Dedicated

To

The Researchers, Clinicians & Health Care Workers of Pakistan

Bismillah Hir Rahman Nir Raheem.
In the name of Allah, the most Beneficent and the most Merciful

Message by the
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&
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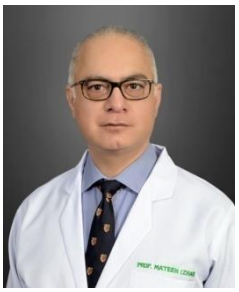

Prof. Mateen Izhar
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(Chief Editor)

Our Jan to March 2022 Edition is being published as the 5th COVID-19 wave with the Omicron variant crests worldwide. Rising infectivity, yet lesser mortality is its hallmark. We pray humanity is relieved of this deadly disease.

We received HEC Y Category for the 2nd year running Alhumdolillah and saw the highest number of article submissions since its inception. A hundred and twelve articles on wide ranging topics were submitted and vetted as Per HEC Guidelines. We have applied for ADL and DOAJ Indexation

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Medical Students' Perception on Learning Anatomy Online During COVID-19 Pandemic in an Integrated Modular System: Comparison of Online and Face to Face SGDs & Interactive Lectures

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ABSTRACT

Introduction: The COVID-19 pandemic influenced education system worldwide. This change was unusual for both students and anatomists. The present study is conducted to perceive online learning experience of 1st& 2nd year MBBS students in a modular system comparing online SGD & lectures with face to face.
Objectives: To assess how medical students of 1st and 2nd year MBBS perceive the experience of learning Anatomy online in an integrated modular system, their attitude towards online small group discussions (SGD) and online Interactive lectures by comparing with face-to-face learning anatomy.
Place and duration of study: The study was conducted in the Department of Anatomy at University College of Medicine, University of Lahore during academic year of 2021 over a period of six months from March 2021.
Material & Methods: This descriptive cross-sectional study was conducted by using a Questionnaire validated by five medical educationists. The sampling was done by nonprobability convenience technique. For a confidence level of 97%, sample size was 197. A total of 202 students from 1st & 2nd year MBBS, were asked to fill in a Questionnaire with Likert scale of 5, anonymously after taking informed consent, regarding online learning Anatomy through Zoom by comparing online SGD and Interactive lectures with face-to-face learning during COVID-19 pandemic. Data was analyzed using SPSS (version 28) software, p value ≤0.05 was considered significant.
Results: A total of 202 responses were received of which 197 responses were complete. Of which 56 were from 1styear while 141 were from 2nd Year MBBS. Some students did not answer some questions. Percentages were calculated against student responses, for missing data the percentages were calculated from total number of responses to each question answered. *p* values calculated were not significant but overall students did not prefer online learning over face to face learning.
Conclusion: Our present study concludes that learning anatomy online is challenging. Overall students did not value online learning more than face-to-face learning in terms of flexibility, freedom, interaction with teachers, lack of hands-on practice and concepts, although they enjoyed the leisure of self-study with whole study material available to them at any time.

Key words: Pandemic, validated, Integrated, Modular, Questionnaire, SGD, Interactive lectures.

INTRODUCTION

It is significant to never forget the vision and reality of the fact that history repeats itself. The COVID-19 pandemic is an unmatched catastrophe that has influenced universal business including education.[1] The hard going of COVID-19 pandemic constrained by social distancing measures disrupted face-to-face learning, limiting educational institutions to convert their mode of education from face to face to online, instantly around the world. Anatomical education is a cornerstone to the majority of health-related fields which is taught traditionally through interactive though mostly teacher-centered lectures, human cadaveric dissection, osteology illustration, Bones and soft tissue Radiology, microscopic slides of Histology, specimens, models of Embryology and Gross anatomy teaching.[2,3,4] The Anatomists around the globe opted for distant online learning without delays to foster Anatomical education.[5] This transformation was unusual for both anatomists as well as students. Anatomists around the world showed concerns about teaching anatomy online and students felt they might have missed the core of the subject without having three-dimensional approaches to Anatomy.[6] To continue medical education uninterrupted in Pakistan, online classes

were implemented as advised by medical universities and Higher Education commission of Pakistan. Different institutes used different teaching platforms like and not limited to Zoom, Google Meet, Microsoft teams, Google classroom, etc., to continue medical education online endlessly.

In the course of medical education in Pakistan, many medical institutes are trying to adopt an integrated modular system[7] in contrast to the traditional system which is the compartmentalization of basic and applied medicine[8] and the traditional system prevailing across most of the medical institutes in the homeland. An Integrated modular system indicates synthesis of knowledge from various disciplines to make a whole, which is purposeful.[9,10] Due to this interconnection and correlation, a medical student can interpret the overall patient scenario in a real life situation.[11]

Hence, the present study was conducted to assess how medical students of MBBS 1st and 2nd year, perceive the experience of learning Anatomy online in an integrated modular system? Furthermore, to explore their attitude towards online small group discussions and online Interactive lectures by comparing with face to face learning Anatomy. The research was aimed to achieve students' responses and feedback to propose improved and more appropriate approaches to direct online teaching policies and methods to help remote Anatomy education.

MATERIAL AND METHODS

The descriptive cross sectional study was conducted in the department of Anatomy at University College of Medicine, University of Lahore during academic year of 2021 over a period of six months from March 2021.Ethical Committee of the University of Lahore, granted clearance with approval no: ERC/12/20/24, Date: 7/12/20. Population was the participants from the 1st year and the 2nd year MBBS students (150+150). For confidence level of 97%, sample size calculated was 184, our response rate was 197.Nonprobability convenience sampling technique was used. The online Anatomy SGDs and Lectures were presented live using Zoom software by screen sharing and breakout rooms. All sessions were monitored and recorded. All study material was shared soon after the classes were over as Power Point slides and recorded sessions on Slate, the official e-learning platform of The University of Lahore; therefore, students had full access to study material any time after the classes for self-study. The lack of anatomical demonstrations was replaced with images, flow sheets, mind maps and diagrams. Along with few anatomical softwares like 3D skull etc., A total of 202 students were asked to fill in a Questionnaire with a Likert scale of 5, anonymously regarding online learning Anatomy during COVID-19 pandemic. Informed consent was witnessed by a second person. Data was collected on campus using the Questionnaire. The validation of the questionnaire was done by five medical educationists. Students' responses were registered in a database using Microsoft Excel.

Statistical analysis:

Descriptive cross sectional analysis was done using IBM SPSS (version 28) software. The Likert score of 5 was used to compare students' responses. The percentages were calculated and compared for face to face and online learning Anatomy from the student's responses. p value ≤ 0.05 was considered significant.

