminated water, soil, and the consumption of crops containing these toxins residues [47]. An investigation demonstrated that MMT modified with Fe, Al, and Ti, has the potential to adsorb deoxynivalenol, which is found in foods and feeds that are contaminated with mildew. It is among the most dangerous mycotoxins, threatening human health as well as animal husbandry [48]. As a mycotoxins adsorbent, octylphenol polyoxyethylene ether modified MMT, a nonionic surfactant, was developed to adsorb polar aflatoxin B1 and weak polar zearalenone, by simulating gastric tract conditions. The surfactant showed the potential to be a useful adsorbent for the simultaneous detoxification of polar and non-polar mycotoxins [49]. Ca-MMT was treated with H_2SO_4, calcination, and organic compounds hexadecyltrimethyl ammonium bromide, cetylpyridinium chloride, and chitosan. The product showed a powerful adsorption performance for mycotoxins [50]. Adsorption role in reducing bioavailability and, consequently, the reported toxicity of monoalkyl trimethyl ammonium salts under environmental circumstances were investigated. The observed toxicity of the compound in the presence of MMT adsorbent was tested on algae. MMT was found to be very effective [51]. The ability of Na-MMT to detoxify two organophosphate pesticides, methyl parathion [O,O-dimethyl O-(p-nitrophenyl) thionophosphate] and tetrachlorvinphos [2-chloro-1-(2,4,5-trichlorophenyl)ethenyl dimethyl phosphate], when treated with N-decyl-N,N-dimethyl-N-(2-aminoethyl) ammonium was found to be adequate [52]. A study focuses on the development of new mycotoxins adsorbents employing zwitterionic surfactants modified MMT for the simultaneous removal of low polar zearalenone and highly polar aflatoxin B1, both of which represent serious health risks. The resulting adsorbent exhibited excellent adsorption performance [53]. An investigation was conducted on Egyptian MMT potential to protect fish from the genotoxicity, histochemical, and biochemical changes caused by aflatoxin B1. It was established that Egyptian MMT could firmly bind aflatoxin in fish guts, hence reducing aflatoxin's bioavailability [54]. The efficacy of Egyptian and Tunisian MMT clays to prevent genotoxicity and histological alterations induced by cadmium chloride

using the Nile tilapia fish as an *in vivo* model was investigated. It was found that both clays might tightly bind $CdCl_2$ and reduce its cytotoxicity and genotoxicity, although Tunisian clay was more effective than Egyptian clay [55]. Na-MMT and Ca-MMT were found to be safe and efficacious binders to microcystins [56], and per- and polyfluoroalkyl [57] that can reach humans or animals through the ingestion of food and drinking water that has been contaminated with cyanobacteria. An investigation was performed to assess the ability of MMT to bind uric acid, which increases uric acid diffusion from the blood to the intestine, prevents uric acid absorption in the intestine, and reduces its levels in the blood. The results showed that uric acid could be adsorbed at various doses of MMT in a concentration-dependent manner. The adsorption process moved fast and in acidic solutions, the adsorptive rate was high, whereas, in alkaline solutions, it was low [58]. It was found that MMT considerably adsorbed creatinine in the simulated intestinal solution in a study that intends to assess the adsorption of creatinine by MMT and the acceleration effect of MMT on creatinine excretion from the intestine [59].

Anticancer therapy application

Cancer is a deadly disease that kills people at an alarming rate all around the world. Chemotherapy, radiotherapy, and surgery are all part of the standard cancer treatment [60]. Conventional cancer therapies are often accompanied by undesired side effects. Therefore, the need for alternative anticancer drug delivery agents has become an important medical issue.

A nanocomposite hydrogel drug delivery systems were developed for oral administration based on polyvinyl alcohol and MMT loaded with capecitabine, as an anticancer drug. The developed nanocomposite hydrogel systems for drug delivery showed adequate efficacy against the 4T1 cancer cell line both *in vitro* and *in vivo*, suggesting them viable candidates for the controlled release of anticancer pharmaceuticals in chemotherapy with improved therapeutic benefits [61]. Other researchers reported the formulations of compatible nanocomposite hydrogel films employing carboxymethyl cellulose-hydroxyethyl cellulose-acrylonitrile-linseed oil polyol (CHAP) plain hydrogel and Na-MMT dispersed CHAP nanocomposite hydrogel films. According to the study, the proposed nanocomposite hydrogel films have looked promising for use in therapeutics, particularly for the delivery of anticancer drugs [62]. A novel nanocomposite has been synthesized from MMT as matrix support, nanoparticles of Fe_3O_4 as filler, and carrageenan as a stabilizer. The nanocomposite exhibited good efficacy against cancer cells [63]. Another magnetic nanocomposite with promising anticancer activity was synthesized from MMT as matrix support, Fe_3O_4 as filler, and *Kappaphycus alvarezii* as a stabilizer [64]. MMT, κ-carrageenan, and chitosan were used to synthesize a composite with prolonged cancer therapy and reduced side effects [65]. A study evaluated the intercalation of tamoxifen in Na-MMT interlayer, which is further combined with poly-(ε-caprolactone), for breast cancer oral chemotherapy. The study proved that MMT functions as a drug delivery matrix and also significantly improve delivery proficiency [66]. *In vitro* tests were performed on supramolecular assemblies made from self-assembling MMT nanosheets modified with β-cyclodextrin. The results indicate that the supramolecular assemblies may serve as the basis for the designing of new cancer drug delivery systems [67]. The intercalation of the anticancer drug 5-fluorouracil, in the interlayer of Na-MMT, with the assistance of chitosan produced a significant value in cancer chemotherapy with fewer side effects [68]. MMT nanoparticles were added to chitosan-agarose hydrogel and then loaded with curcumin to prepare a curcumin-loaded nanocomposite hydrogel. The product exhibited good therapeutic effects [69]. The antineoplastic drug, 6-mercaptopurine, was intercalated into Na-MMT interlayer and was further entrapped in poly (L-lactide) matrix to form microcomposite spheres to improve pharmacokinetic proficiency and *in vitro* release and reduce cell toxicity. The produced microcomposite has great potential for anticancer therapy [70].

Diarrhea treatment application

The cause of diarrhea is multiple pathogens and multiple factors, which are primarily brought on by a variety of infections and causes. It is clinically characterized by changes in stool consis-

tency and frequency, and in certain cases, fever, vomiting, and abdominal pain may occur [71].

A typical medicinal substance used to treat pediatric diarrhea is MMT. It can immobilize and inhibit viruses, bacteria, and toxins that may produce in the digestive tract [72]. MMT can reduce stomach pain or discomfort in irritable bowel syndrome patients who experience constipation more frequently [73]. It was observed that giving children with the diarrheal disease a preparation containing MMT, vitamin AD, and zinc could increase short-term efficacy, shorten the time it takes for symptoms to disappear, reduce the level of inflammatory factors, and increase T-lymphocyte levels without raising the incidence of adverse drug reactions. MMT can coat the mucosa of the digestive tract and bind mucosal glycoproteins, repairing and enhancing the mucosal barrier defense against outside influences [74]. The powder of MMT if combined with probiotics could enhance the treatment of diarrhea in children and shorten the time of clinical symptoms, and improve immune function [75]. In addition, the combination of MMT and ZnO was used in reducing diarrhea and enhancing mucosal barrier integrity, and intestinal microflora of weaned pigs [76]. It was also found that the use of berberine in combination with MMT reduced hospitalization time [77]. The duration of acute watery diarrhea can be shortened with MMT and acetorphan [78]. Children's diarrhea can be efficiently reduced with the help of combined zinc gluconate-MMT therapy with no noticeable side effects [79]. In a separate study, it was found that MMT could improve the symptoms of diarrhea and decrease the recovery time of autumn diarrhea in children of different ages [80]. Animal studies have taken an important aspect in this regard. Cu-MMT was found to be as effective in reducing diarrhea and inflammation and improving mucosal barrier integrity and intestinal microflora in weanling pigs [81]. On the other hand, a study suggested that MMT may be a helpful alternative to conventional antibiotics in the treatment and prevention of Salmonella-related animal diarrhea [82].

Obesity prevention application

Being overweight and obese are among the important problems suffered by a large number of people around the world, which have a significant impact on health [83]. Obesity has been linked to several health diseases, including type 2 diabetes, dyslipidemia, nonalcoholic fatty liver disease, cardiovascular problems, and cancer, which are the main causes of death [84-86].

According to recent research, porous colloids including MMT may help prevent weight gain and promote anti-obesity effects [87]. The everyday diet frequently includes fatty foods, and it is generally known that fats play a major role in obesity. Therefore, it is crucial to avoid obesity by immobilizing ingested lipids and raising lipid excretion to lessen fat absorption in the digestive system. MMT as a natural adsorbent clay mineral can adsorb dietary lipids and increase fecal lipid excretion, thus preventing obesity. It was found that MMT crystals can adsorb dietary lipids both *in vitro* and *in vivo*. This ability improves lipid excretion during bowel movements, preventing obesity and its associated comorbidities [88]. Results showed that MMT immobilizes fatty acids and endotoxins via the adsorption-excretion axis in the digestive tract, and MMT could potentially be employed as a prebiotic to prevent intestinal dysbiosis and obesity-associated metabolic problems in obese people [89]. It was also shown that acid-modified MMT can lower lipids by decreasing intestinal absorption and enhancing lipid excretion, so avoiding hyperlipidemia, obesity, and fatty liver [90]. MMT particles with considerable dietary lipid and digestion byproduct adsorptive capabilities were successfully created. These particles might lead to the development of novel, less harmful anti-obesity treatments [91]. According to another research, MMT reduces the dietary lipids and sterols that are absorbed through the gastrointestinal tract, reducing the risk of obesity, hyperlipidemia, and hypercholesterolemia [92].

Wound healing application

Wound healing is a complex and dynamic process that consists of hemostasis, inflammation, proliferation, and remodeling. Different types of cells are involved in this biological process, including neutrophils, macrophages, lymphocytes, keratinocytes, fibroblasts, and endothelial cells [93,94].

Recent research on nanocomposites made of clay minerals and pharmaceuticals shows that they can interact with biological structures and provide new options for tissue engineering, particularly in the area of wound healing. Due primarily to their potential to reduce water activity, clay minerals may present a variety of opportunities for the development of systems that might facilitate the antimicrobial action of loaded antibacterial drugs, and cell adhesion, growth, and neotissue development [95-98]. Researchers have developed a poly aldehyde dextran MMT composite for controlling large hemorrhages that exhibits excellent tissue adhesion, antimicrobial, and wound healing capabilities [99]. A graphene-MMT composite sponge was also synthesized under a hydrothermal reaction to accelerate wound healing [100]. A nanocomposite made of bacterial cellulose and silver-modified MMT was synthesized as a promising scaffold for healing wounds [101]. To improve wound healing in infected skin lesions, researchers have developed a norfloxacin-MMT nanocomposite as a powder for cutaneous application. The composite appeared to be a useful treatment for burns, diabetic foot ulcers, and other chronic ulcers or skin wounds that are prone to infection [102]. Researchers have also developed electrospun scaffolds, based on biopolymers-MMT intended as a 3D foundation for skin regeneration and repair [103]. To provide a sustained release of chlorhexidine, chitosan-MMT composite films containing chlorhexidine were prepared. All of the produced films demonstrated effective antibacterial wound healing [104]. Using the freezing-thawing method, bionanocomposite hydrogels based on polyvinyl alcohol and egg white as the matrix and MMT nanoclay as the reinforcement were prepared. Clindamycin, an efficient antibiotic, was added to the obtained bionanocomposite hydrogels to provide novel potential wound dressings for treating infected wounds. The bionanocomposite exhibited good effective results concerning infected wounds [105]. Scaffolds were produced from nanocomposites of polycaprolactone and quaternary ammonium salt-modified MMT using the electrospinning technique. The cytotoxicity assessment revealed minimal toxicity and confirmed the efficacy of polycaprolactone-MMT nanocomposite scaffolds as wound dressings [106]. The wound healing property of bacterial cellulose was combined with the antimicrobial activity of MMT to produce novel artificial substitutes for burns. The product showed improved tissue regeneration during wound healing [101]. Nanocomposite hydrogels based on egg white and polyvinyl alcohol and MMT nanoclay were prepared by a facile cyclic freezing-thawing technique. The nanocomposite employed seemed to work well for treating burns and wounds [107]. When combined with a bone-derived polypeptide, the ciprofloxacin-MMT composite demonstrated a promising wound healing progression [108]. As a wound dressing composite, a biopolymer membrane made of chitosan, collagen, and organo-MMT loaded with *Callicarpa nudiflora* was designed. The composite membrane with a porous layered structure exhibited a high swelling ratio, low degradation ratio, and excellent moisture permeability properties [109].

Bone treatment application

With the rise in traffic accidents, there has been a growth in the demand for defective tissues, particularly bone tissues, in recent years. Although autologous bone transplantation remains the best method for treating bone defects, its applicability is somewhat restricted due to its restricted material options, higher surgical trauma, and sensitivity to infection at the bone site [110,111].

MMT is frequently used to reinforce bone scaffolds mechanically [112]. To achieve the nanostrengthening of the composite bone scaffold, it can be converted into graphene-like nanosheets with increased specific surface area through interlayer exfoliation behavior [113]. On the other hand, the MMT crystal structure rich cations between the layers have substantial ion exchange capacity, which can enable intercalation to cause the insertion of ionic polymer molecules into the interlayer areas among its sandwich structure [114]. It is anticipated that employing MMT as the nanofiller phase can significantly improve the mechanical properties of polymer bone scaffolds [115]. By mixing 5 wt% MMT with chitosan solution in an acidic environment, researchers prepared composite materials. They found that the Si-O-Si group of MMT formed hydrogen bonds with the hydroxyl and amino groups of chitosan. The effective transfer of interfacial tension

is enhanced by this strong interfacial interaction. As a result, chitosan-MMT has more tensile strength than pure chitosan [116]. Another study found that when the MMT content was less than 4.5 wt%, the tensile strength of the Poly-l-lactic acid/MMT composite increased with the increase of the content and reaches 44.1% [117]. Several studies have been conducted to improve the mechanical properties of MMT intercalated with gelatin and chitosan. It was established that adding MMT made the scaffold pore wall thicker and increased its tensile strength [118]. According to a study on the addition of MMT to nano-hydroxyapatite nanocomposites, adding 10% MMT increased the composite flexural strength and compressive strength by 18.9% and 107.9%, respectively [119]. The effects and mechanism of nano-MMT on osteoblast and osteoclast differentiation were investigated by researchers *in vivo* and *in vitro*. In Ca-deficient ovariectomy rats, the osteogenic effects of high calcium content (3.66 wt%) nano-MMT on alkaline phosphatase activity, mineralization, bone microarchitecture, and expression level of osteoblast and osteoclast associated genes were examined. Nano-MMT was found to attenuate the low-Ca-associated changes in trabecular and cortical bone mineral density. It improved the activity and mineralization of alkaline phosphatase, as well as the expression of genes associated with osteoblast and osteoclast differentiation [120].