RESULTS

A total of 202 responses were received of which 197 responses were complete while the remaining 5 responses were incomplete. Of which 56 were from the first-year while 141 were from the second-year MBBS. From 1st year 31 respondents were male while 25 were female. From 2nd year 74 respondents were male, while 67 were female. Of 197 responses some students did not answer some of the questions therefore for missing data the percentages for those questions were calculated by counting total number of responses to those question. *P* value was calculated by Chi Square, which were not significant (more than 0.05), in terms of percentages obtained from students of 1st year and 2nd year responses regarding perception on learning anatomy online versus face to face. The percentages for each question answered by students are given in Table-1.

	Item #	Questionnaire Items	Strongly Agree	Partly Agree	Neutral	Partly Disagree	Strongly Disagree
		Online Learning Anatomy					
1st year	1	**The transition to online learning Anatomy was smooth**	8.9%(5)	35.7%(20)	12.5% (7)	19.6% (11)	23.2% (13)
2nd year			12.1%(17)	29.3%(41)	20% (28)	23.6%(33)	15% (21)
Total			11.2%(22)	31.1%(61)	17.9% (35)	22.4% (44)	17.3% (34)
1st year	2	**Online learning Anatomy was more flexible than face to face learning in terms of space and time management**	10.9%(6)	3.6%(2)	23.6%(13)	23.6% (13)	38.2% (21)
2nd year			12.8%(18)	9.2%(13)	19.1%(27)	33.3% (47)	25.5% (36)
Total			12.2%(24)	7.7%(15)	20.4%(40)	30.6% (60)	29.1% (57)
1st year	3	**It is easier to communicate with teacher in online environment**	23.2% (13)	14.3%(8)	17.9%(10)	17.9% (10)	26.8% (15)

2nd year			15.6%(22)	15.6%(22)	19.1%(27)	24.8% (35)	24.8% (35)
Total			17.8%(35)	15.2%(30)	18.8%(37)	22.8% (45)	25.4% (50)
1st year	4	**It is challenging to learn Anatomy online**	53.6%(30)	19.6%(11)	7.1% (4)	7.1% (4)	12.5% (7)
2nd year			45.3%(63)	19.4%(27)	15.1%(21)	12.2% (17)	7.9% (11)
Total			47.7%(93)	19.5%(38)	12.8%(25)	10.8% (21)	9.2% (18)
1st year	5	**The online course has helped me to improve my IT skills.**	18.5%(10)	25.9%(14)	22.2%(12)	16.7% (9)	16.7% (9)
2nd year			18.6%(26)	30%(42)	29.3%(41)	10.7% (15)	11.4% (16)
Total			18.6%(36)	28.9%(56)	27.3%(53)	12.4% (24)	12.9% (25)
1st year	6	**I value the online learning environment more than a face-to-face format**	8.9%(5)	8.9%(5)	17.9%(10)	17.9% (10)	46.4% (26)
2nd year			10.1%(14)	10.8%(15)	21.6%(30)	21.6% (30)	36.0% (50)
Total			9.7%(19)	10.3%(20)	20.5%(40)	20.5% (40)	39.0% (76)
1st year	7	**Online learning Anatomy helped me in creating better understanding of topic**	10.7%(6)	7.1%(4)	19.6%(11)	21.4% (12)	41.1% (23)
2nd year			10.7%(15)	11.4%(16)	20%(28)	27.1% (38)	30.7% (43)
Total			10.7%(21)	10.2%(20)	19.9%(39)	25.5% (50)	33.7% (66)
1st year	8	**Online learning Anatomy helped me in being more interactive with my teachers**	16.1%(9)	23.2%(13)	12.5%(7)	16.1% (9)	32.1% (18)
2nd year			10.1%(14)	13%(18)	23.9%(33)	23.9% (33)	29.0% (40)
Total			11.9%(23)	16%(31)	20.6%(40)	21.6% (42)	29.9% (58)
1st year	9	**Online learning Anatomy makes topic more interesting**	8.9%(5)	12.5%(7)	16.1%(9)	17.9% (10)	44.6% (25)
2nd year			12.8%(18)	10.6%(15)	20.6%(29)	24.8% (35)	31.2% (44)
Total			11.7%(23)	11.2%(22)	19.3%(38)	22.8% (45)	35% (69)
1st year	10	**Online teaching has changed my attitude toward learning Anatomy**	19.6%(11)	23.2%(13)	19.6%(11)	16.1% (9)	21.4% (12)
2nd year			14.9%(21)	19.9%(28)	24.1%(34)	19.9% (28)	21.3% (30)
Total			16.2%(32)	20.8%(41)	22.8%(45)	18.8% (37)	21.3% (42)
1st year	11	**The misconception and confusion regarding topic were easy to resolve in online learning Anatomy**	11.1%(6)	16.7%(9)	24.1%(13)	22.2% (12)	25.9% (14)
2nd year			10.8%(15)	17.3%(24)	35.3%(49)	19.4% (27)	17.3% (24)
Total			10.9%(21)	17.1%(33)	32.1%(62)	20.2% (39)	19.7% (38)
1st year	12	**I would benefit if there were more Online Anatomy courses**	14.8%(8)	14.8%(8)	16.7%(9)	18.5% (10)	35.2% (19)
2nd year			11.7%(16)	10.2%(14)	22.6%(31)	24.8% (34)	30.7% (42)
Total			12.6%(24)	11.5%(22)	20.9%(40)	23% (44)	31.9% (61)
1st year	13	**I prefer online learning Anatomy than face to face learning**	11.1%(6)	7.4%(4)	14.8%(8)	20.4% (11)	46.3% (25)
2nd year			12.9%(18)	7.2%(10)	12.2%(17)	25.2% (35)	42.4% (59)
Total			12.4%(24)	7.3%(14)	13%(25)	23.8% (46)	43.5% (84)
1st year	14	**Online teaching Anatomy enables me to attend classes more frequently than face to face mode**	27.8%(15)	11.1%(6)	18.5%(10)	9.3% (5)	33.3% (18)
2nd year			27.9%(38)	19.1%(26)	16.2%(22)	16.2% (22)	20.6% (28)
Total			27.9%(53)	16.8%(32)	16.8%(32)	14.2% (27)	24.2% (46)
1st year	15	**Online learning Anatomy helps me in utilizing my time more efficiently**	22.2%(12)	14.8%(8)	18.5%(10)	13% (7)	31.5% (17)
2nd year			21.9%(30)	17.5%(24)	21.9%(30)	19.7% (27)	19.0% (26)
Total			22%(42)	16.8%(32)	20.9%(40)	17.8% (34)	22.5% (43)
1st year	16	**It is easier to revise online shared Anatomy material than taking notes in face-to-face teaching environment**	30.2%(16)	22.6%(12)	15.1%(8)	17% (9)	15.1% (8)
2nd year			21%(29)	19.6%(27)	22.5%(31)	15.2% (21)	21.7% (30)
Total			23.6%(45)	20.4%(39)	20.4%(39)	15.7% (30)	19.9% (38)
1st year	17	**More teaching software are required in online Anatomy course**	44.4%(24)	14.8%(8)	27.8%(15)	7.4% (4)	5.6% (3)
2nd year			34.8%(48)	27.5%(38)	19.6%(27)	7.2% (10)	10.9% (15)
Total			37.5%(72)	24%(46)	21.9%(42)	7.3% (14)	9.4% (18)
		Small Group Discussion (SGD)					
1st year	18	**I would benefit if there were more online SGDs on Anatomy**	27.8%(15)	7.4%(4)	24.1%(13)	9.3% (5)	31.5% (17)
2nd year			14.5%(20)	17.4%(24)	25.4%(35)	15.2% (21)	27.5% (38)
Total			18.2%(35)	14.6%(28)	25%(48)	13.5% (26)	28.6% (55)
1st year	19	**I am satisfied with the duration of online Anatomy SGD**	25.9%(14)	13%(7)	27.8%(15)	9.3% (5)	24.1% (13)
2nd year			17.3%(24)	23%(32)	25.2%(35)	13.7% (19)	20.9% (29)
Total			19.7%(38)	20.2%(39)	25.9%(50)	12.4% (24)	21.8% (42)
1st year	20	**I prefer Learning Anatomy through online SGDs**	15.4%(8)	9.6%(5)	21.2%(11)	21.2% (11)	32.7% (17)
2nd year			12.4%(17)	12.4%(17)	19.7%(27)	25.5% (35)	29.9% (41)
Total			13.2%(25)	11.6%(22)	20.1%(38)	24.3% (46)	30.7% (58)
1st year	21	**I would benefit more if online SGD were merge with live demonstration of bones, models, prosected models, and specimens.**	55.6%(30)	18.5%(10)	13%(7)	3.7% (2)	9.3% (5)
2nd year			36%(50)	22.3%(31)	17.3%(24)	10.8% (15)	13.7% (19)
Total			41.5%(80)	21.2%(41)	16.1%(31)	8.8% (17)	12.4% (24)
		Interactive Lecture					
1st year	22	**I would benefit if there were more online Interactive lectures on Anatomy**	25.9%(14)	20.4%(11)	14.8%(8)	11.1% (6)	27.8% (15)
2nd year			17.3%(24)	16.5%(23)	25.9%(36)	18% (25)	22.3% (31)
Total			19.7%(38)	17.6%(34)	22.8%(44)	16.1% (31)	23.8% (46)
1st year	23	**I am satisfied with the duration of online interactive lectures of Anatomy**	24.1%(13)	14.8%(8)	22.2%(12)	14.8% (8)	24.1% (13)
2nd year			20.1%(28)	20.9%(29)	30.9%(43)	7.9% (11)	20.1% (28)
Total			21.2%(41)	19.2%(37)	28.5%(55)	9.8% (19)	21.2% (41)
1st year	24	**Online Anatomy Interactive lectures offer me less advantage**	34% (18)	24.5%(13)	17%(9)	13.2% (7)	11.3% (6)
2nd year			20.3%(28)	21%(29)	27.5%(38)	15.9% (22)	15.2% (21)
Total			24.1%(46)	22%(42)	24.6%(47)	15.2% (29)	14.1% (27)
1st year	25	**I prefer Learning Anatomy through Interactive online lectures.**	25.9%(14)	9.3%(5)	9.3%(5)	22.2% (12)	33.3% (18)
2nd year			13.7%(19)	11.5%(16)	25.9%(36)	20.9%(29)	28.1% (39)
Total			17.1%(33)	10.9%(21)	21.2%(41)	21.2%(41)	29.5% (57)