Drug carrier application

Drug extended release for patients who require medicinal treatment round the clock is very necessary. MMT generally sustains drug release in various formulations. It also speeds up the absorption and solubility of hydrophobic drugs. To regulate and/or enhance the pharmacological properties of drugs, such as solubility, dissolution rate, and absorption, MMT could be used to develop a variety of drug delivery systems [121].

A study was conducted on the intercalation of timolol maleate into the MMT interlayer at different pH values and concentrations. The drug was successfully intercalated into the interlayer of MMT and its controlled release from MMT-timolol maleate hybrid has been observed during *in vitro* release experiments [122]. Another study was conducted on the preparation and characterization of irinotecan nanocomposite beads based on MMT and sodium alginate as drug carriers. After the incorporation of irinotecan into MMT, the resulting hybrid was compounded with alginate, and irinotecan-MMT-alginate nanocomposite beads were obtained by ionotropic gelation technique. According to the results of the *in vitro* drug release experiments, MMT and MMT in combination with alginate were able to control the release of irinotecan by making it sustained by lowering the released amount and release rate [123]. For topical drug delivery to the eye, MMT and brimonidine (an eye drop to reduce the intraocular pressure) brimonidine-MMT hybridized as a delivery carrier. Via an ion-exchange reaction, the brimonidine molecules were intercalated in the MMT interlayer space to develop the brimonidine-MMT hybrid, which was subsequently combined with polyvinyl alcohol to produce a dry tablet. In *in vitro* conditions, the brimonidine-MMT@polyvinyl alcohol hybrid drug released brimonidine in a sustained manner for more than 5 h. When the hybrid drug was delivered into rabbit eyes *in vivo*, 43% and 18.5% of brimonidine-MMT stayed on the preocular surface for 10 and 60 minutes, respectively. Thus, the brimonidine-MMT@polyvinyl alcohol hybrid drug showed a prolonged decrease in intraocular pressure for 12 h, which was approximately twice as long as that seen with the brimonidine eye drop that is available commercially [124]. A study amid to develop and investigate a drug delivery system formed by intercalation of bromopride with Na-MMT. The results showed that bromoprid was successfully intercalated with the lamellar silicate. In assays, the Na-MMT/bromoprid molecular complex displays a sustained release [125]. A successful one-pot fabrication of ZIF-8-encapsulated medicine is used to develop an MMT-enveloped zeolitic imidazolate framework (M-ZIF-8), which is then followed by MMT coating to produce a core-shell nanoplatform for gastrointestinal (GI) drug delivery. ZIF-8 encapsulated drugs can keep their natural structure, while the MMT layer enhances mucosal adherence and maximizes medicine release. The M-ZIF-8 shows a significant advancement in GI drug delivery [126]. MMT was found to be a suitable material for changing how the tobramycin and norfloxacin drugs are released. The inter-

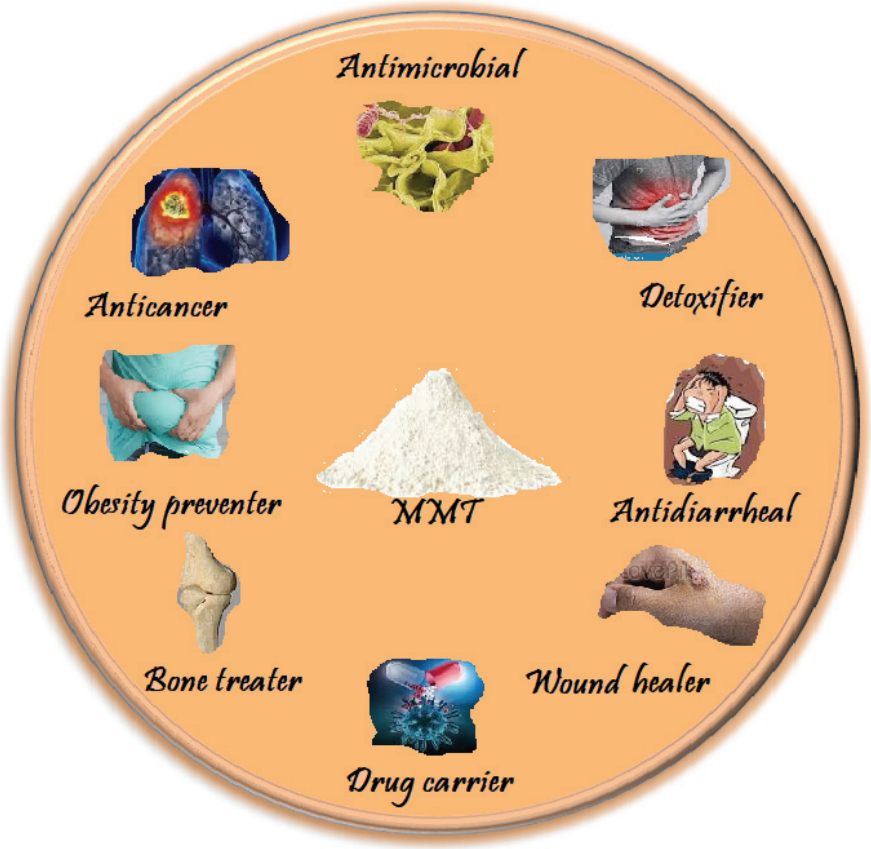

Figure 2. Some of the important medical applications of MMT.

calation of tobramycin or norfloxacin between MMT layers was used to produce drug delivery systems. It was found that the pH of the medium influences the release rates and the percentage of release rises as the pH does [127]. For controlled drug delivery, ring-shaped nanocomposite hydrogel rings made of polyacrylamide, sodium carboxymethyl cellulose, and MMT nanoparticles were developed. The nanocomposite rings were able to prolong release for 15 days in the vaginal fluid simulant, which mimics the vaginal conditions at a pH of 4.2 and a temperature of 37°C. This was demonstrated in an *in vitro* release experiment using methylene blue as a hydrophilic model drug [128]. **Figure 2** summarizes some of the important applications of MMT in the medical field.

Conclusions

MMT is one of the types of clay mineral, which is composed of an octahedral sheet sandwiched between two tetrahedral sheets. This type of clay has received important interest as an additive in polymers and products for enhanced effects. MMT forms composites of different species that can be used in a wide range of therapeutic cases. By significantly adsorbing drug molecules, MMT maintains the release of many pharmaceutical formulations. Pharmaceutical drugs benefit from better drug entrapment and sustained release owing to adsorption capability. The majority of clay mineral investigations focused on how effectively they functioned against toxic substances. There are many important medical applications of MMT such as antimicrobial agents, detoxification agents, cancer therapy agents, preventive obesity agents, as well as treatment of diarrhea and treatment of wounds.

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Conflict of interest statement
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References

1. Suresh R, Borkar SN, Sawant VA, Shende VS, Dimble SK. Nanoclay drug delivery system. Int J Pharm Sci. Nanotechnol, 2010;3(2):901-5.
2. Edelman CH, Favejee JL. On the Crystal structure of montmorillonite and halloysite. Z Kristallogr Krist. 1940;102(1-6):417-31. doi: 10.1524/zkri.1940.102.1.417.
3. Uddin F. Montmorillonite: An introduction to properties and utilization. London, IntechOpen, 2018. doi: 10.5772/intechopen.77987.
4. Segad M, Jonsson B, Åkesson T, Cabane B. Ca/Na Montmorillonite: Structure, forces and swelling properties. Langmuir. 2010;26(8):5782-90. doi: 10.1021/la9036293.
5. Zhu R, Chen Q, Zhou Q, Xi Y, Zhu J, He H. Adsorbents based on montmorillonite for contaminant removal from water: A review. App Clay Sci. 2016;123:239-258. doi: 10.1016/j.clay.2015.12.024.
6. Akpomie KG, Dawodu FA. Acid-modified montmorillonite for sorption of heavy metals from automobile effluent. Beni-Suef univ J Basic Appl Sci. 2016;5(1):1-12. doi: 10.1016/j.bjbas.2016.01.003.
7. Tyagi B, Chudasama CD, Jasra RV. Determination of structural modification in acid activated montmorillonite clay by FT-IR spectroscopy. Spectrochim Acta A Mol Biomol Spectrosc. 2006;64(2):273-8. https://doi.org/10.1016/j.saa.2005.07.018.
8. Wu L, Liu J, Liu Y, Huang R, Tang N, Wang X, Hu L. In situ growth of Fe3O4 on montmorillonite surface and its removal of anionic pollutants. RSC Adv. 2021;11(53):33399-407. doi: 10.1039/d1ra06318a.
9. Yu WH, Zhu B, Tong DS. Deng K, Fu CP, Huang TH, Zhou CH. Tuning the acidity of montmorillonite by H3PO4-activation and supporting WO3 for catalytic dehydration of glycerol to acrolein. Clays Clay Miner. 2022;70:460-79. doi: 10.1007/s42860-022-00193-6.
10. Khajeh M, Ghaemi A. Exploiting response surface methodology for experimental modeling and optimization of CO2 adsorption onto NaOH-modified nanoclay montmorillonite. J Environ Chem Engin. 2020;8(2):103663. doi: 10.1016/j.jece.2020.103663.
11. Szewczuk-Karpisz K, Bajda T, Tomczyk A, Kuśmierz M, Komaniecka I. Immobilization mechanism of Cd2+/HCrO4-/CrO42- ions and carboxin on montmorillonite modified with Rhizobium leguminosarum bv. trifolii exopolysaccharide. J Hazard Mater. 2022;428(8):128228. doi: 10.1016/j.jhazmat.2022.128228.
12. Acisli O, Khataee A, Karaca S, Sheydaei M. Modification of nanosized natural montmorillonite for ultrasound-enhanced adsorption of Acid Red 17. Ultrason Sonochem. 2016;31:116-21. doi: 10.1016/j.ultsonch.2015.12.012.
13. Li P, Zhang H, Xia M, Wang F, Zhu S, Lei W. The synergistic effect and microscopic mechanism of co-adsorption of three emerging contaminants and copper ion on gemini surfactant modified montmorillonite. Ecotoxicol Environ Saf. 2019;184:109610. doi: 10.1016/j.ecoenv.2019.109610.
14. Wang L, Wang A. Adsorption properties of Congo Red from aqueous solution onto surfactant-modified montmorillonite. J Hazard Mater. 2008;160(1):173-80. doi: 10.1016/j.jhazmat.2008.02.104.
15. Rathinam K, Atchudan R, Edison TN. Zirconium oxide intercalated sodium montmorillonite scaffold as an effective adsorbent for the elimination of phosphate and hexavalent chromium ions. J Environ Chem Eng. 2021;9(5):106053. doi: 10.1016/j.jece.2021.106053.
16. Luo W, Ouyang J, Antwi P, Wu M, Huang Z, Qin W. Microwave/ultrasound-assisted modification of montmorillonite by conventional and gemini alkyl quaternary ammonium salts for adsorption of chromate and phenol: Structure-function relationship. Sci Total Environ. 2019;655:1104-12. doi: 10.1016/j.scitotenv.2018.11.329.
17. Gould IM, Bal AM. New antibiotic agents in the pipeline and how they can help overcome microbial resistance. Virulence. 2013;4(2):185-91. doi: 10.4161/viru.22507.
18. Golkar Z, Bagasra O, Pace DG. Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. J Infect Dev Ctries. 2014;8(2):129-36. doi: 10.3855/jidc.3573.
19. Sengupta S, Chattopadhyay MK, Grossart HP. The multifaceted roles of antibiotics and antibiotic resistance in nature. Front Microbiol. 2013;4:47. doi: 10.3389/fmicb.2013.00047.
20. Bertagnolli C, Kleinübing SJ, da Silva MGC, Preparation and characterization of a Brazilian bentonite clay for removal of copper in porous beds. Appl Clay Sci. 2011;531:73-9. doi: 10.1016/j.clay.2011.05.002.
21. Bujdakova H, Bujdáková V, Májeková-Koščová H, Gaálová B, Bizovská V, Boháč P. Bujdak J. Antimicrobial activity of organoclays based on quaternary alkylammonium and alkylphosphonium surfactants and montmorillonite. Appl Clay Sci. 2018;158:21-8. doi: 10.1016/j.clay.2018.03.010.
22. Yang S, Ji Y, Deng F, Sun X, Ning C. Co-exchanged montmorillonite: a potential antibacterial agent with good antibacterial activity and cytocompatibility. J Mater Chem B. 2022;10(19):3705-15. doi: 10.1039/d2tb00032f.
23. Yamamoto Y, Yoshihara K, Nagaoka N, Van Meerbeek B, Yoshida Y. Novel composite cement containing the anti-microbial compound CPC-Montmorillonite. Dent Mater. 2022;38(1):33-43. doi: 10.1016/j.dental.2021.10.009.
24. Matsuo K, Yoshihara K, Nagaoka N, Makita Y, Obika H, Okihara T, Matsukawa A, Yoshida Y, Van Meerbeek B. Rechargeable anti-microbial adhesive formulation containing cetylpyridinium chloride montmorillonite. Acta Biomater. 2019;100:388-97. doi: 10.1016/j.actbio.2019.09.045.
25. Naoe T, Hasebe A, Horiuchi R, Makita Y, Okazaki Y, Yasuda K, Matsuo K, Yoshida Y, Tsuga K, Abe Y, Yokoyama A. Development of tissue conditioner containing cetylpyridinium chloride montmo-