Table-1: Questionnaire with students' responses.

DISCUSSION

Healthcare students are generally not that tech savvy. For healthcare workers to embrace evidence-based practice, it is essential for them to be computer literate. Angelina reported no statistically significant difference in computer literacy based on gender and age.[12] Okanath et al. reported that there were difficulties in the implementation of online classes for higher education students;[13] this assertion does not correspond with the findings reported in our study where the majority of the students reported that their transition to online classes was smooth.

The students found the transition to online learning Anatomy to be smooth (42.3% vs 39.7%), the students (59.7%) did not consider the online learning anatomy to be more flexible than face to face learning in terms of space and time management. Also the students didn't find it easier to communicate with teachers in the online environment as compared to face-to-face classes (48.2%), similarly, (59.5%) students did not value the online learning environment more than the face-to-face environment. Likewise (59.2%) Students did not believe that online learning anatomy helped them to have a better understanding of the topic. (51.5%) of Students did not find it more helpful to interact frequently with their teachers. Therefore (67.3%) of Students did not prefer online learning compared to face to face learning. Overall students preferred face to face learning than online learning Anatomy.

James et al. reported that adult learners prefer flexibility when it comes to learning programs.[14] Online education provided the flexibility that the adult learners crave. Azlan et al. reported that students felt that the e-learning and study from home, [15] online classes provided much flexibility. Our study findings revealed that the students didn't consider our online classes to be flexible; this was possibly because our institution followed the same timetable for the synchronous online classes. Thus, the students did not experience the freedom and flexibility that the asynchronous online sessions afford. Social connection is a basic need of humans.[16] Students in an online class may feel lonely and isolated.[17,18] And this feeling of loneliness and isolation may hinder their learning.[19] One of the most important traits of an effective teacher is the rapport that the teacher builds with his or her students in the class.[20,21] Thus, in an online class where students lack the opportunity to interact with their instructors' face-to-face, students are bound to feel isolated, and this may hinder their learning. Our study findings revealed that students found it difficult to communicate with their teachers in an online class. Our results are in line with findings reported by Mohammad et al. who wrote that the majority of the students reported that the communication between the instructors and the students has become difficult in online classes.[22] Our study findings also revealed that our students found online anatomy learning to be challenging. This is in stark contrast to the findings reported by Kalpana & Robert in their article where the majority of their respondents reported online anatomy learning to be interesting and enjoyable.[23] COVID-19 pandemic transformed medical education as there is increased individual and collective awareness and acceptance that technology can augment and enhance the delivery of medical education.[24,25] Our students also reported that their IT skills have improved because of online classes. Our study revealed that students don't value the online learning environment more than the face-to-face environment; this finding is supported by the data published by Derar where he reported that students had a negative attitude towards online learning in comparison to face-to-face learning.[26] Jyoti et al. wrote that students reported the understanding of the topic was better in face-to-face classes then in live online classes.[27] Rudi Klein et al. wrote that students reported better understanding of anatomy topics when taught online.[28] Similarly, our respondents didn't believe that they developed better concepts during online classes as compared to face-to-face sessions. Abhinandan & Anupama wrote that students reported receiving adequate support and resources from their teachers.[29] This contrasts with our findings where the students did not believe that online classes didn't help them in being more interactive with their teachers. Trifonet al. reported that students ranked the traditional face-to-face teaching of anatomy as more effective than online methods of teaching[30] which is congruent with our findings. They also reported that online teaching methods have improved the student's participation in anatomy lessons[30] which is not congruent to our findings. Xiaoqian suggested that there is a link between attendance and a student's academic performance.[31]Darici et al. reported higher attendance in online classes as compared to on-site classes.[32] In our study 1st year students, who were experiencing the online teaching for the first time, did not believe that the online classes enabled them to attend classes more frequently than they would have been able to in face-to-face sessions while the 2nd year students who had prior experience of online classes were more positive in their outlook. 1st year students didn't feel that the online learning helped them in utilizing their time effectively, this finding is

supported by the results obtained by Anjali & Agamin their study, where majority of the students found it difficult to manage their time during online sessions.[33] While 2nd year students in our study were divided on this issue. Our study revealed that students found it easier to revise a topic that they had learned online, possibly because both the recorded video of the online lecture and the PowerPoint presentation were shared with them once the session was over. Alexandra & Sunhea reported that the majority of their respondents felt that online radiological anatomy helped students in anatomy revision.[34] Our findings revealed that the majority of our respondents reported that more resources are required in online anatomy classes. Fareeha et al. mentioned several challenges that arose during online medical education.[35] Naturally for online sessions students need the Gmail accounts and Zoom software, while these issues don't arise in a face-to-face class.

Students generally prefer online lectures over face to face classes.[36, 37] In our study only first year students preferred online lectures while the second year students did not.

Ambreen et al. reported that even the teachers expressed their reservations about online teaching as they feared that it might compromise the level of teaching for subjects like anatomy that require hands-on practice with bones and models.[38] While online teaching, lectures and tutorials, in preclinical years can be effective,[39] it cannot replace hands-on experience.[40] The level of collaboration in online sessions cannot match the effectiveness of collaboration in face-to-face sessions.[41] These findings match our results. However online medical education has had some success during COVID-19 induced lock-downs but it is prudent to not get carried away by that success.[40] A hybrid system of both online and face-to-face is more preferable.[40]

CONCLUSION

Our present study concludes that learning anatomy online is challenging. Overall students did not value online learning more than face-to-face learning in terms of flexibility, freedom, interaction with teachers, hands-on practice and concepts, although they enjoyed the leisure of self-study with whole study material available to them at any time. In future a hybrid system of both face to face and online with more anatomy resources can be helpful.

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Frequency of Leukoerythroblastic Picture and Hematological Parameters in COVID-19 Patients and Association With Disease Severity

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ABSTRACT

Introduction: Patients infected by COVID-19 can present with severe lung damage and acute respiratory distress syndrome (ARDS) and have a significant mortality risk involving all body system such as cardiovascular (CVS), gastrointestinal (GIT), neurological (CNS), immune system and haemopoietic system.
Aims & Objectives: To determine frequency of Leukoerythroblastic picture, compare complete blood counts and cell ratios (NLR, PLR, LMR) of positive COVID-19 patients with suspected COVID-19 patients and their association with severity as stratified by mode of admission and clinical status.
Place and duration of study: King Edward Medical University, Mayo Hospital Lahore from December 2020 to January 2021.
Material &Methods: 75 RT-PCR confirmed COVID-19 and 75 suspected patients of both genders and aged above 18 years were included. Severity was classified by mode of admission and patient status. CBC samples of all patients were analyzed for counts, differential and LE picture on Sysmex XE-1000 automatic blood analyzer. NLR, LMR and PLR were calculated in both groups. Peripheral smear findings were noted for leukoerythroblastic picture in all patients. Data was analyzed using SPSS version 25, p value ≤0.05 was considered significant.
Results: Each group consist of equal (75) number of patients with male predominance. Mean age of patient in confirmed and suspected groups were 58±14 and 61±15 respectively in both groups. Majority were admitted in HDU and ICU compared to isolated wards (P: .000). Frequency of LE picture in both groups was (2.7%) (P:0.12) by Chi-square. Mean± SD were determined, of hematological parameters and ratios in confirmed and suspected groups. ANC (P:0.022), ALC (P:0.032), NLR (P:0.002) were significantly different when compared in both groups.
Median (IQR) of Hb (p: 0.05), WBC (P: 0.000), platelet (P: 0.008), ANC (P: 0.000), NLR (P: 0.000), LMR (P: 0.016) were significantly correlated with severity, when analyzed by independent-sample Kruskal Wallis Test.
Conclusion: Leukoerythroblastic picture is not associated with severity in COVID-19. Neutrophil to lymphocyte ratio is important prognostic factor in suspected and confirmed COVID-19 patients.

Key words: COVID-19, Neutrophil lymphocyte ratio (NLR), platelet lymphocyte ratio (PLR), lymphocyte monocyte ratio, (LMR), Leukoerythroblastic reaction.

INTRODUCTION

Corona virus disease starting in 2019 in Wuhan, Hubei Province, China involved all countries and became pandemic in 2020.
Patients infected by COVID-19 can present with severe lung damage and acute respiratory distress syndrome (ARDS) and have a significant mortality risk involving all body system such as cardiovascular (CVS), gastrointestinal (GIT), neurological (CNS), immune system and haemopoietic system.[1,2]
This infection is caused by the severe acute respiratory syndrome coronavirus 2 strain (SARS-CoV-2). Patient is labeled as COVID 19 positive when PCR report shows viral detection. Suspected is the term denoted to patients with positive contact history, any 2 clinical features (fever, respiratory symptoms and positive radiological findings) and awaited/negative PCR as per institution protocol.[3]
Clinical course of disease differs in patients; some patients develop mild symptoms with good prognosis while others present with difficult treatment and high mortality. Classification of disease severity is very important to guide the right treatment and care.[1,2]
Identification of routine laboratory parameters that can guide the disease categorization between mild and severe COVID-19 cases could help to predict patients at risk.

CBC parameters and ratios abnormalities in COVID-19 are correlated with disease advancement, stringency and mortality.[4]

Leukoerythroblastic (LE) picture is defined as nucleated red cells and left shift of myeloid series circulating in the blood. It can be seen in conditions like bone marrow fibrosis, myeloproliferative disorders, and infiltration of bone marrow by metastatic diseases. Viral infections such as parvovirus can be the rare cause. A case report published in 2020 showed presence of LE picture in COVID-19 patient.[5]

The case report was based on findings noted in start of pandemic and author described the LE picture in a COVID-19 patient which improved and disappeared with treatment. Author suggested further studies to see exact frequency and role of LE picture in a disease severity and progression.