rillonite as new antimicrobial agent: Pilot study on antimicrobial activity and biocompatibility. J Prosthodont Res. 2020;64(4):436-43. doi: 10.1016/j.jpor.2019.12.002.
26. Akbar N, Siddiqui R, Khamis M, Ibrahim T, Khan NA. A novel montmorillonite clay-cetylpyridinium chloride complex as a potential antiamoebic composite material in contact lenses disinfection. Exp Parasitol. 2022;240:108330. doi: 10.1016/j.exppara.2022.108330.
27. Roy A, Joshi M, Butola BS, Malhotra S. Antimicrobial and toxicological behavior of montmorillonite immobilized metal nanoparticles. Mater Sci Eng C Mater Biol Appl. 2018;93:704-15. doi: 10.1016/j.msec.2018.08.029.
28. Horue M, Cacicedo ML, Fernandez MA, Rodenak-Kladniew B, Torres Sánchez RM, Castro GR. Antimicrobial activities of bacterial cellulose - Silver montmorillonite nanocomposites for wound healing. Mater Sci Eng C Mater Biol Appl. 2020;116:111152. doi: 10.1016/j.msec.2020.111152.
29. Roy A, Joshi M, Butola BS. Antimicrobial performance of polyethylene nanocomposite monofilaments reinforced with metal nanoparticles decorated montmorillonite. Colloids Surf B Biointerfaces. 2019;178:87-93. doi: 10.1016/j.colsurfb.2019.02.045.
30. Tunç S, Duman O, Polat TG. Effects of montmorillonite on properties of methyl cellulose/carvacrol based active antimicrobial nanocomposites. Carbohydr Polym. 2016;150:259-68. doi: 10.1016/j.carbpol.2016.05.019.
31. Rapacz-Kmita AR, Pierchała MK, Tomas-Trybuś A, Szaraniec B, Karwot J. The wettability, mechanical and antimicrobial properties of polylactide/montmorillonite nanocomposite films. Acta Bioeng Biomech. 2017;19(4):25-33.
32. Abdel Aziz MS, Salama HE. Effect of vinyl montmorillonite on the physical, responsive and antimicrobial properties of the optimized polyacrylic acid/chitosan superabsorbent via Box-Behnken model. Int J Biol Macromol. 2018;116:840-8. doi: 10.1016/j.ijbiomac.2018.05.081.
33. Kimna C, Deger S, Tamburaci S, Tihminlioglu F. Chitosan/montmorillonite composite nanospheres for sustained antibiotic delivery at post-implantation bone infection treatment. Biomed Mater. 2019;14(4):044101. doi: 10.1088/1748-605X/ab1a04.
34. Nouri A, Yaraki MT, Ghorbanpour M, Agarwal S, Gupta VK. Enhanced antibacterial effect of chitosan film using montmorillonite/CuO nanocomposite. Int J Biol Macromol. 2018;109:1219-31. doi: 10.1016/j.ijbiomac.2017.11.119.
35. Giannakas A, Vlacha M, Salmas C, Leontiou A, Katapodis P, Stamatis H, Barkoula NM, Ladavos A. Preparation, characterization, mechanical, barrier and antimicrobial properties of chitosan/PVOH/clay nanocomposites. Carbohydr Polym. 2016;140:408-15. doi: 10.1016/j.carbpol.2015.12.072.
36. Rapacz-Kmita A, Bućko MM, Stodolak-Zych E, Mikołajczyk M, Dudek P, Trybus M. Characterisation, in vitro release study, and antibacterial activity of montmorillonite-gentamicin complex material. Mater Sci Eng C Mater Biol Appl. 2017;70(Pt 1):471-8. doi: 10.1016/j.msec.2016.09.031.
37. Wang X, Du Y, Yang J, Tang Y, Luo J. Preparation, characterization, and antimicrobial activity of quaternized chitosan/organic montmorillonite nanocomposites. J Biomed Mater Res A. 2008;84(2):384-90. doi: 10.1002/jbm.a.31326.
38. Hong SI, Rhim JW. Antimicrobial activity of organically modified nano-clays. J Nanosci Nanotechnol. 2008; 8(11):5818-24. doi: 10.1166/jnn.2008.248.
39. Shameli K, Ahmad MB, Zargar M, Yunus WM, Rustaiyan A, Ibrahim NA. Synthesis of silver nanoparticles in montmorillonite and their antibacterial behavior. Int J Nanomedicine. 2011;6:581-90. doi: 10.2147/IJN.S17112.
40. Lobato-Aguilar HA, Lizama-Uc G, Uribe-Calderon JA, Cauich-Rodriguez J, Rodriguez-Fuentes N, Cervantes-Uc JM. Antibacterial properties and release kinetics of chlorhexidine diacetate from montmorillonite and palygorskite clays. J Biomater Appl. 2020;34(8):1052-8. doi: 10.1177/0885328219891710.
41. Xu Z, Jiang X, Li Y, Ma X, Tang Y, Li H, Yi K, Li J, Liu Z. Antifungal activity of montmorillonite/peptide aptamer nanocomposite against Colletotrichum gloeosporioides on Stylosanthes. Int J Biol Macromol. 2022; 217:282-90. doi: 110.1016/j.ijbiomac.2022.07.034.
42. de Oliveira LH, Trigueiro P, Souza JSN, de Carvalho MS, Osajima JA, da Silva-Filho EC, Fonseca MG. Montmorillonite with essential oils as antimicrobial agents, packaging, repellents, and insecticides: an overview. Colloids Surf B Biointerfaces. 2022;209(Pt 2):112186. doi: 10.1016/j.colsurfb.2021.112186.
43. Sohrabnezhad Sh, Rassa M, Dahanesari EM. Spectroscopic study of silver halides in montmorillonite and their antibacterial activity. J Photochem Photobiol B. 2016;163:150-5. doi: 10.1016/j.jphotobiol.2016.08.018.
44. Damato A, Vianello F, Novelli E, Balzan S, Gianesella M, Giaretta E, Gabai G. Comprehensive review on the interactions of clay minerals with animal physiology and production. Front Vet Sci. 2022;9:889612. doi: 10.3389/fvets.2022.889612.
45. Hearon SE, Wang M, Phillips TD. Strong adsorption of dieldrin by parent and processed montmorillonite clays. Environ Toxicol Chem. 2020;39(3):517-25. doi: 10.1002/etc.4642.
46. Wang M, Safe S, Hearon SE, Phillips TD. Strong adsorption of Polychlorinated Biphenyls by processed montmorillonite clays: Potential applications as toxin enterosorbents during disasters and floods. Environ Pollut. 2019;255(Pt 1):113210. doi: 10.1016/j.envpol.2019.113210.
47. Wang M, Rivenbark KJ, Phillips TD. Adsorption and detoxification of glyphosate and aminomethylphosphonic acid by montmorillonite clays. Environ Sci Pollut Res Int. 2023;30:11417-30. doi: 10.1007/s11356-022-22927-8.
48. Zhang Q, Zhang Y, Liu S, Wu Y, Zhou Q, Zhang Y, Zheng X, Han Y, Xie C, Liu N. Adsorption of deoxynivalenol by pillared montmorillon-

ite. Food Chem. 2021;343:128391. doi: 10.1016/j.foodchem.2020.128391.
49. Wang G, Lian C, Xi Y, Sun Z, Zheng S. Evaluation of nonionic surfactant modified montmorillonite as mycotoxins adsorbent for aflatoxin B1 and zearalenone. J Colloid Interface Sci. 2018;518:48-56. doi: 10.1016/j.jcis.2018.02.020.
50. Mao J, Zhou Y, Lv G, Zhou R. Simultaneous detoxification of aflatoxin B1, zearalenone and deoxynivalenol by modified montmorillonites. Molecules. 2022;27(1):315. doi: 10.3390/molecules27010315.
51. van Wijk D, Gyimesi-van den Bos M, Gartteneer-Arends I, Geurts M, Kamstra J, Thomas P. Bioavailability and detoxification of cationics: I. Algal toxicity of alkyltrimethyl ammonium salts in the presence of suspended sediment and humic acid. Chemosphere. 2009;75(3):303-9. doi: 10.1016/j.chemosphere.2008.12.047.
52. Groisman L, Rav-Acha C, Gerstl Z, Mingelgrin U. Sorption and detoxification of toxic compounds by a bifunctional organoclay. J Environ Qual. 2004;33(5):1930-6. doi: 10.2134/jeq2004.1930.
53. Wang G, Xi Y, Lian C, Sun Z, Zheng S. Simultaneous detoxification of polar aflatoxin B1 and weak polar zearalenone from simulated gastrointestinal tract by zwitterionic montmorillonites. J Hazard Mater. 2019;364:227-37. doi: 10.1016/j.jhazmat.2018.09.071.
54. Hassan AM, Kenawy AM, Abbas WT, Abdel-Wahhab MA. Prevention of cytogenetic, histochemical and biochemical alterations in Oreochromis niloticus by dietary supplement of sorbent materials. Ecotoxicol Environ Saf. 2010;73(8):1890-5. doi: 10.1016/j.ecoenv.2010.07.041.
55. Mahrous KF, Hassan AM, Radwan HA, Mahmoud MA. Inhibition of cadmium- induced genotoxicity and histopathological changes in Nile tilapia fish by Egyptian and Tunisian montmorillonite clay. Ecotoxicol Environ Saf. 2015;119:140-7. doi: 10.1016/j.ecoenv.2015.04.054.
56. Wang M, Rivenbark K, Gong J, Wright FA, Phillips TD. Application of edible montmorillonite clays for the adsorption and detoxification of microcystin. ACS Appl Bio Mater. 2021;4(9):7254-65. doi: 10.1021/acsabm.1c00779.
57. Wang M, Orr AA, Jakubowski JM, Bird KE, Casey CM, Hearon SE, Tamamis P, Phillips TD. Enhanced adsorption of per- and polyfluoroalkyl substances (PFAS) by edible, nutrient-amended montmorillonite clays. Water Res. 2021;188:116534. doi: 10.1016/j.watres.2020.116534.
58. Ma Z, Long LH, Liu J, Cao YX. Montmorillonite adsorbs uric acid and increases the excretion of uric acid from the intestinal tract in mice. J Pharm Pharmacol. 2009;61(11):1499-504. doi: 10.1211/jpp/61.11.0009.
59. Zhang YT, Wang XF, Long LH, Liu T, Cao YX. Montmorillonite adsorbs creatinine and accelerates creatinine excretion from the intestine. J Pharm Pharmacol. 2009;61(4):459-64. doi: 10.1211/jpp/61.04.0007.
60. Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res. 2008;25(9):2097-116. doi: 10.1007/s11095-008-9661-9.
61. Taleblou N, Sirousazar M, Hassan ZM, Khaligh SG. Capecitabine-loaded anti-cancer nanocomposite hydrogel drug delivery systems: in vitro and in vivo efficacy against the 4T1 murine breast cancer cells. J Biomater Sci Polym Ed. 2020;31(1):72-92. doi: 10.1080/09205063.2019.1675225.
62. Kouser R, Vashist A, Zafaryab M, Rizvi MA, Ahmad S. Na-montmorillonite-dispersed sustainable polymer nanocomposite hydrogel films for anticancer drug delivery. ACS Omega. 2018;3(11):15809-20. doi: 110.1021/acsomega.8b01691.
63. Yew YP, Shameli K, Mohamad SE, Lee KX, Teow SY. Green synthesized montmorillonite/carrageenan/Fe3O4 nanocomposites for pH-responsive release of protocatechuic acid and its anticancer activity. Int J Mol Sci. 2020;21(14):4851. doi: 10.3390/ijms21144851.
64. Yew YP, Shameli K, Mohamad SEB, Nagao Y, Teow SY, Lee KX, Mohamed Isa ED. Potential anticancer activity of protocatechuic acid loaded in montmorillonite/Fe3O4 nanocomposites stabilized by seaweed Kappaphycus alvarezii. Int J Pharm. 2019;572:118743. doi: 10.1016/j.ijpharm.2019.118743.
65. Jafari H, Atlasi Z, Mahdavinia GR, Hadifar S, Sabzi M. Magnetic κ-carrageenan/chitosan/montmorillonite nanocomposite hydrogels with controlled sunitinib release. Mater Sci Eng C Mater Biol Appl. 2021;124:112042. doi: 10.1016/j.msec.2021.112042.
66. Kevadiya BD, Thumbar RP, Rajput MM, Rajkumar S, Brambhatt H, Joshi GV, Dangi GP, Mody HM, Gadhia PK, Bajaj HC. Montmorillonite/poly-(ε-caprolactone) composites as versatile layered material: reservoirs for anticancer drug and controlled release property. Eur J Pharm Sci. 2012;47(1):265-72. doi: 10.1016/j.ejps.2012.04.009.
67. Yu M, Pan L, Sun L, Li J, Shang J, Zhang S, Liu D, Li W. Supramolecular assemblies constructed from β-cyclodextrin-modified montmorillonite nanosheets as carriers for 5-fluorouracil. J Mater Chem B. 2015;3(46):9043-52. doi: 10.1039/c5tb01513h.
68. Kevadiya BD, Patel TA, Jhala DD, Thumbar RP, Brahmbhatt H, Pandya MP, Rajkumar S, Jena PK, Joshi GV, Gadhia PK, Tripathi CB, Bajaj HC. Layered inorganic nanocomposites: a promising carrier for 5-fluorouracil (5-FU). Eur J Pharm Biopharm. 2012;81(1):91-101. doi: 10.1016/j.ejpb.2012.01.004.
69. Haseli S, Pourmadadi M, Samadi A, Yazdian F, Abdouss M, Rashedi H, Navaei-Nigjeh M. A novel pH-responsive nanoniosomal emulsion for sustained release of curcumin from a chitosan-based nanocarrier: Emphasis on the concurrent improvement of loading, sustained release, and apoptosis induction. Biotechnol Prog. 2022;e3280. doi: 10.1002/btpr.3280.
70. Kevadiya BD, Chettiar SS, Rajkumar S, Bajaj HC, Gosai KA, Brahmbhatt H. Evaluation of clay/poly (L-lactide) microcomposites as anticancer drug, 6-mercaptopurine reservoir through in vitro cytotoxicity,