Neutrophils, lymphocytes, thrombocytes have important role in regulation of infection, inflammation so ratios of these parameters are important as early inflammatory markers. Neutrophil lymphocyte ratio (NLR) is ratio of absolute neutrophil to absolute lymphocyte count. Normally it is below 3, but ratio above 3 is noted especially in stress, sepsis according to research studies.[2]

Lymphocyte to monocyte ratio was calculated by absolute lymphocyte to absolute monocyte count. The range is 3-9 according to research studies.[2]

Platelet to lymphocyte ratio is calculated by absolute lymphocyte count (%) to absolute platelet count (%). Normal value is between 50-150.[2]

Disseminated intravascular coagulation (DIC) is reported in COVID-19 patients characterized by lymphopenia, thrombocytopenia, coagulopathy.[4]

Cell ratios including Neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), lymphocyte to monocyte ratio (LMR) can be easily determined through a CBC report and have been shown to be useful prognostic marker. Raised NLR, Raised PLR and decreased value of LMR are indicators of severe disease and bad prognosis.[6-11]

In this study we wanted to correlate CBC and peripheral smear findings with this disease. Our aim was to determine frequency of LE picture in COVID-19 patients and its relation with severity of disease. We desired to compare complete blood counts and cell ratios (NLR, PLR, LMR) of positive confirmed COVID-19 patients with suspected COVID-19 patients to see whether these can be beneficial in adding to diagnostic and treatment criteria. We also wanted to study the association of these parameters with severity on basis of severity stratification by clinical status of patients in both groups.

MATERIAL AND METHODS

A Retrospective Cross sectional was carried out at King Edward Medical University, Mayo Hospital Lahore from December 2020 to January 2021. Approval was obtained from the Ethical Review Board of King Edward Medical University (Institutional Review Board Approval letter no: 196/RC/KEMU).

During this study, 150 patients in total were included in study, 75 COVID-19 PCR positive and 75 suspected patients. Patients above 18 years of age and of either sex were included. 75 COVID-19 PCR positive patients and 75 suspected patients were studied. Patients with known Hematological disorders (Acute or chronic Leukemia, Myeloproliferative neoplasms) were excluded from the study.

Epidemiological data of both groups including age and sex, clinical data including symptoms at presentation and severity status of COVID-19 was classified by mode of admission as patients with mild disease in wards/rooms, moderate in high dependency units (HDU), severe/critical in Intensive care unit (ICU) and the choice of oxygen depend on patient status and its availability (whether on oxygen, nasal catheter, NRM, at CPAP or at ventilator) was collected. CBC samples of all patients of both groups was taken and analyzed for counts, differential and LE picture on Sysmex XE-1000 automatic blood analyzer. NLR, LMR and PLR in confirmed and suspected patients is included. Peripheral smear was stained with field Giemsa stain and examined under microscope. Frequency of leukoerythroblastic picture in confirmed, suspected patients and its association with severity of disease was noted.

Data was stratified according to age, gender, severity of disease, suspected and confirmed cases for COVID-19. Haematological parameters were compared between confirmed and suspected cases as well as in subgroups on basis of clinical status. Quantitative variables WBC, HB, platelets, ANC (absolute neutrophil count), ALC (absolute lymphocyte count), NLR and LMR were analyzed by mean and standard deviation in confirmed and suspected cases, while median (IQR) interquartile range is calculated mild, moderate and severely ill groups. Categorical variables like gender, status and severity of patients, frequency of leukoerythroblastic (LE) picture in each group by frequency and percentage. Continuous variables were analyzed by independent t-test in both groups and Kruskal Wallis test in severity based groups, while categorical variables by Chi-square test.

Statistical analysis:
Data was analyzed in SPSS version 25. p-value ≤ 0.05 is significant.

RESULTS

Each group consisted of (75) patients, with 75% male predominance as compared to 25% females.
Mean age of patients(yrs) in confirmed and suspected groups were 55±11, 58±14 and 61±15 respectively in both groups. Frequency (%) of patients on the basis of clinical status, indoor location and treatment instituted in confirmed and suspected cases is presented in Table-1. Mean±SD of hematological parameters were determined in confirmed and suspected groups in Table-2. These were analyzed by independent t-test for significance.

Groups		Status					total	P value
CON	severity	FM	Oxy	CPAP	NRM	Ven	75	.000
	ICU	0	16	4	1	6	27	
	HDU	5	35	0	1	1	41	
	WARD	5	2	0	0	0	7	
SUS	ICU	0	23	7	5	3	17	.000
	HDU	2	33	0	5	0	40	
	WARD	8	12	0	0	0	18	

P: (.000) significant
Table-1: Subgrouping of Confirmed/Suspected COVID 19 cases as per Clinical *severity *status

Mild: In ward/isolated group Moderate: In HDU (high dependency unit) Severe/critical: ICUFM: facemask, Oxy: oxygen, ven: ventilator

Groups	Parameters	Values	p-value
Confirmed	Age	55±11	0.4
suspected		58±14	
Confirmed	HB	13.8±7.5	
Suspected		13.4±7.09	0.75
Confirmed	WBC	16.1±6.4	
Suspected		14.9±8.2	.31
Confirmed	PLR	232.2±127	.16
Suspected		260±121	
Confirmed		17±17	
Suspected	ANC	12.1±6.5	.022*
Confirmed	ALC	6.2±19	
Suspected		1.3±.1.7	.032*
mono confirmed	Mono	3.7±2.1	.61
Suspected		2.6±2	
NLR confirmed	NLR	35.4±43	.002*
Suspected		18±14.7	
Confirmed	LMR	3.3±11	
Suspected		2.6±2.5	.07
Confirmed	PLR	80±135	.07
Suspected		49±52	

Table-2: Hematological parameters according in confirmed and suspected groups.

Haematological parameters and ratios (NLR, LMR, PLR) according to severity stratification are shown in Table-3. Median (IQR) of hematological parameters were determined according to severity stratification Their association were analyzed by independent-sample Kruskal Wallis Test, while frequency of leukoerythroblastic picture in suspected and confirmed group was observed 1 (1%) in confirmed and 3 (2%) in suspected cases (p-value: 0.12) was found to be insignificant by Chi-square test.

Severity	Wards/ isolated Rooms (mild)	HDU (moderate)	ICU Sever/ critical)	p-value
Hb (g/dl) Median (IQR)	9.5(15.2)	12(3.8)	13.9(2.6)	.055*
WBC (X 109/L) Median (IQR)	14(7.9)	14.9(10.2)	18.5(7.2)	.000*
PLT X (109/L) Median (IQR)	304(252)	206(111)	204(168)	.008*
ANC X 109/L) Median (IQR)	11(5.7)	13(10)	14.7(9.93)	.000*
ALC X 10^3/UL) Median(IQR)	1.4(1.05)	0.6(0.7)	0.6(.8)	.695*
NLR X 10^3/UL) Median (IQR)	8.6(16.8)	18(43.8)	30(23.6)	.000*
LMR Median (IQR)	2(0.6)	1(1.2)	1.3(0.84)	.016*
PLR Median (IQR)	45(80)	35(96)	35(110)	.094*
Total	25	81	44	.028^

The significance level is 0.05
* (Krusal independent test), ^ (Chi square test)
Table-3: Hematological parameters according to severity of COVID-19