oxidative stress markers and in vivo pharmacokinetics. Colloids Surf B Biointerfaces. 2013;112:400-7. doi: 10.1016/j.colsurfb.2013.07.008.
71. Zhang Y, Yin S, Jia Y, Qin L. Safety and efficacy of apatinib combined with iodine-125 in chemotherapy-refractory advanced lung cancer: a case report. Medicine (Baltimore) 2020; 99(33):e21600. doi: 10.1097/MD.0000000000021600.
72. Bham S, Saeed F, Khan M, Khan R, Siraj F, Afsar S, Mehmood T. P368 Frequency of diarrhea and pneumonia in vaccinated and unvaccinated children under 5 years of age: a single center study. Arch Dis Childhood 2019;104:A303. doi: 10.1136/archdischild-2019-epa.714.
73. Ducrotte P, Dapoigny M, Bonaz B, Siproudhis L. Symptomatic efficacy of beidellitic montmorillonite in irritable bowel syndrome: a randomized, controlled trial. Aliment Pharmacol Ther. 2005;21(4):435-44. doi: 10.1111/j.1365-2036.2005.02330.x.
74. Wang Z, Wang Y, Hu Y, Chen M, Li T. Efficacy of montmorillonite and vitamin AD combined with zinc preparation in children with diarrheal disease and its effect on inflammatory factors. Am J Transl Res. 2021;13(5):5428-35.
75. Jin Y, Ying L, Shi L. Probiotics combined with montmorillonite for treatment of diarrhea in children: Effect on clinical symptoms, immune function, and adverse reactions. World Chin J Dig. 2016;24(27):3925. doi: 10.11569/wcjd.v24.i27.3925.
76. Hu CH, Gu LY, Luan ZS, Song J, Zhu K. Effects of montmorillonite-zinc oxide hybrid on performance, diarrhea, intestinal permeability and morphology of weanling pigs. Anim Feed Sci Technol. 2012; 177(1-2):108-15; doi: 10.1016/j.anifeedsci.2012.07.028.
77. Yu M, Jin X, Liang C, Bu F, Pan D, He Q, Ming Y, Little P, Du H, Liang S, Hu R. Berberine for diarrhea in children and adults: a systematic review and meta-analysis. Therap Adv Gastroenterol. 2020;13:1756284820961299. doi: 10.1177/1756284820961299.
78. Chen J, Wan CM, Gong ST, Fang F, Sun M, Qian Y, Huang Y, Wang BX, Xu CD, Ye LY, Dong M, Jin Y, Huang ZH, Wu QB, Zhu CM, Fang YH, Zhu QR, Dong YS. Chinese clinical practice guidelines for acute infectious diarrhea in children. World J Pediatr. 2018;14(5):429-36. doi: 10.1007/s12519-018-0190-2.
79. Chunyong MU. Clinical analysis of montmorillonite powder combined with zinc gluconate in the treatment of 74 children with diarrhea. Chin J Prim Med Pharm. 2014;12:1498-9.
80. Liu XZ, Zeng Z. [Umbilical therapy combined with moxibustion for autumn diarrhea in children]. Zhongguo Zhen Jiu. 2019;39(8):832-6. doi: 10.13703/j.0255-2930.2019.08.009.
81. Song J, Li YL, Hu CH. Effects of copper-exchanged montmorillonite, as alternative to antibiotic, on diarrhea, intestinal permeability and proinflammatory cytokine of weanling pigs. Appl Clay Sci. 2013;77-78:52-5. doi: 10.1016/j.clay.2013.01.016.
82. Yao DW, Yu ZZ, Li N, Hou YN, Xu JR, Yang DJ. Copper-modified palygorskite is effective in preventing and treating diarrhea caused by Salmonella typhimurium. J Zhejiang Univ Sci B. 2017;18(6):474-80. doi: 10.1631/jzus.B1600133.
83. Stevens G, Mascarenhas M, Mathers C. Global health risks: progress and challenges. Bull World Health Organ. 2009;87(9):646. doi: 10.2471/blt.09.070565.
84. Emerging Risk Factors Collaboration, Wormser D, Kaptoge S, Di Angelantonio E, Wood AM, Pennells L, Thompson A, Sarwar N, Kizer JR, Lawlor DA, Nordestgaard BG, Ridker P, Salomaa V, Stevens J, Woodward M, Sattar N, Collins R, Thompson SG, Whitlock G, Danesh J. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. Lancet. 2011;377(9771):1085-95. doi: 10.1016/S0140-6736(11)60105-0.
85. Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon DA, Smeeth L. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5•24 million UK adults. Lancet. 2014; 384(9945):755-65. doi: 10.1016/S0140-6736(14)60892-8.
86. Arnold M, Pandeya N, Byrnes G, Renehan PAG, Stevens GA, Ezzati PM, Ferlay J, Miranda JJ, Romieu I, Dikshit R, Forman D, Soerjomataram I. Global burden of cancer attributable to high body-mass index in 2012: a population-based study. Lancet Oncol. 2015;16(1):36-46. doi: 10.1016/S1470-2045(14)71123-4.
87. Joyce P, Dening TJ, Meola TR, Wignall A, Ulmefors H, Kovalainen M, Prestidge CA. Contrasting anti-obesity effects of smectite clays and mesoporous silica in sprague-dawley rats. ACS Appl Bio Mater. 2020;3(11):7779-88. doi: 10.1021/acsabm.0c00969.
88. Xu P, Dai S, Wang J, Zhang J, Liu J, Wang F, Zhai Y. Preventive obesity agent montmorillonite adsorbs dietary lipids and enhances lipid excretion from the digestive tract. Sci Rep. 2016;6:19659. doi: 10.1038/srep19659.
89. Xu P, Hong F, Wang J, Cong Y, Dai S, Wang S, Wang J, Jin X, Wang F, Liu J, Zhai Y. Microbiome remodeling via the montmorillonite adsorption-excretion axis prevents obesity-related metabolic disorders. EBioMedicine. 2017;16:251-61. doi: 10.1016/j.ebiom.2017.01.019.
90. Wang Q, Shen J, Mo E, Zhang H, Wang J, Hu X, Zhou J, Bai H, Tang G. A versatile ultrafine and super-absorptive H+-modified montmorillonite: application for metabolic syndrome intervention and gastric mucosal protection. Biomater Sci. 2020;8(12):3370-80. doi: 10.1039/d0bm00474j.
91. Dening TJ, Joyce P, Kovalainen M, Gustafsson H, Prestidge CA. Spray dried smectite clay particles as a novel treatment against obesity. Pharm Res. 2018;36(1):21. doi: 10.1007/s11095-018-2552-9.
92. Gutman R, Rauch M, Neuman A, Khamaisi H, Jonas-Levi A, Konovalova Y, Rytwo G. Sepiolite Clay attenuates the development of hypercholesterolemia and obesity in mice fed a high-fat high-cholesterol diet. J Med Food. 2020;23(3):289-96. doi: 10.1089/jmf.2019.0030.
93. Jahromi MAM, Zangabad PC, Basri SMM, Zangabad KS, Ghamarypour A, Aref AR, Karimi M, Hamblin MR. Nanomedicine and advanced technologies for

burns: Preventing infection and facilitating wound healing. Adv Drug Deliv Rev. 2018;123:33-64. doi: 10.1016/j.addr.2017.08.001.
94. Mascarenhas-Melo F, Peixoto D, Aleixo C, S Gonçalves MB, Raza F, Pawar KD, Veiga F, Liu M, Paiva-Santos AC. Nanoclays for wound management applications. Drug Deliv Transl Res. 2022. doi: 10.1007/s13346-022-01279-3.
95. Bramhill J, Ross S, Ross G. Bioactive nanocomposites for tissue repair and regeneration: a review. Int J Environ Res Public Health. 2017;14:1-21. doi: 10.3390/ijerph14010066.
96. Sandri G, Bonferoni MC, Ferrari F, Rossi S, Aguzzi C, Mori M, Grisoli P, Cerezo P, Tenci M, Viseras C, Caramella C. Montmorillonite-chitosan-silver sulfadiazine nanocomposites for topical treatment of chronic skin lesions: in vitro biocompatibility, antibacterial efficacy and gap closure cell motility properties. Carbohydr Polym. 2014;102:970-7. doi: 10.1016/j.carbpol.2013.10.029.
97. Naumenko EA, Guryanov ID, Yendluri R, Lvov YM, Fakhrullin RF. Clay nanotube-biopolymer composite scaffolds for tissue engineering. Nanoscale. 2016;8:7257-71. doi: 10.1039/C6NR00641H
98. Sandri G, Aguzzi C, Rossi S, Bonferoni MC, Bruni G, Boselli C, Cornaglia AI, Riva F, Viseras C, Caramella C, Ferrari F. Halloysite and chitosan oligosaccharide nanocomposite for wound healing. Acta Biomater. 2017;57:216-24. doi: 10.1016/j.actbio.2017.05.032.
99. Liu C, Liu C, Yu S, Wang N, Yao W, Liu X, Sun G, Song Q, Qiao W. Efficient antibacterial dextran-montmorillonite composite sponge for rapid hemostasis with wound healing. Int J Biol Macromol. 2020;160:1130-43. doi: 10.1016/j.ijbiomac.2020.05.140.
100. Li G, Quan K, Liang Y, Li T, Yuan Q, Tao L, Xie Q, Wang X. Graphene-montmorillonite composite sponge for safe and effective hemostasis. ACS Appl Mater Interfaces. 2016;8(51):35071-80. doi: 10.1021/acsami.6b13302.
101. Sajjad W, Khan T, Ul-Islam M, Khan R, Hussain Z, Khalid A, Wahid F. Development of modified montmorillonite-bacterial cellulose nanocomposites as a novel substitute for burn skin and tissue regeneration. Carbohydr Polym. 2019;206:548-56. doi: 10.1016/j.carbpol.2018.11.023.
102. García-Villén F, Faccendini A, Aguzzi C, Cerezo P, Bonferoni MC, Rossi S, Grisoli P, Ruggeri M, Ferrari F, Sandri G, Viseras C. Montmorillonite-norfloxacin nanocomposite intended for healing of infected wounds. Int J Nanomedicine. 2019;14:5051-60. doi: 10.2147/IJN.S208713.
103. Sandri G, Faccendini A, Longo M, Ruggeri M, Rossi S, Bonferoni MC, Miele D, Prina-Mello A, Aguzzi C, Viseras C, Ferrari F. Halloysite- and montmorillonite-loaded scaffolds as enhancers of chronic wound healing. Pharmaceutics. 2020;12(2):179. doi: 10.3390/pharmaceutics12020179.
104. Ambrogi V, Pietrella D, Nocchetti M, Casagrande S, Moretti V, De Marco S, Ricci M. Montmorillonite-chitosan-chlorhexidine composite films with antibiofilm activity and improved cytotoxicity for wound dressing. J Colloid Interface Sci. 2017;491:265-72. doi: 10.1016/j.jcis.2016.12.058.
105. Delir S, Sirousazar M, Kheiri F. Clindamycin releasing bionanocomposite hydrogels as potential wound dressings for the treatment of infected wounds. J Biomater Sci Polym Ed. 2020;31(12):1489-514. doi: 10.1080/09205063.2020.1764161.
106. Sadeghianmaryan A, Yazdanpanah Z, Soltani YA, Sardroud HA, Nasirtabrizi MH, Chen X. Curcumin-loaded electrospun polycaprolactone/montmorillonite nanocomposite: wound dressing application with anti-bacterial and low cell toxicity properties. J Biomater Sci Polym Ed. 2020;31(2):169-87. doi: 10.1080/09205063.2019.1680928.
107. Jahani-Javanmardi A, Sirousazar M, Shaabani Y, Kheiri F. Egg white/poly (vinyl alcohol)/MMT nanocomposite hydrogels for wound dressing. J Biomater Sci Polym Ed. 2016;27(12):1262-76. doi: 10.1080/09205063.2016.1191825.
108. Kevadiya BD, Rajkumar S, Bajaj HC, Chettiar SS, Gosai K, Brahmbhatt H, Bhatt AS, Barvaliya YK, Dave GS, Kothari RK. Biodegradable gelatin-ciprofloxacin-montmorillonite composite hydrogels for controlled drug release and wound dressing application. Colloids Surf B Biointerfaces. 2014;122:175-83. doi: 10.1016/j.colsurfb.2014.06.051.
109. Yu X, Guo L, Liu M, Cao X, Shang S, Liu Z, Huang D, Cao Y, Cui F, Tian L. Callicarpa nudiflora loaded on chitosan-collagen/organomontmorillonite composite membrane for antibacterial activity of wound dressing. Int J Biol Macromol. 2018;120(Pt B):2279-84. doi: 10.1016/j.ijbiomac.2018.08.113.
110. Pan S, Yu H, Yang X, Yang X, Wang Y, Liu Q, Jin L, Yang Y. Application of nanomaterials in stem cell regenerative medicine of orthopedic surgery. J Nanomater. 2017;2017. doi: 10.1155/2017/1985942.
111. Rahyussalim AJ, Marsetio AF, Saleh I, Kurniawati T, Whulanza Y. The needs of current implant technology in orthopaedic prosthesis biomaterials application to reduce prosthesis failure rate. J Nanomater. 2016;2016. doi: 10.1155/2016/5386924.
112. Peña-Parás L, Sánchez-Fernández JA, Vidaltamayo R. Nanoclays for biomedical applications. In: Martínez LMT, editore. Handbook of ecomaterials. Springer International Publishing AG; 2018; p. 3453-71. doi: 10.1007/978-3-319-68255-6_50.
113. Zhu TT, Zhou CH, Kabwe FB, Wu QQ, Li CS, Zhang JR. Exfoliation of montmorillonite and related properties of clay/polymer nanocomposites. Appl Clay Sci. 2019;169:48-66. doi: 10.1016/j.clay.2018.12.006.
114. Demir AK, Elçin AE, Elçin YM. Strontium-modified chitosan/montmorillonite composites as bone tissue engineering scaffold. Mater Sci Eng. C. 2018;89:8-14. doi: 10.1016/j.msec.2018.03.021.
115. Li D, Li P, Xu Y, Guo W, Li M, Chen M, Wang H, Lin H. Progress in montmorillonite functionalized artificial bone scaffolds: Intercalation and interlocking, nanoenhancement, and controlled drug release. J Nanomater. 2022;2022. doi: 10.1155/2022/7900382.
116. Paluszkiewicz C, Stodolak E, Hasik M, Blazewicz M. FT-IR study of montmorillonite-chitosan nanocomposite materials. Spectrochim Acta A Mol Bio-

mol Spectrosc. 2011;79(4):784-8. doi: 10.1016/j.saa.2010.08.053.
117. Shuai C, Li Y, Feng P, Yang W, Zhao Z, Liu W. Montmorillonite reduces crystallinity of poly-l-lactic acid scaffolds to accelerate degradation. Polym Adv Technol. 2019;30(9):2425-35. doi: 10.1002/pat.4690.
118. Zheng JP, Xi LF, Zhang HL, Yao KD. Correlation between reaction environment and intercalation effect in the synthesis of gelatin/montmorillonite hybrid nanocomposite. J Mater Sci Lett. 2003;22:1179-81. doi: 10.1023/A:1025332029976.
119. Nawang R, Hussein MZ, Matori KA, Abdullah CA, Hashim M. Physicochemical properties of hydroxyapatite/montmorillonite nanocomposite prepared by powder sintering. Results Phys. 2019;15:102540. doi: 10.1016/j.rinp.2019.102540.
120. Kim GJ, Kim D, Lee KJ, Kim D, Chung KH, Choi JW, An JH. Effect of nano-montmorillonite on osteoblast differentiation, mineral density, and osteoclast differentiation in bone formation. Nanomaterials (Basel). 2020;10(2):230. doi: 10.3390/nano10020230.
121. Park JH, Shin HJ, Kim MH, Kim JS, Kang N, Lee JY, Kim KT, Lee JI, Kim DD. Application of montmorillonite in bentonite as a pharmaceutical excipient in drug delivery systems. J Pharm Investig. 2016;46(4):363-75. doi: 10.1007/s40005-016-0258-8.
122. Joshi GV, Kevadiya BD, Patel HA, Bajaj HC, Jasra RV. (2009). Montmorillonite as a drug delivery system: Intercalation and in vitro release of timolol maleate. Int J Pharm. 2009;374(1-2):53-7. doi: 10.1016/J.IJPHARM.2009.03.004.
123. Iliescu RI, Andronescu E, Ghitulica CD, Voicu G, Ficai A, Hoteteu M. Montmorillonite-alginate nanocomposite as a drug delivery system--incorporation and in vitro release of irinotecan. Int J Pharm. 2014;463(2):184-92. doi: 10.1016/j.ijpharm.2013.08.043.
124. Park CG, Choi G, Kim MH, Kim SN, Lee H, Lee NK, Choy YB, Choy JH. Brimonidine-montmorillonite hybrid formulation for topical drug delivery to the eye. J Mater Chem B. 2020;8(35):7914-20. doi: 10.1039/d0tb01213k.
125. Bello ML, Junior AM, Freitas CA, Moreira MLA, da Costa JP, de Souza MA, Santos BAMC, de Sousa VP, Castro HC, Rodrigues CR, Cabral LM. Development of novel montmorillonite-based sustained release system for oral bromopride delivery. Eur J Pharm Sci. 2022;175:106222. doi: 10.1016/j.ejps.2022.106222.
126. Zhao H, Ye H, Zhou J, Tang G, Hou Z, Bai H. Montmorillonite-enveloped zeolitic imidazolate framework as a nourishing oral nano-platform for gastrointestinal drug delivery. ACS Appl Mater Interfaces. 2020;12(44):49431-41. doi: 10.1021/acsami.0c15494.
127. Kenawy ER, Shaker NO, Azaam M, Lasheen AS, Lin J, Zakaria MB. Montmorillonite intercalated norfloxacin and tobramycin for new drug-delivery systems. J Nanosci Nanotechnol. 2020;20(8):5246-51. doi: 10.1166/jnn.2020.18532.
128. Sharifzadeh G, Hezaveh H, Muhamad II, Hashim S, Khairuddin N. Montmorillonite-based polyacrylamide hydrogel rings for controlled vaginal drug delivery. Mater Sci Eng C Mater Biol Appl. 2020;110:110609. doi: 10.1016/j.msec.2019.110609.