DISCUSSION

In our study on 150 patients, majority of patients were males (72% in confirmed and 65% in suspected group). Male predominance in COVID-19 patients has also been seen in studies by Asgher, Usul etal.[12] No significant difference in severity for gender difference was found in our study however studies by Taj etal,[13] Terpos etal[4] showed male gender a risk factor for severity of disease. This difference can be due to different time period as their study was based on data obtained in first wave whereas our data is based on 3rd wave.
Mean age was above 50 years in mild, moderate and severe condition in both groups, and age was not risk factor for severity in our study (P value:0.19). The study by Usul etal also had similar findings while

studies by Taj S. etal [13] and Terpos etal [4] showed that older age is risk factor for severity of disease. Old age was associated with increased mortality in first wave while in second wave patients of all age groups are seen.[12,13,4]

Mode of admission in our study was mainly HDU and ICU (78.7% in confirmed and 89% in suspected group (p-value .000). In contrast to our study, Asgher etal showed in their study that most of the patients presented with mild to moderate symptoms so admitted in isolation wards (60%) while rest of patients (30%) were admitted in ICU. Since Mayo hospital is biggest hospital in government setup with extensive ICU facilities and patients are referred here so mode of admission in our study was HDU and ICU predominantly.

Our study was aimed to find frequency of LE picture in COVID-19 (confirmed and suspected) patients. It was seen in 4 patients (2.7%). Precursors of leukocytes are reported in a study in Covid -19 patients and seen mostly in patients developing DIC. LE picture has not been reported elsewhere and is found to be insignificant in our study also. This negative association is important as it rules out LE picture association with COVID-19 infection and when present is mostly due to causes other than this infection. Since according to exclusion criteria all other causes have been excluded, presence of LE picture in few patients indicated stress on bone marrow and was not associated specifically with this virus infection.

Current study showed no significant difference for HB, WBC, platelet and monocyte counts on CBC in comparison of suspected with confirmed group.

Decreased Lymphocyte count (lymphopenia) was observed in all patients as depicted by low ALC. It was significant when compared in confirmed group and suspected groups. LMR is not clinically significant different in suspected and confirmed groups. The probable reason can be that lymphopenia develops progressively in suspected group and becomes stable in confirmed group.

In this study on suspected and confirmed COVID-19 has not showed significant difference on PLR, it can be due to observation that platelet count remained in normal range and was not associated with disease severity. S. Blomme showed no relation of thrombocytopenia with disease and it remained normal in range in COVID-19. [17]

This is similar to study conducted by Taj S etal. In contrast to our study Asgher M S etal, showed high PLR in severe cases as compared to recovered patients and isolation ward.[13,14] Another study conducted by Liao D etal revealed significant thrombocytopenia in severe disease.[16] So the platelet can remain normal, low and high in COVID-19 as different strains of virus are evolving in different waves. However PLR is not good prognostic marker.

Our data showed leukocytosis, neutrophilia and increased NLR in confirmed group as compared to suspected group. As it is hypothesized that Covid -19 has effect on T-cell lymphocytes and leads to reduction of lymphocytes resulting in high NLR in confirmed group. As evidence suggest that PCR test remaining negative within 4-5 days after symptoms is due to low viral load, it can be the cause of above findings.[15]

In summary the hematological parameters do not differ much in confirmed and suspected group except for NLR. So management on strong clinical suspicion of Covid 19 disease will be beneficial and is being practiced.

Our study showed, hemoglobin was slightly high (≥13 g/dl) in severe/critical cases of COVID-19 positive patients than moderate and mild cases (≤13 g/dl) (p value 0 .05). This difference may be due to presence of co morbidities, dehydration, smoking habits and varies from patient to patient or due to hypoxia induced compensated erythrocytosis. Similar findings were seen in study by Usul E etal.[12] In contrast to our results a study was by S Blomme etal showed mild anemia but no statically significant difference in sub groups on the basis of severity.[17] Taj etal showed no association of anemia Hb, MCV, HCt parameters with severity of COVID-19. [13].Whereas Fagihdinvari etal found an association of anaemia and outcome of COVID 19. [18] In the present study LMR was significantly correlated for disease severity (p value 0.016). And it is poor prognostic marker as shown by Asgher MS.[14] In addition to above ratios the most significant poor prognostic marker was NLR due to leukocytosis with neutrophilia on differential. This fact has been proved by number of studies. Ahmed MAS et al showed leukocytosis, high neutrophil to lymphocyte ratio in critically ill (ICU) patients when compared with patients in isolation wards. Taj S et al also observed leukocytosis, high Neutrophil to lymphocyte ratio in critical COVID-19 patients. Pervaiz A, Pasha U, et al observed that that NLR in patients with COVID-19 is predictor of mechanical ventilation.[19]

CONCLUSION

Leukoerythroblastic picture is not associated with severity in COVID-19. NLR is important independent prognostic factor in suspected and confirmed COVID-19 patients.

Limitations:

This study had limitations. Firstly, this was a retrospective study, secondly, we had no data of death and recovery. Finally, radiological findings, and
comorbidities like hypertension, diabetes were not included.

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Efficacy of Paediatric Preinduction Anxiety Distraction Techniques During Oncologic Procedures

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ABSTRACT

Introduction: Children undergoing oncologic procedures as part of their treatment may suffer from anxiety. It could be related to parental separation, pain/ bodily harm or a previous bad experience of procedure. Pre-procedure anxiety may result in adverse clinical outcomes such as emergence delirium, pulmonary complications and behavioral issues. Preoperative anxiety must be assessed to deal with using appropriate preinduction distraction techniques.
Aims & Objectives: The objective of audit is to determine efficacy of preinduction distraction techniques used in our clinical set up (to meet RCoA standards) in reducing anxiety and need of restrain for children at time of induction.
Place and duration of study: Shaukat Khanum Memorial Cancer Hospital & Research Centre, Lahore Pakistan. 14th of January 2020 to 15th of March 2020 (8 Weeks).
Material & Methods: It is a prospective outcome-based audit study of 101 children (2-8 years) undergoing intrathecal chemotherapy and bone marrow biopsy at Shaukat Khanum Cancer Hospital, Lahore. All children had non-pharmacological preinduction distraction techniques (Parental/legal guardian presence and/or play car) to reduce anxiety, cry and need for restraint. Anxiety levels as assessed by modified Yale Preoperative Anxiety Scale (mYPAS), cry and restrain were benchmarked with Royal College of Anesthetists (RCoA) standards.
Results: A total of 101 children with a median age of 4 years (2-8 years), had 100% parental/legal guardian presence at induction. In our audit, 52% of children cried and 43% were found to be anxious. However, only 21% children required use of restraint (holding still in laps) by accompanying parent/legal guardian. This is acceptable for restraint but not for cry/ anxiety as per RCoA benchmark.
Conclusion: Preinduction distraction technique of parental presence and/or toy car, showed only limited benefit in terms of cry, restraint and anxiety levels. Our audited results met benchmark set by RCoA only in terms of restraint but not for anxiety/cry.

Key words: Distraction, Preinduction, Anxiety, Pediatric, Premedication, Pakistan

INTRODUCTION

Paediatric oncologic procedures result m anxiety which is often exhibited in these children's behavior. It is observed as a change in the form of interaction with parents, interest in playing or surroundings, facial expressions, crying, vocalization (quiet or screaming) and lack of cooperation with others, including parents. Preinduction anxiety is relatively common up to 60% amongst children. [1]

Children may have a one-off procedure or repeated ones depending upon their treatment course. A bad experience of induction for procedure, makes subsequent induction episodes even more traumatic. The child suffers from short and long term consequences[2,3,4,5] such as: emergence delirium, increased pain relief need, separation anxiety, aggressive behavior, temper tantrums, bed wetting, nightmares, sleep and appetite disturbances.

The anxiety experienced by children also gets reflected into their parents and vice versa [6,7,8]. To minimize the risk of adverse clinical outcomes, an individualized pre-induction technique to reduce anxiety should be adopted.

Preinduction distraction usually includes: premedication, parental presence, behavior therapy (video games/cartoon, play therapy clowns or motor vehicles). The practice of preinduction technique varies from anesthetist to anesthetist, however the overall outcome should meet the standards set by the Royal College of Anesthetists (RCoA) [9] whereby 75% of children should go through the procedure without crying or need of restraint.

The purpose of this prospective outcome-based audit study was to determine if the current clinical practices of preinduction distraction technique in our clinical set up are effective (to meet RCoA standards) in reducing anxiety and need of restraint for children at the time of induction.

MATERIAL AND METHODS

This is a prospective, outcome-based audit of 101 sample size based on convenience sampling to include all children aged two to eight years who underwent general anesthesia for intrathecal chemotherapy and bone marrow biopsy.

Among these children, those who were induced for a combined procedure (such as CT scan or central line insertion/ removal), immobile (bed/ wheelchair bound) or blind were excluded. The Shaukat Khanum Institutional Review Board (IRB) exemption and Audit Review Committee approval was granted on September 17, 2019.

Data was collected from Anesthesia records/ clinical notes from electronic hospital information system (HIS) and clinical assessment as per proforma.

Children scheduled for the procedure had routine preparation of day cases including preoperative anesthesia assessment and informed consent. Children waited for their turn for procedure in the preoperative holding bay with their parents/ legal guardian. As a preinduction distraction technique, they were offered a remote-controlled play car to play in the preoperative holding bay and to be driven into the procedure room, which was either accepted or refused by children depending on their own free choice. No premedication drug was administered to allay pre-procedure anxiety.

At the time of their procedure, the child either in the play car or lap of their parent/ legal guardian, was brought into the procedure room. After WHO safety checklist Sign-in a child would have inhalational induction with a clear facemask in the play car or in the lap of an accompanying adult. The endpoint is good parental/ legal guardian separation at which s/he is awake but calm (not anxious or crying). The child is observed for preoperative anxiety using the Modified Yale Preoperative Anxiety Scale (mYPAS) which also includes crying and need for restraint by accompanying parent/ legal guardian. The rest of anesthetic conduct is as per Anesthetists discretion.

Anxiety is a psychological state of stress and uneasiness in face of unclearly perceived danger. To determine the degree of anxiety a behavior observation tool 'Modified Yale Preoperative Anxiety Scale' (mYPAS) is used by an observer in young children. It is a validated tool which is widely used in research with good interobserver reliability and validity. It has five main domains and a total of 22 elements in it. The total score is the sum of each domain divided by the number of its elements, multiplied by 20 (Total score= (A/4+B/6+C/4+D/4+E/4)* 100/5). The cut off score of 57 is set for a significant level of anxiety in children.[5]

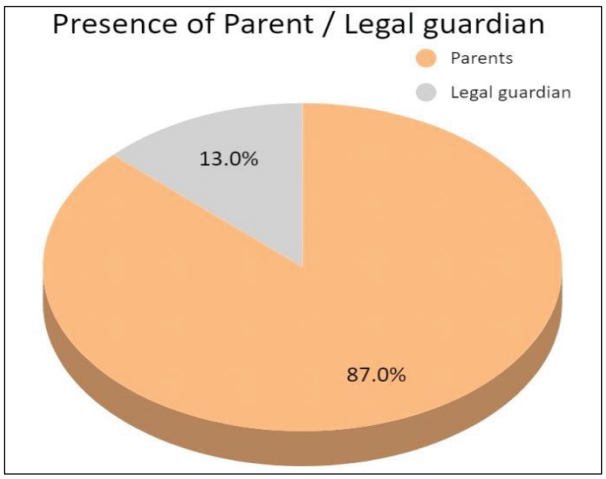
Statistical analysis:

The data was presented in average, median and percentage on Microsoft Excel 2010.

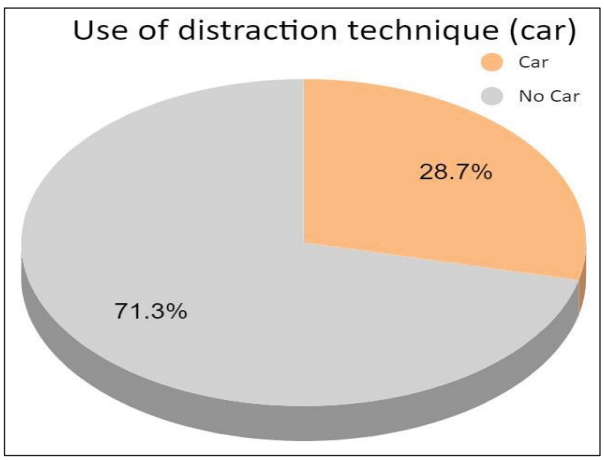
RESULTS

The collected data of One hundred and one children undergoing intrathecal chemotherapy and/or bone marrow biopsy ranged in age from 2 to 8 years with a median age of 4 years. There were 69.3% under the age of five years (preschool).

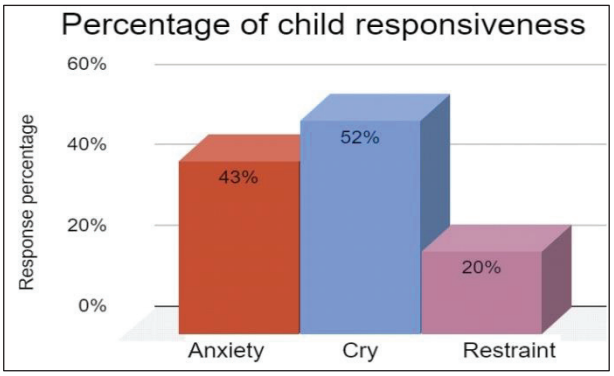
To reduce the anxiety, all children had either a parent (87%) or legal guardian (13%) present at the time of induction (Graph-1). As a preinduction distraction technique, remote controlled play cars were availed by only 28.7% to play in the preoperative holding bay and to drive in for induction to the procedure room (Graph-2).