REVIEW PAPER

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Data distribution analysis – a preliminary approach to quantitative data in biomedical research

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ABSTRACT

Statistical analysis is an integral part of medical research. It helps transform raw data into meaningful insights, supports hypothesis testing, optimises study design, assesses risk and prognosis, and facilitates evidence-based decision-making. The statistical analysis increases research findings' reliability, validity and generalisability, ultimately advancing medical knowledge and improving patient care. Without it, meaningful analysis of the data collected would be impossible. The conclusions drawn would be unsubstantiated and misleading.

Many health professionals are unfamiliar with statistical analysis and its basic concepts. The analysis of clinical data is an integral part of medical research. Identifying the data type (continuous, quasi-continuous or discrete) and detecting outliers are the first and most important steps. When analysing the data distribution for normality, graphical and numerical methods are recommended. Depending on the type of data distribution, appropriate non-parametric or parametric tests can be used for further analysis. Data that are not normally distributed can be normalised using various mathematical methods (e.g., square root or logarithm) and analysed using parametric tests in the next steps.

This review provides essential explanations of these concepts without using complex mathematical or statistical equations but with several graphical examples of various statistical terms.

Introduction

Statistical analysis is essential in medical research. It transforms raw data into meaningful insights, supports hypothesis testing, optimises study design, assesses risk and prognosis, and facilitates evidence-based decision-making. The

statistical analysis increases research findings' reliability, validity and generalisability, ultimately advancing medical knowledge and improving patient care. Without it, meaningful analysis of the data collected would be impossible. The conclusions drawn would be unsubstantiated and misleading.

Many medical professionals are unfamiliar with statistical analysis and its basic concepts, starting with the types of quantitative data, such as continuous and discrete data, or their distribution analysis. This review provides essential explanations of these concepts without using complex mathematical or statistical equations but with several graphical examples of various statistical terms.

Data types

Different types of data are collected in biomedical research. The most common are quantitative, qualitative and descriptive (textual).

Quantitative or numerical data can take any numerical value and are represented as numbers. Some values are less than, equal to, or greater than others, for example, age 15, 21, and 35 years; length 22, 19, and 10 cm; area 2, 2, and 2.5 cm2; weight 78, 82, and 95.3 kg; power 224, 248, and 301 watts; or a ratio of two variables such as serum triglycerides to HDL concentrations of 1.2, 3.5, and 4.9. Quantitative data may or may not have units.

Qualitative data are usually non-numerical and are described by labels or qualitative characteristics. Many qualitative variables can only be categorised, labelled, but never ranked, ordered or graded, such as gender (male, female), ethnicity (e.g. African American, European, Latin American) or colour (red, yellow, green, black). For example, red is not bigger or smaller than blue. However, other qualitative observations can be ranked in a natural order based on qualitative analysis. However, the distances between the categories are unknown. One object may be larger than another. One person may be nicer than another. One heart failure patient in New York Heart Association (NYHA) functional class 2 has less severe symptoms than another in NYHA 3. Some examples of ordinal data for which relative, subjective or arbitrary scales should be used are warm, warmer and warmest, or primary, high school, college, graduate and postgraduate for educational level. Similarly, the effect of pharmacotherapy on a patient's symptoms can be subjectively rated as no change, slightly better, really better compared to previous treatment, or best of all medications taken before. For qualitative data, signs (+, ++, +++ or -, --, ---) and letter codes (A, B, C) are often used instead of longer words. As some statistical software does not accept text, numbers are used as codes in such cases. The numerical codes entered should be treated as nominal (preferably) or ordinal (if they can be ranked) data. Otherwise, numbers replacing text may be treated as continuous and become a source of error.

Descriptive data are typically textual and consist of words, abbreviations, phrases and sentences, e.g. medical notes, observations, test summaries, open-text comments and opinions. Specialised analysis tools are required to quantify and/or describe such data. These tools can be natural language processing techniques, Qualitative Text Analysis (QCA) or other methods such as the Generative Pre-trained Transformer (GPT), which is part of the family of Large Language Models (LLMs) analysed by artificial intelligence (ChatGPT).

Regardless of the type, all data are collected in databases. Data can be stored in spreadsheets or dedicated databases. Spreadsheets provide a tabular format with rows and columns to store and manage data. Most people find them easier to use for entering, manipulating and analysing data effectively, performing calculations, applying formulas, formatting and exporting to external statistical software. Unfortunately, spreadsheets have limitations compared to dedicated database management systems for large and more complex data sets. Dedicated and specially designed database management systems such as MySQL, Oracle, and Microsoft SQL Server, the Redcap Project are better solutions for such tasks. They offer features such as data indexing, data relationships, integrity constraints, normalisation and transaction management.

Regardless of the form of the database, various characteristics or parameters (variables) describing people, objects, animals, samples, etc., are stored and prepared for further statistical analysis.

This review focuses on quantitative data. We will explain the most common terms, how to analyse and interpret their distribution and present graphical examples.

Basic definitions

Several basic terms related to the types of quantitative data, their characterisation and the analysis of the distribution of data are presented in **Table 1**.

Types of quantitative data

Continuous data can have infinite possible values within a given range, including fractions, decimals or integers. Between any two values, there is always another. The reporting of each value depends on the precision of the measurement, which may determine whether the data are continuous. For example, a precision of 1 in 100 is considered continuous data, as opposed to 1 in 10, which makes the data quasi-continuous (almost continuous) or sometimes discrete because it appears to be stepped.

Medical examples of continuous data include cardiac cycle duration (910.9, 920.0, 920.1 ms), age (31.85, 31.86, 31.87 years), body temperature (36.58, 36.59, 36.60 °C), body mass index (BMI) (27.27, 27.28, 27.29 kg/m^2), blood glucose concentration (11.64, 11.65, 11.66 mmol/L).

Quasi-continuous data represent values that have been rounded or grouped into intervals. Using the same examples above, the rounded values will be 920 ms for the cardiac cycle, 32 years for age, 36.6°C for body temperature, 27.3 kg/m2 for body mass index and 11.7 mmol/L for blood glucose concentration. Some values in clinical practice are always rounded and given in whole numbers, such as heart rate – 63, 86, 105 beats/minute, blood pressure – 122/78, 124/84, 152/95 mmHg, body mass – 56, 78, 113 kg, etc. Quasi-continuous data, however, should be considered continuous for statistical analysis.

Discrete data can only take specific values and have no value between two adjacent values. Typical examples are the number of pregnancies (there cannot be 3.35 pregnancies) and the number of hospitalisations (it is impossible to be hospitalised 5.173 times). The main difference between continuous and discrete data is that continuous data can take any value within a specific range, whereas discrete data can take only certain values. Continuous data are measured and expressed more accurately than discrete data.

Possible data transformation at a cost of precision loss

BMI as			
Original data	Transformed data		
continous	quasi-continous	discrete	ordinal categories
17.63148	17.6	18	1 – underweight
21.24743	21.2	21	2 – normal weight
23.22671	23.2	23	2 – normal weight
25.37863	25.4	25	3 – overweight
26.04873	26	26	3 – overweight
27.24712	27.2	27	3 – overweight
33.56914	33.6	34	4 – class 1 obesity
35.14975	35.1	35	5 – class 2 obesity
41.38937	41.4	41	6 – class 3 obesity

Impossible data transformation

Figure 1. A sample of different original body mass indices derived from height and mass. These data are transformed from continuous through quasi-continuous, discrete to ordinal. Each further step involves a loss of accuracy. Transforming data to a less precise category often involves grouping observations into predefined ranges or bins. This results in a loss of information and granularity. Subtle differences between individuals may be obscured, making it harder to see fine patterns or relationships in the data or showing weak or no associations between variables. The reverse process of recovering the original information (continuous data) from all the transformed data is mathematically unfeasible.

Mathematical manipulation with continuous and discrete data is possible; e.g., measuring height and weight makes it easy to calculate BMI. Similarly, converting continuous or discrete data into categorical data is also straightforward. All such data belong to an interval or ratio scale.

Based on BMI and known criteria, a person is categorised as underweight, normal weight, overweight or obese category 1, 2 or 3. However, this is a one-way process. Retrieving backward information on BMI from one of these categories is impossible (see **Figure 1**).

Outliers

An outlier is a value significantly different from other values in the dataset. Measurement inaccuracies, data entry errors, natural variation, or truly unusual observations are the leading causes of

Table 1. Basic terms related to the types of quantitative data, characterisation and analysis of distribution.

Term	Definition
Descriptive statistics	Analyses designed to describe and summarise the data set.
Continuous data	Data that can take on any value within a range, e.g., body temperature, serum sodium concentration, white blood cells count, and time.
Quasi-continuous data	Data nearly continuous or continuous data that were rounded for some purposes, e.g. age in years, body weight in kilograms, blood pressure in mmHg, heart rate in beats/minute.
Discrete data	Data that can only take on specific values, e.g., the number of children in a family, the number of fingers and toes, and the number of epilepsy attacks.
Distribution	It displays the rate or probability of different values occurring in a given data set.
Histogram	Graphical representation of the distribution of numerical data binned into neighbouring bars.
Density plot	Graphical visualisation of the distribution of continuous data as a smooth curve with continuous data representing the data distribution.
Q-Q plot	Short term for the quantile-quantile plot. Graphical visualisation of assessing whether data follow a normal distribution.
Outlier	An observation or data point with an extreme value that lies far away from most data points.
Minimum	The smallest value in a dataset.
Maximum	The largest value in a dataset.
Percentile	A measure used to indicate the value below which a given percentage of observations in a group of observations falls. For example, the 5th percentile indicates that 5% of the values in a dataset are less than or equal to that value.
Lower quartile (Q1)	25^{th} percentile, a value below or equal to which 25% of the values in the dataset are located.
Upper quartile (Q3)	75^{th} percentile, a value below or equal to which 75% of the values in the dataset are located.
Interquartile range (IQR)	The range between a dataset's first quartile (25^{th} percentile) and the third quartile (75^{th} percentile).
Range	Distance between the maximum and minimum values of a data set.
Central Tendency	Various measures indicating the middle or centre of a distribution.
Median	Middle value (50^{th} percentile) in a dataset ranked from minimum to maximum values.
Mode	The most common value in a dataset.
Mean	The average value of a dataset calculated by adding up all the values and dividing by the number of values.
Trimmed mean	A statistical measure calculated by removing a certain percentage of the largest and smallest values in a dataset and then calculating the mean of the remaining values. It is done to reduce the impact of outliers on the mean, for instance, removing 5% of the measurements reduces 2.5% of the largest and 2.5% of the smallest values.
Normal distribution	A bell-shaped curve represents the distribution of many biological phenomena and data.
Skewness	A measure of how asymmetrical a distribution is.
Kurtosis	A measure of how peaked or flat a distribution is compared to a normal distribution.
Deviation	The distance between the mean and a particular data point in a given distribution.
Standard deviation (SD)	A measure of how much the data deviates from the mean.
Variance	A measure of how spread out the data is from the mean.
Coefficient of variation (CV)	A relative and unitless measure of the dispersion of data points around the mean. It allows comparing variability between disparate groups and characteristics. A smaller CV indicates that the data points are more tightly clustered around the mean, while a larger coefficient of variation indicates that the data points are more spread out.
Standard error of the mean (SEM)	A measure of how much the sample mean is likely to differ from the true population mean to assess the precision of the sample mean. It is derived by dividing the standard deviation by the root of the sample size.
Confidence Interval (CI)	A range of values likely to contain the true population parameter with a certain confidence level. CI is usually expressed as a percentage, such as 95% or 99%
Z-score	A statistical measure that determines the relative distance of a given value from the mean, using standard deviation as the measure of that distance. In other words, the Z-score represents the number of standard deviations a data point is from the mean of the distribution. It is calculated as the difference between the given value and the mean divided by the standard deviation. By using the Z-score, data points from different distributions can be standardised and compared on the same scale. A positive z-score indicates that the data point is above the mean, while a negative z-score indicates that it is below the mean. A Z-score of 0 means that the data point is exactly at the mean.

these extreme values. Outliers can significantly impact statistical analyses and distort the results or conclusions drawn from the data. They can affect some measures of central tendency, such as the mean, and estimates of variability, such as the standard deviation. Outliers can violate statistical methods assuming normal data distribution. In contrast, non-parametric methods and descriptors such as median, interquartile range (IQR) or mode are insensitive to outliers.

Identifying outliers is essential to ensure the integrity and validity of data analysis. It involves examining the data distribution and looking for values unusually far from most observations. Outliers can be detected using various methods, including graphical techniques (e.g. box plots, scatter plots, violin plots), statistical tests and computational algorithms. The decision to deal with outliers depends on the specific research context, the nature of the outliers and the analysis objectives. In research, it is essential to document all procedures for identifying and dealing with outliers. This ensures transparency and reproducibility.

Types of data distribution

The normal distribution is very common in biomedical research. It is also known as the Gaussian distribution or the bell curve. The normal distribution is symmetrical about the mean and has a characteristic bell shape. Many biological and physical phenomena follow a normal distribution. For example, the heights of adult humans follow a normal distribution.

Skewed distributions are another type of distribution. They occur when the data do not have symmetrical distribution around the mean. There are two types of skewed distribution: positively skewed (to the right) and negatively skewed (to the left). In a positively skewed distribution, the curve's tail is longer on the right than on the left. In a negatively skewed distribution, the curve's tail is longer on the left than on the right. A common example of a positively skewed distribution is income data, where many people have low incomes, and a few have very high incomes. The age distribution of patients admitted to a hospital with neonatal and paediatric wards is different and skewed to the right compared to another hospital where only adults, especially older people, are admitted. The mean serum creatine concentration is higher, and the distribution is skewed to the left in nephrology patients compared to general medical ward patients.

Bimodal distributions occur when there are two peaks in the data. This happens when two different subgroups in the same data differ and emerge. For example, in a combined data set, the average muscle mass for men and women differs. It naturally separates – such data distributions show two peaks.

Multimodal distributions occur when there are more than two peaks in the data. They occur when more than two data groups have different characteristics. For example, the distribution of the height of men, women and children in the same database shows three peaks in the data. Often this represents unbalanced data collection, such as more young people or more women than men.

Distribution analysis

Examining distributions is an integral part of data analysis. It involves comparing the characteristics of two or more distributions to determine whether they are different or similar. Several graphical and numerical methods can be used to compare distributions.

Graphical data visualisation used to analyse data distributions

In medical research, data visualisation is invaluable for analysing all forms of quantitative data. Various data features can be identified using appropriate visualisations, such as central tendency, dispersion, minimum and maximum values, outliers, data distribution and shape. These visualisations make interpreting data, communicating findings, and drawing conclusions easier. Presenting complex data in visual formats simplifies identifying differences, associations, trends and patterns.

1. A *histogram* is used to estimate the probability distribution of all forms of quantitative data, preferably continuous and quasi-continuous. Although they are not best suited to discrete data, histograms can provide insight into the number of cases with certain discrete values.

To construct a histogram, the data values are first binned into ranges, i.e. the whole range is divided into a series of narrower intervals. The number of values falling within each bin is then counted (see **Figure 2**). The bins represent successive, adjacent, non-overlapping intervals of a variable and are often (but not necessarily) of equal size. Histograms show the empirical shape of the distribution, central tendency (mean, median, mode), dispersion (variance, standard deviation) and outliers.

2. A *density plot* also shows the distribution of continuous data, similar to a histogram. However, the density plot uses a smooth theoretical curve instead of bars to represent the distribution. While they may not be the perfect way to visualise discrete data, density plots can give some insight into their distribution.

The smoothness of density plots refers to the degree of smoothing applied to the density curve drawn over the real data. With low smoothness, usually, more than one peak is visible. Higher smoothing degrees provide only one peak and may resemble a normal or skewed distribution curve (see **Figure 3**).

These plots are particularly useful for identifying the shape of the distribution, including whether it is symmetric, skewed or bimodal. They also provide information about the central tendency, dispersion and outliers present in the data.

Using a theoretical plot fitted to real data has its consequences. Very often, such a plot crosses the real data at the lower and upper limits, and sometimes the density plots show values that are not possible. There are no negative values for concentration, length or weight. A height of 300 cm is humanly impossible. These are artificial effects of the smoothing algorithms, which can stretch the estimated density curve to values that do not make sense for a particular dataset.

To deal with such a problem, a density plot can be truncated at zero to avoid negative val-

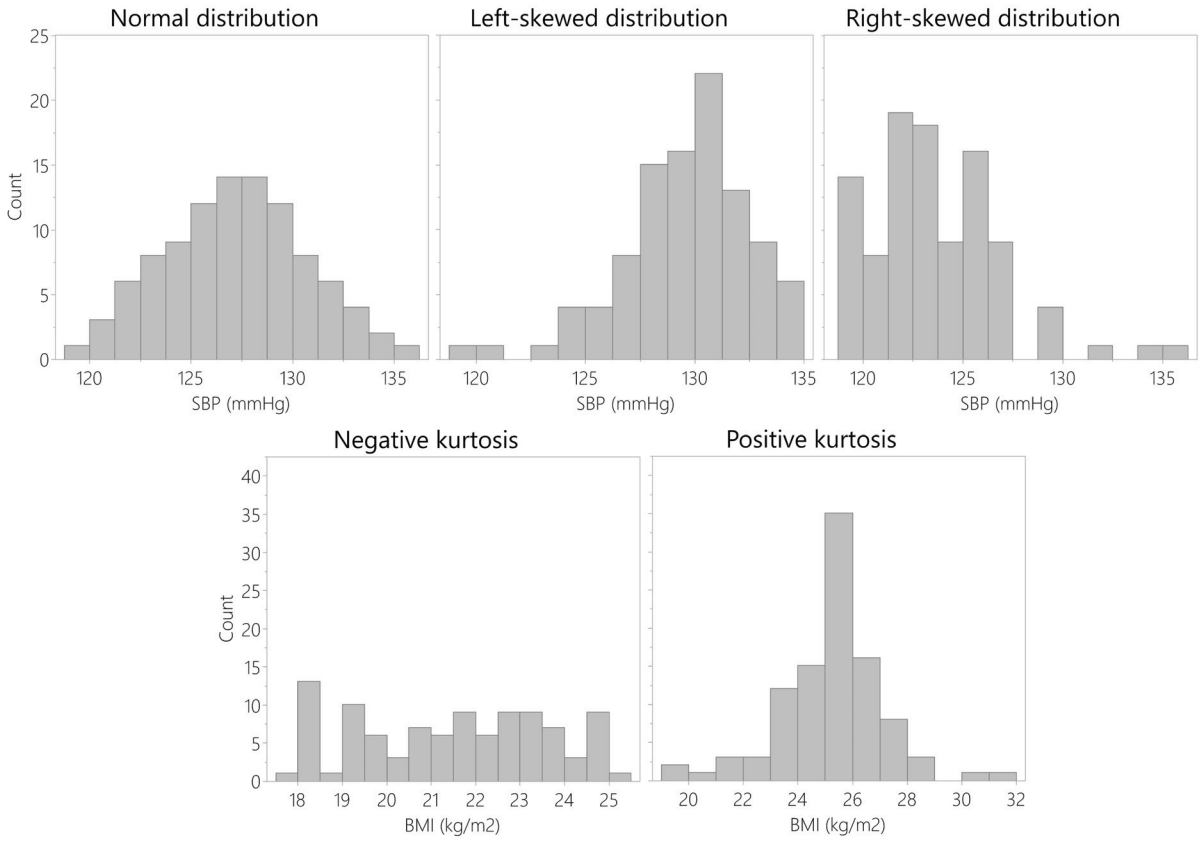

Figure 2. Examples of different histograms with systolic blood pressure (SBP) results in the upper panels and body mass index (BMI) in the lower panels. The first histogram with SBP shows a normal data distribution. The next two examples present data skewed to the left and right. The two BMI examples at the bottom display distributions with negative kurtosis (flattened shape) and positive kurtosis (narrower and higher shape).

ues – all negative values are set to zero. The upper range of these values should be defined for extremely high values.

3. A *box plot* or *box-and-whisker plot* shows the distribution of all types of quantitative data. It summarises vital statistical characteristics and highlights potential outliers in the data set (see **Figure 4**).

The following statistical measures are typically used to construct a box plot. – Median, represented by a horizontal line inside a box, dividing it in half. – Quartiles, i.e. the lower

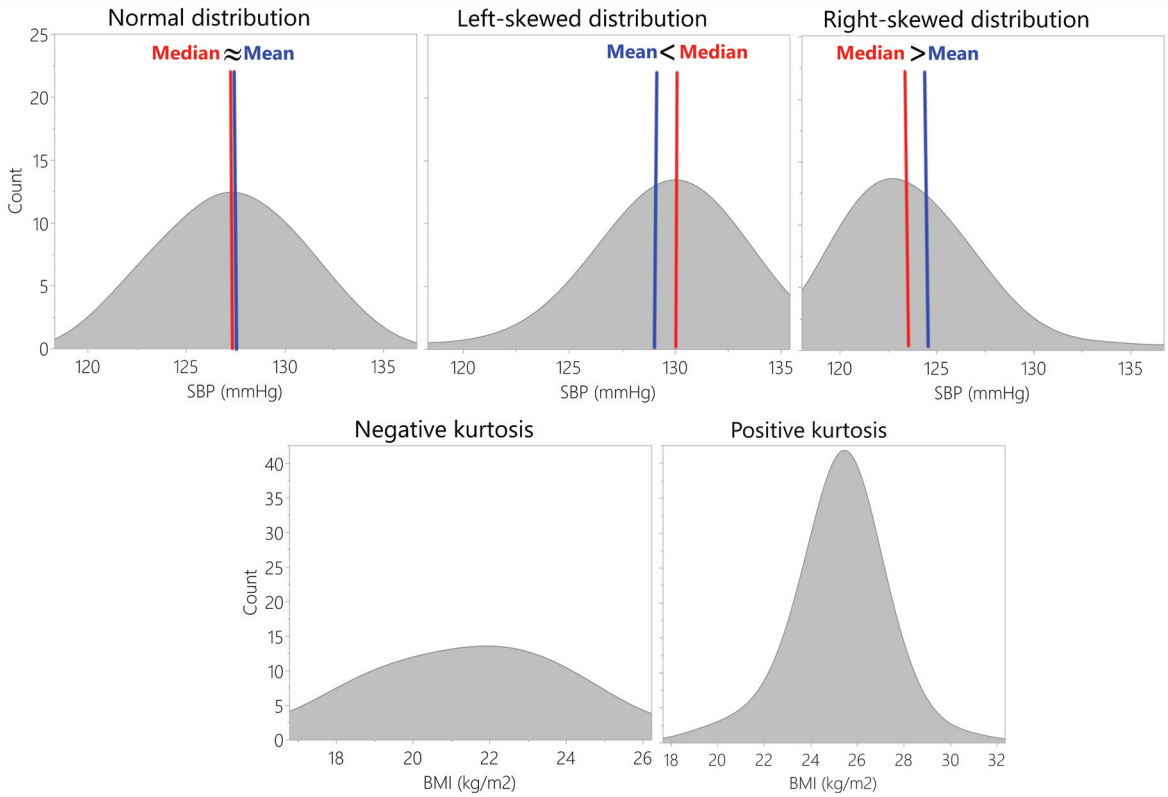

Figure 3. For this figure, the same data were used as in Figure 2. The histograms are replaced by density plots showing the data with a normal distribution, skewed to the right and the left, and then with positive and negative kurtosis (leptokurtic and platykurtic distribution). The mean and median are usually overlapping or very close to each other for the normal distribution. In contrast, for skewed data, the mean and median are separated. Negative or positive kurtosis does not affect the position of the mean and median.

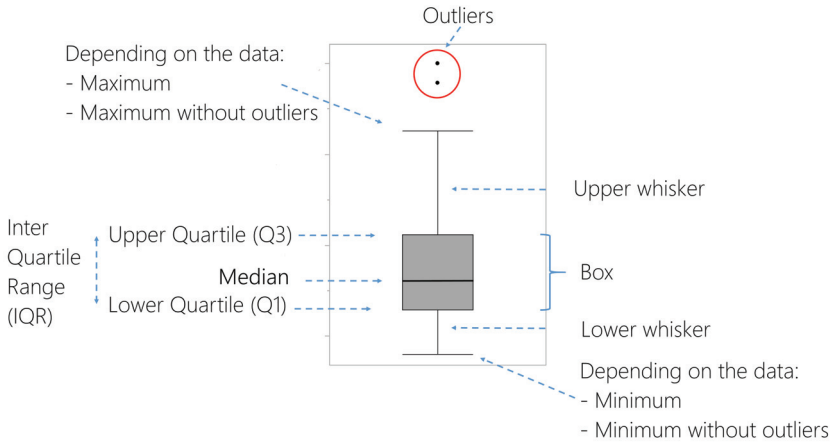

Figure 4. A general explanation of the box-whisker plot. The median represents the central tendency, while minima, maxima, outliers, whiskers and quartiles are different ways of expressing the dispersion of the data. The unequal distances between the median and Q1 and Q3, or the top and bottom whiskers, reflect whether the data are skewed or not. In this example, the data are right-skewed.

quartile (Q1) for the 25th percentile and the upper quartile (Q3) for the 75th percentile. The distance between Q1 and Q3 helps identify the data's spread. In the box plot, Q1 and Q3 are represented by the lower and upper boundaries of the box, respectively. This distance is called the interquartile range (IQR) and covers the middle half (50%) of all values in the data set. – Whiskers that extend from the box indicate the dataset's range. The lower whisker typically represents the minimum non-outlying value within 1.5 times the IQR below Q1, while the upper whisker represents the maximum non-outlying value within 1.5 times the IQR above Q3. Values outside the whiskers are considered outliers and are plotted individually. – Outliers, shown as individual data points or asterisks, are outside the whiskers (more on outliers in a separate section). They are considered to be potential anomalies in the data set.

Box plots are a flexible way of presenting data and may display the mean, SD, SEM or 95% CI. In this situation, the statistical analysis uses the Z-score to identify outliers or unusual values.

4. A *violin plot* combines features of a box plot and a kernel density plot to provide a comprehensive representation of the shape, central tendency, dispersion and multimodality of the data.

   The width of the violin at each point represents the density of the data at that value. In contrast, the body of the violin plot shows the density distribution, indicating the relative concentration of data at different values along the x-axis. Wider sections indicate higher density, while narrower sections indicate lower density (see **Figure 5**). Similarly to the box plot, the violin plot can include lines representing the data's median, Q1 and Q3 (IQR) and the outliers. Unlike box plots, violin plots show the shape and distribution of the data, indicating whether it is symmetrical, skewed, unimodal, bimodal or multimodal, with multiple peaks or modes representing different subgroups or patterns within the data.
5. A *scatter plot* displays individual data points as dots along a number line or axis. It shows the data's distribution and helps identify patterns or outliers.

   To create a scatter plot, each data point is plotted as a dot at its corresponding value on the number line. The dots are stacked vertically for multiple data points with the same value (non-unique or tied values). This stacking shows the frequency or density of data at each unique value (see **Figure 6**).

   Unlike other plots that aggregate data, scatter plots show each data point. This allows the entire raw data set to be observed and specific values or patterns of interest to be identified. This makes it easy to follow the spread and concentration of data, with gaps or clusters of dots indicating areas of high or low density, uneven data distribution. Scatter

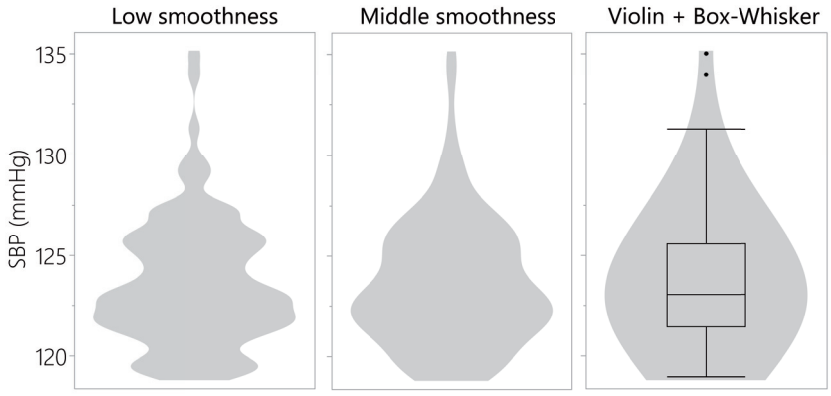

Figure 5. Identical systolic blood pressure (SBP) values are presented in three violin plots with different degrees of smoothness (low, medium and high). Low smoothness gives more information about the number of local peaks. With a more aggressive high level of smoothness, the violin is unimodal. Medians, Q1, Q3 or outliers can be added to all charts. Violin plots help to see if the data is skewed – the plots shown are right-skewed.

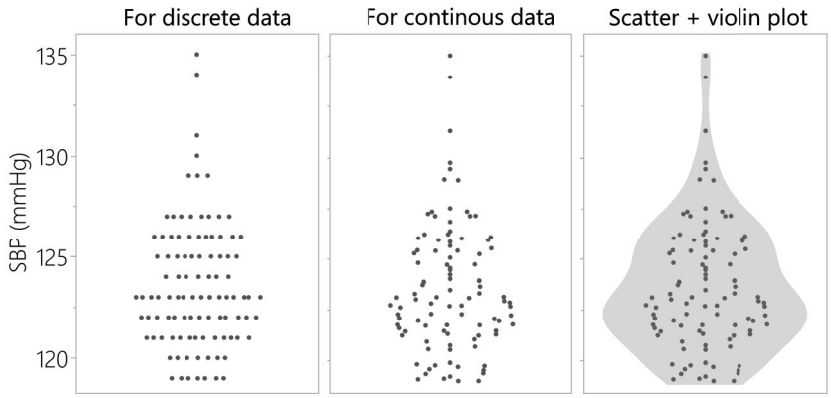

Figure 6. Systolic blood pressure (SBP) values presented as discrete data (left panel) and next (middle panel) as continuous data. Finally, a scatter plot is combined with a violin plot (right panel). In all cases, the data are randomly distributed around the centre of the scatterplot, but the shape of the scatterplot follows the data distribution. All forms of scatterplot can be supplemented with additional graphs, such as violin or box-whiskers plots, or measures of central tendency (Median, Mode, Mean) and dispersion (SD, Q1, Q3). Skewness can also be visualised using scatter plots.

plots can show measures of central tendency, such as the mean or median, SD or Q1 and Q3, and outliers.

6. A *line plot* presents quantitative data by connecting successive points that change over time. Many measurements are taken repeatedly to study their changes, e.g. blood glucose concentration before and after meals, blood pressure each morning and evening, and body weight during a weight loss programme. They show trends, patterns, and fluctuations over the observed period. The line plot is an example of a time series plot.

To construct a line plot, data points are plotted on the y-axis, representing the studied variable against time. Connected data with straight lines highlight the changes and trends over the observed period (see **Figure 7**).

By checking line plots, it is possible to reveal overall trends or patterns in the data, and the slope provides information about the direction and magnitude of the change, whether it is increasing, decreasing, or staying relatively constant over time. These plots help identify seasonal or periodic patterns, recurring fluctuations or cycles. As outliers deviate

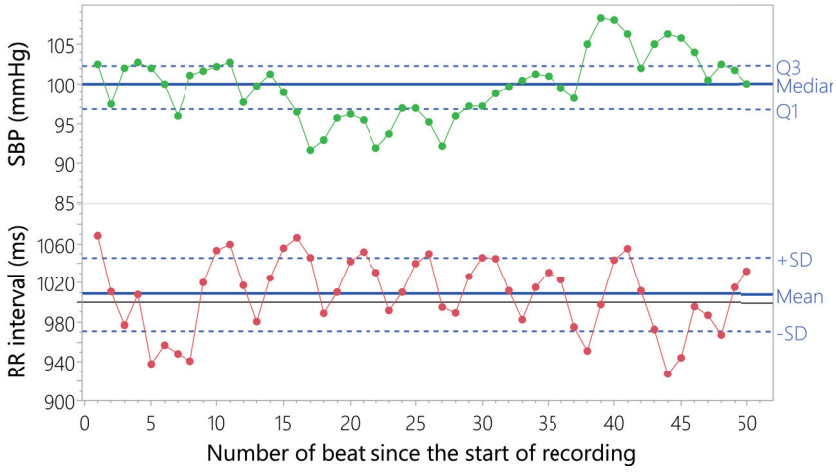

Figure 7. Two samples of line plots of synchronised beat-to-beat recordings of the duration of each cardiac cycle (RR intervals from ECG) and systolic blood pressure (SBP) from the finger arterial pressure waveform from a 25-year-old healthy woman in a supine position. For SBP (the upper panel), the median and Q1 and Q3 values are shown, while the mean and +/- SD values are displayed for the RR intervals.

significantly from the general trend, it is easy to spot them. Line plots can help make predictions or forecasts based on historical data. The line plots can be accompanied by shaded areas or error bars around the lines representing, for instance, the 95% confidence intervals and showing the dispersion of values around the trend lines.

7. A *Q-Q (quantile-quantile) plot* is a graphical tool that examines whether a data set follows a particular theoretical distribution, such as a normal distribution. It compares the quantiles of the observed data with the quantiles of the expected theoretical distribution.

   To construct a Q-Q plot, the values of the observed data set are first sorted in ascending order. Next, the corresponding quantiles of the expected distribution are calculated. These quantiles represent the values that would be expected if the observed data followed the specified distribution perfectly. The Q-Q plot then displays the observed quantiles on the x-axis and the expected quantiles on the y-axis. Each data point represents a pair of observed and expected quantiles (see **Figure 8**).

   If the observed data closely follow the expected distribution, the points on the plot will fall approximately on a straight identity line that follows the function x = y. This identity line assumes that the estimated points (on the y-axis) are the same as the observed points (on the x-axis). Departures from a straight line indicate deviations from the expected distribution.

   These plots help to assess the normality assumption of a data set. It suggests that the data follows a normal distribution if the data points on the plot closely follow the identity line. However, if the points diverge from the line, this indicates deviations from normality, such as skewness or heavy tails. Q-Q plots can also be used to compare two sets of data. Plotting the quantiles of one data set against the quantiles of another makes it easy to see if the two data sets have similar distributions.

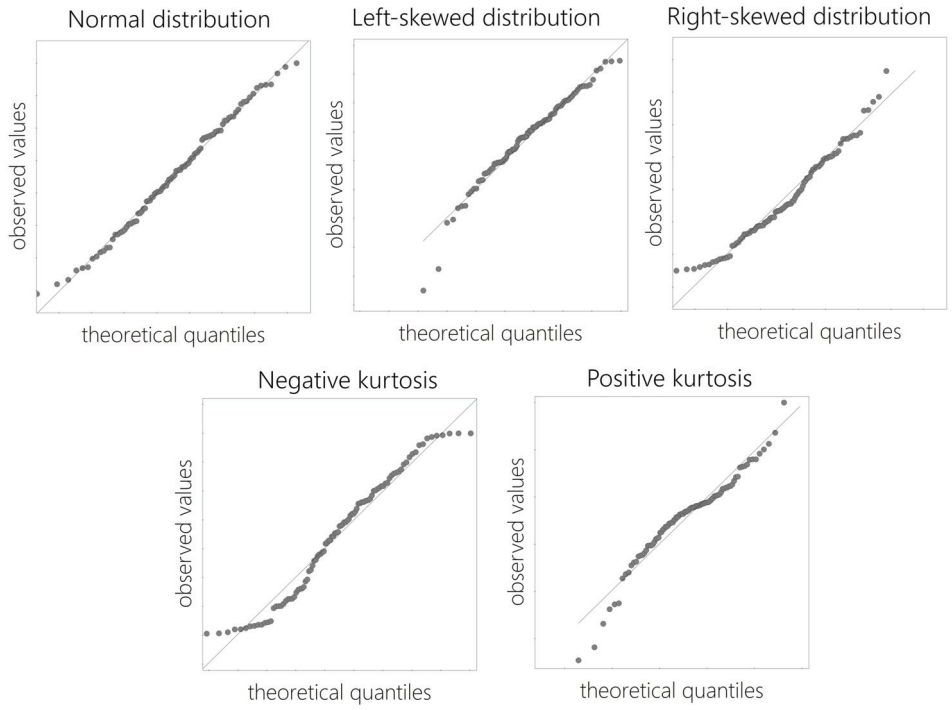

Figure 8. An example of a quantile-quantile (Q-Q) plot comparing quantiles representing the observations and their distribution with quantiles corresponding to the theoretical normal distribution. The points form a line along the identity line (y = x) if both sets of quantiles come from the same distribution. Gaussian and other distributions such as uniform, exponential or Pareto can be compared using these plots. Q-Q plots are more diagnostic than comparing sample histograms, density plots, scatter plots, box-whisker plots or violin plots. With Q-Q plots, skewness and kurtosis are immediately visible. They are easy to examine. The multimodality of distributions can also be found. See an example in Figure 9.

Q-Q plots do not show measures of central tendency, but it is easier to see how the data deviate from normal distributions, whether skewed or have kurtosis.

Standard numerical tests for normality testing

Many tests are used in medical research to analyse data distribution. The most common are:

1. *Kolmogorov-Smirnov test* [1]. This test determines whether a sample comes from a normal distribution. It compares the empirical data distribution with the cumulative distribution function of a theoretical normal distribution. Advantages: It is sensitive to differences in both location and shape between the sample and the normal distribution. Disadvantages: It is less powerful than other tests when the sample size is small.
2. *Shapiro-Wilk test* [2]. This test determines whether sample data come from a normal distribution based on the correlation between the observed data and the expected normal values. The test was originally proposed by Shapiro for small sample sizes. It is now used for data sets ranging from 3 to 5,000 samples [3,4]. Advantages: It is more potent than other tests when the sample size is small. Disadvantages: It is less powerful than other tests when the sample size is large.
3. *Shapiro-Francia test* [5]. This is similar but simpler than the Shapiro-Wilk test but has better power for small samples. It measures the deviation of the sample data from normality by comparing the sample distribution to a normal distribution with the same mean and variance. Advantages: It is more potent than other normality tests when the sample size is small and less sensitive to outliers than other normality tests. Apart from being less popular (not well known), there are no methodological disadvantages when used with small data sets.
4. *D'Agostino-Pearson (D'Agostino's K-squared) test* [6,7]. This test determines whether a sample comes from a normal distribution. It is based on the skewness and kurtosis of the sample as measures of deviation from normality. The D'Agostino-Pearson test provides a formal statistical test to support or challenge the visual assessment made, for example, by Q-Q plots. Both methods provide a more complete analysis of normality. Advantages. It is a powerful test of normality. Disadvantages: It may not be sensitive to forms of non-normality other than skewness and kurtosis, such as multi-modality or heavy tails.
5. *Anderson-Darling test* [8]. This parametric test uses the sample data to estimate the normal distribution parameters. The test statistic is based on the difference between the observed and expected cumulative distribution functions. Advantages: It is more powerful than other tests when the sample size is large. Disadvantages: It is less powerful than other tests when the sample size is small.
6. *Cramer-von Mises test* [9]. Similar to the Anderson-Darling test, but gives more weight to differences in the tails of the distributions. Advantages: It is a powerful test of normality for larger sample sizes. Disadvantages: It is sensitive to sample size.
7. *Jarque-Bera test* [10]. This test determines whether a sample comes from a normal distribution. It uses skewness and kurtosis as measures of deviation from normality. Advantages: It complements graphical methods such as Q-Q plots. Disadvantages: Its ability to identify certain types of non-normal distribution is limited as it primarily looks for deviations from the normal pattern based on skewness and kurtosis.
8. *Lilliefors test (Kolmogorov-Smirnov-Lilliefors test)* [11,12]. It is an extension of the Kolmogorov-Smirnov test but adjusted when the mean and variance of the data are also estimated. Advantages: It has better power than the original Kolmogorov-Smirnov test to detect deviations from normality. This is especially true for moderate sample sizes. Disadvantages: It can be overly conservative and not appropriate for small samples.
9. *Lobato-Velasco test* [13]. This test measures skewness and kurtosis and their correlation coefficients for observations. While assessing the normality of the data distribution, the Lobato-Velasco test provides consistent results for data that are correlated over time. Advantages: This test considers the specificity of dependent data and provides consistent results for data that are correlated over time.

Disadvantages: It is sensitive to deviations from the assumption of stationarity.

For smaller sample sizes < 50, it is advisable to employ the Shapiro-Wilk test or Shapiro-Francia test for their higher power in detecting deviations from normality in such cases [9,14]. If skewness or kurtosis are more important, the D'Agostino-Pearson or Jarque-Bera test and other tests focusing more on skewness and kurtosis work better. For sample sized > 50, other methods, particularly graphical like Q-Q plots, histograms, density plots, box-and-whiskers and other tests of normality can be used.

For very large samples, most normality tests are too sensitive and will detect even small deviations from normality [15]. It is advisable to use graphical tools to avoid prematurely labelling data as non-normal for small deviations that are unlikely to affect the interpretation of the data. These methods visualise the true distribution. They help to identify multimodality, asymmetry or excessive variance.

Multimodality is one of several reasons for the lack of normality in the distribution. In a multimodal distribution, clusters or subgroups of values are separated from each other. Statistical analysis and inference should take account of such clustered values and, where appropriate, apply specific tests for cluster analysis or multimodal modelling. These may facilitate under-

Table 2. Data distribution evaluation based on measures/coefficients, statistical tests and graphs.

Analysis type	Result of analysis	Result interpretation
Normality distribution tests	P-value < 0.05	Not a normal distribution
Skewness assessment		
Skewness coefficient	Positive (especially greater than 2*)	Right-skewed distribution
	Negative (especially less than -2*)	Left-skewed distribution
Tests for assessing skewness	P-value < 0.05	Skewed distribution
Histogram and density plots	Long right tail	Right-skewed distribution
	Long left tail	Left-skewed distribution
Box-whiskers, violin and dot plots	Extended top of the chart (an upper whisker)	Right-skewed distribution
	Extended lower part of the graph (a lower whisker)	Left-skewed distribution
Q-Q plot	Right and left tails significantly departing above the identity line	Right-skewed distribution
	Right and left tails significantly departing above the identity line	Left-skewed distribution
Mean versus Median	Mean distinctively above median	Right-skewed distribution
	Mean distinctively below median	Left-skewed distribution
Kurtosis assessment		
Kurtosis coefficient	Positive (especially greater than 4*)	Sample distribution is narrower than a normal distribution
	Negative (specifically less than -4*)	Sample distribution is flatter and wider than a normal distribution
Tests for assessing kurtosis	P-value < 0.05	Kurtosis atypical for normal distribution
Q-Q plot	Left tail above and right tail below the identity line	The distribution is more flattened than a normal distribution
	Left tail well below and right tail well above the fit line	Distribution is narrower than a normal distribution
Histograms and density plots	"Heavy" tails	The distribution is more flattened than a normal distribution
Multimodality assessment		
Histogram and density plot	Distinct clusters of bars (density) of similar height representing separate groups	Multimodality occurs
Q-Q plot	Multiple groups of points deviating in different directions from the fit line	
Violin and Point plot	Occurring in alternating wide and narrow shapes, separated clusters of multiple points represent different value groups with different centers	

* Limits proposed for the district of significant deviation from normality [14].

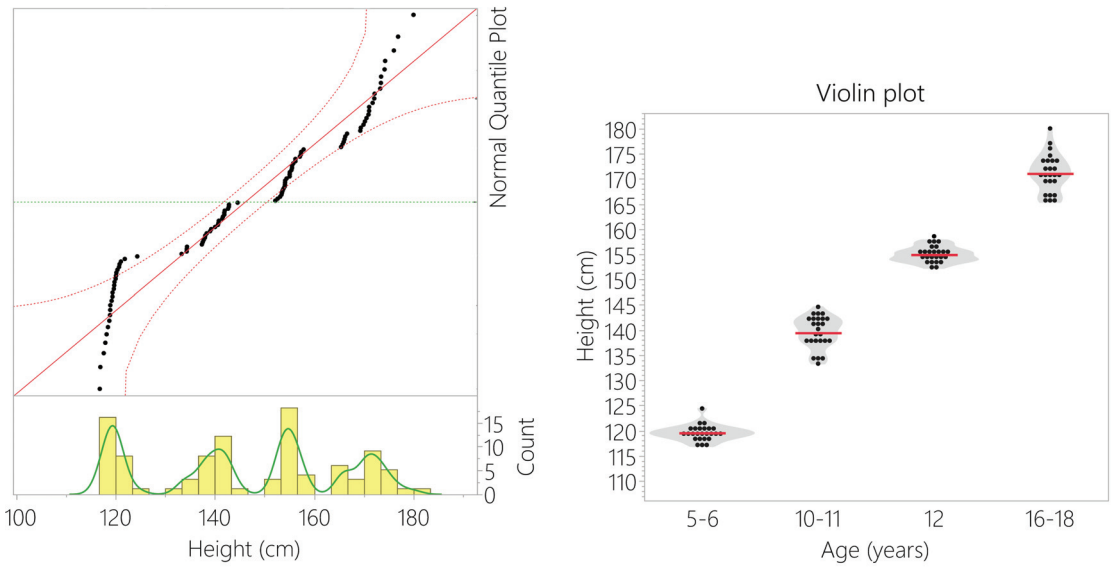

Figure 9. An example of Q-Q plots, histograms and density plots (left panel) with the results of height measurements collected in a group of healthy children aged between 5 and 18 years. Four distinct peaks of lumped height values (local maxima) appear. An additional analysis (right panel) examining the height distribution against age explains that the four local maxima correspond to four different age groups of the children studied. Categorical, continuous and discrete data can all form multimodal distributions and can be analysed in this way.

standing of differences between two or more subgroups.

When the statistical mean is the primary measure describing the data, it is important to ensure that the data are normally distributed and not skewed. This is because the central limit theorem states that the sample mean of large samples approaches the true population mean. In other words, the means of such samples satisfy the normality of the distribution. However, this theorem does not define whether the data have a normal distribution. The theorem does not exempt the researcher from investigating how the data are distributed.

Figure 10 shows over 20000 measurements of 1-minute total heart rate variability (SDNN) for sinus rhythm and for atrial fibrillation. In both cases, statistical tests detected significant (p-value < 0.0001) deviations from the normality of the distribution. However, only one graph shows data that are significantly out of normal distribution. For sinus rhythm, this is due to the high skewness of the data (mean 35.5 ms and median 53.8 ms). For atrial fibrillation, the distribution is less skewed (mean 137.5 ms and median 136.1 ms). Only on the left side is the proportion of observed values above the expected value slightly higher. The atrial fibrillation data can therefore be assumed to have a normal distribution.

With large data sets, the normality tests have too much power and may detect even minimal deviations from normality as significant. In such cases, graphical analysis is always essential and may be decisive.

However, only one graph shows data that are significantly out of normal distribution. For sinus rhythm, this is due to the high skewness of the data (mean 35.5 ms and median 53.8 ms). For atrial fibrillation, the distribution is less skewed (mean 137.5 ms and median 136.1 ms). Only on the left side is the proportion of observed values above the expected value slightly higher. The atrial fibrillation data can therefore be assumed to have a normal distribution.

Discussion

Assessing the normality of a distribution is the first step in many statistical analyses. It should always start with a visual assessment, for example, using histograms or density plots. Unfortunately, due to the required time and uncertainty of interpreting such plots, statistical tests become the only tool for testing the normality of data distributions. Normality tests are central to statistical analysis. However, they should complement, not replace, graphical assessment of normality.

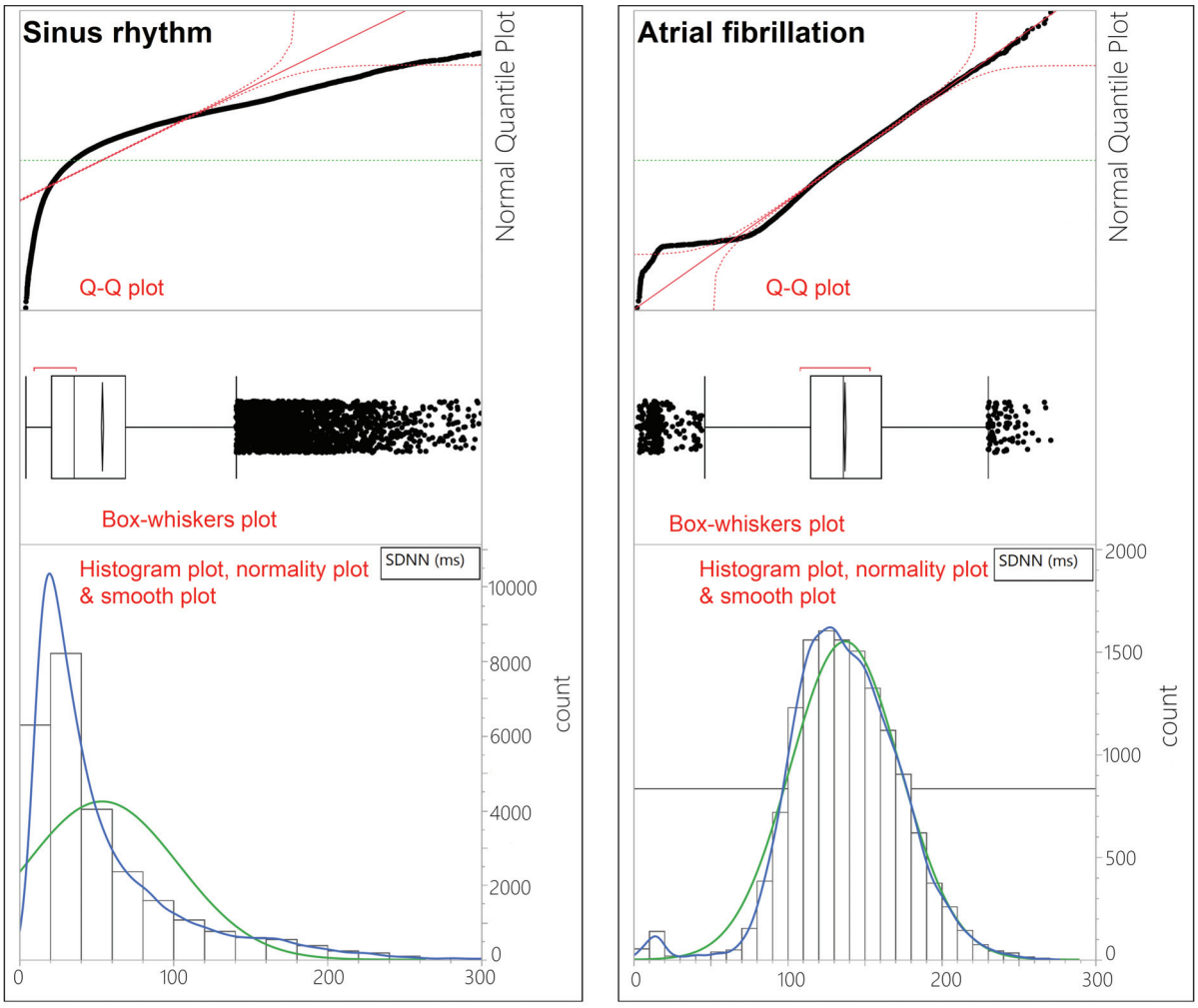

Figure 10. Q-Q plots, box and whisker plots, histograms and normal density plots showing analysis of SDNN calculated for 1 minute beat-to-beat values of RR interval duration. Left panel shows plots for normal sinus rhythm, right for AF. Each panel summarises the finding for more than 20,000 separate 1-minute files of RR intervals. For sinus rhythm, the data distribution is not normal, which can be seen in the Q-Q plot, box-whisker plot – greater distance between the median and Q3 and the right whisker, and a clear clustering of outliers outside this whisker. The histogram is also highly skewed. The distribution analysis of SDNN for AF appears to be Gaussian.

Is normality testing necessary?

Normality tests aim to determine whether a data set is well-modelled by a normal distribution. A single normality test is usually sufficient. If the results are uncertain or borderline, other tests can be used to confirm or reject the normality of the distribution of the data being analysed. In such tests, the null hypothesis is that the distribution is normal, confirmed if the p-value exceeds 0.05. If $p < 0.05$, normality is rejected.

A normal distribution is symmetric, so data conforming to this distribution can be summarised with mean and SD and later analysed with parametric tests. True normality is considered a myth because real data, including medical data, usually deviate from the ideal normal distribution to some extent. For skewed non-normal data, mean and SD may be misleading and confusing because of potential over- or underestimation. The median, Q1 and Q3 are required for data with a non-Gaussian distribution. It is also convenient for readers to see both the mean and the median to decide whether the distribution is normal.

To date, statisticians have not reached a consensus on a single best test for assessing the normality of distribution for all possible data and situations. Normality tests with small group sizes often confirm a normal distribution, while tests with large groups tend to reject this assumption. Circumstances in which all tests agree in judging the normal distribution are straightforward. The

problem arises when different tests give different assessments of the distribution. What do you do when the statistical tests disagree with your assessment? This is another reason to return to graphical methods for assessing normal distribution. It is worth looking at the presence of outliers and whether errors are hidden among them, using additional graphical techniques such as Q-Q plots.

Assessing the distribution's normality should help select the best methods for further analysis. Choosing the right normality test can significantly impact the reliability and validity of the statistical analysis. Normality tests help determine whether parametric tests are appropriate for further statistical analysis. Parametric tests, such as t-test and ANOVA for comparisons or Pearson's correlation test and regression models based on least squares estimation, rely heavily on the normality assumption. Sample size estimation for design studies would not be possible without proper test selection.

Determining whether the data show a serious departure from normality is crucial. If there is any doubt about the normality of the data distribution, it is better to use non-parametric tests in further analyses. If the data are normally distributed in one subgroup but not in another, it is recommended that non-parametric tests be used for the subgroup that does not have normally distributed data.

Nonparametric tests do not assume that the data are normally distributed. Non-parametric methods should be used in further analyses for data that are not normally distributed. The simplest examples are the Mann-Whitney or Kruskal-Wallis tests for comparisons or the Spearman correlation test. They are more resistant to violations of this assumption. There are also robust statistical methods used in medical research to analyse data that may have outliers or other anomalies and to deal with such problems [16]. However, some statistical power is lost by using non-parametric tests rather than parametric tests. Alternatively, the data can be normalised by transforming them with some mathematical functions (e.g. logarithm, square root). Another solution is to treat the results as exploratory rather than conclusive.

Consistency in the presentation and interpretation of data is important, and the choice of a particular approach should stand if the validity of the statistical method used has been established. Unwarranted changes from parametric to non-parametric tests or vice versa during the process may raise concerns about the reliability of the statistical analysis and affect the final result.

Summary

Exploring clinical data is an integral part of medical research. One of the first steps is to distinguish whether the data is continuous, quasi-continuous or discrete. Since outliers of different origins can affect the final results, it is important to notice them and decide what to do about them. When analysing the data distribution, graphical and numerical methods should be used after adequately identifying whether the data have a normal distribution; non-parametric or parametric tests should be used in further analysis.

Reliable and correct statistical analysis is crucial in medical research for many reasons, including accurate data interpretation, findings validation, evidence-based decision-making, and generalisability of results. It underpins the credibility and impact of medical research, leading to advances in healthcare and improved patient outcomes.

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Conflict of interest statement
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References

1. Sulla ANK. Determinazione empirica di una legge didistribuzione. Giorn Dell'inst Ital Degli Att. 1933;4:89-91. doi: 10.12691/ajams-1-1-2.
2. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika. 1965;52:591-611. doi: 10.2307/2333709.

3. Royston P. Approximating the Shapiro-Wilk W-test for non-normality. Statistics and computing. 1992;2:117-119. doi: 10.1007/BF01891203.
4. Royston P. A Toolkit for Testing for Non-Normality in Complete and Censored Samples. The Statistician. 1993;42:37. doi: 10.2307/2348109.
5. Shapiro SS, Francia RS. An approximate analysis of variance test for normality. Journal of the American statistical Association. 1972;67:215-216. doi: 10.1080/01621459.1972.10481232.
6. D'Agostino R, Pearson ES. Tests for departure from normality. Empirical results for the distributions of b 2 and√ b. Biometrika. 1973;60:613-622. doi: 10.2307/2335012.
7. D'Agostino RB, Belanger A, D'Agostino Jr RB. A suggestion for using powerful and informative tests of normality. The American Statistician. 1990;44:316-321. doi: 10.2307/2684359.
8. Anderson TW, Darling DA. A test of goodness of fit. Journal of the American statistical association. 1954;49:765-769. doi: 10.2307/2281537.
9. Thode HC. Testing For Normality. New York: CRC Press; 2002.
10. Jarque CM, Bera AK. Efficient tests for normality, homoscedasticity and serial independence of regression residuals. Economics letters. 1980;6:255-259. doi: 10.1016/0165-1765(80)90024-5.
11. Lilliefors HW. On the Kolmogorov-Smirnov Test for Normality with Mean and Variance Unknown. Journal of the American Statistical Association. 1967;62:399-402. doi: 10.1080/01621459.1967.10482916.
12. Lilliefors HW. On the Kolmogorov-Smirnov Test for the Exponential Distribution with Mean Unknown. Journal of the American Statistical Association. 1969;64:387-389. doi: 10.1080/01621459.1969.10500983.
13. Lobato IN, Velasco C. A simple test of normality for time series. Econ Theory [Internet]. 2004 [cited 2023 Jun 8];20. doi: 10.1017/S0266466604204030.
14. Mishra P, Pandey CM, Singh U, Gupta A, Sahu C, Keshri A. Descriptive Statistics and Normality Tests for Statistical Data. Ann Card Anaesth. 2019;22:67-72. doi: 10.4103/aca.ACA_157_18. Cited: in:: PMID: 30648682.
15. Demi R S. Comparison of Normality Tests in Terms of Sample Sizes under Different Skewness and Kurtosis Coefficients. International Journal of Assessment Tools in Education. 2022;9:397-409. doi: 10.21449/ijate.1101295.
16. Farcomeni A, Ventura L. An overview of robust methods in medical research. Stat Methods Med Res. 2012;21:111-133. doi: 10.1177/0962280210385865.

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Books

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1. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. Edinburgh: Churchill Livingstone; 2003.

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2. Beers MH, Porter RS, Jones TV, Kaplan JL, Berkwits M (editors). The Merck manual of diagnosis and therapy. 18th ed. Whitehouse Station (NJ): Merck Research Laboratories; 2006.

Chapter in the book

1. Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465–478.

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