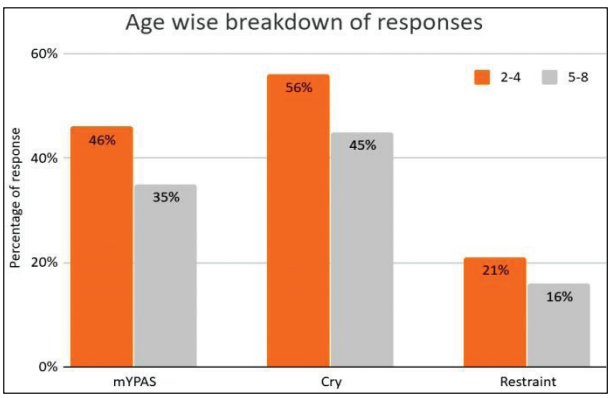
Graph-1: Presence of parent or legal guardian.



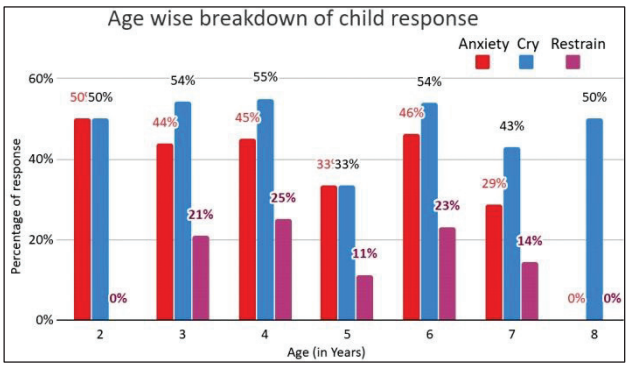
Graph-2: Percentage of children who had play car, as a preinduction positive distraction technique.



Graph-3: Percentage of child responsiveness



Graph-4: Age wise breakdown of child responses (2-4 year versus 5-8 year).



Graph-5: Age wise breakdown of child response.

The behavior observation revealed that a little more than half (52%) of children cried, one fifth required restraint in the form of their parents or legal guardians holding them firmly in their laps to facilitate the induction of general anesthesia. Using the mYPAS scale, two-fifths (43%) children were found to be anxious at time of induction (Graph-3).

The sub group analysis of anxiety, cry and restraint, based on age was carried out to depict any difference in behavior in younger children. In the less than five year old (69.3%) group of children, 56% cried, 21% required restraint (in the form of their parents or legal guardians holding them firmly in their laps) and 46% were anxious at time of induction (Graph-4). These behavior parameters are higher in the younger age group (2-4 years) than that of the older age group of children five years and above. However, a more detailed breakdown of age for behavior shows a decrease in older group (except 6 year old), with only worth mentioning the difference of not requiring any restraint in two and eight year olds (Graph-5).

The accepted standard set by Royal College of Anesthetists (RCoA) [9] is to have 75% children should have induction in anesthesia room without crying/anxiety or need to restraint. In this context we had not met the standard for anxiety/ crying, however it was met with a good margin for restraint (21% versus 25%).

The purpose of this prospective outcome-based audit was to determine if the currently adopted measures of preinduction distraction technique were effective in reducing anxiety to meet RCoA standard. Children's anxiety as exhibited by behavioral changes agitation requiring physical restraint was effectively reduced to 21%, (RCoA standard 25%).

DISCUSSION

Preinduction distraction techniques to reduce a child's anxiety vary amongst different hospitals and anesthetists within a hospital. However, the cornerstone of preinduction technique is to individualize it to the child considering his/her age, temperament, previous experiences and anxiety level to achieve desired results.[10] The desired result is a child who is not crying, anxious or requiring physical restraint before induction of General anesthesia.

Preinduction techniques are divided into pharmacological (sedative drugs) and non-pharmacological management to relieve anxiety.[11,12] In this cohort of children no sedative premedication was used, parental/ legal guardian presence and remote controlled play car was used as per routine practice of the clinical area.

Parental presence at anesthesia induction (PPIA) is widely practiced [13] but studies show conflicting evidence of its value in reducing anxiety. It was found to be of value in some studies if parents are calm/ relaxed and if the child is having repeated exposure to procedures.[14,15] However, the Cochrane Review in 2009 and in 2015 along with other studies have shown a very limited role of parental presence on a child's anxiety level and co-operation during induction.[16,17] In our audit although all children were accompanied by a family member but not necessarily by parents (13% by legal guardians). It is probably due to repeated hospital visits during the course of illness that other family members were also involved in accompanying the child. There are also varying family dynamics in which a child and parent are

comfortable with another relative to take this responsibility. It is a unique occurrence on which we found no literature for comparison. However, we did find that in parental presence, anxiety level was lower in children (40.2% versus 53.8%) along with crying (51.7% versus 53.8%) which indicates that the child's comfort level may decrease with legal guardians as the time for induction approaches in the procedure room. However, the restraint is higher with parents (20.7% versus 15.4%) which could be due to parental readiness to intervene earlier.

The distraction technique using various toys helps in relieving anxiety.[18,19] It helps to comfort and create a positive experience of procedure. In our audit the other distraction technique used, is a remote-controlled play car used by 28.7% children. It had no major difference in cry (48.3% versus 54.1%) and restraint (17.2% versus 20.83%). However, the anxiety levels were higher (51.7% versus 38.9%) in children using a play car. It could be related to the lack of movement control in an unfamiliar environment.

As our audit included children aged 2 to 8 year, there could be a difference in anxiety/ behavior of younger children compared to older ones. Sadeghi A et al (2017) and Caprilli et al. (2004) results showed younger children to be more anxious compared to their older counterparts, this is also observed in our audit whereby anxiety (46% versus 35%), crying (56% versus 45%) and restrain (21% versus 16%) was observed.[20,21,22]

Strength of this audit study is that most of the distraction interventions used and observational tool mYPAS is validated for inhalational induction, which is the predominant practice in this group of children. The observational tool mYPAS is also used by Lim E et al for pediatric intravenous induction therefore it is valid for both groups of induction method.[23] The anxiety level of a child in the procedure room before induction is related to recovery complications,[24] we also assessed the child's behavior at this most relevant point of time.

This audit shows a good reliance on coping promoting behavior (including distraction techniques). The main weakness is that it is a non-interventional audit of current practices that shows the results in comparison to the standard set by RCoA. However, it does not prove (and is not the objective of the audit study) superiority of any particular technique (preinduction distraction).

The results indicate that there is a room for improvement in adopting a multi-pronged strategy. It includes increasing awareness of anesthetists, nurses and parents in terms of expectations and benefits. It would help create a demand from other health professionals as well parents and end users. RCoA identified lack of training or judgment about assessing child's anxiety and need for appropriate preinduction distraction technique. This could be overcome by empowering team members to be able to identify the need and be able to discuss with anesthetist. Departmental protocols and regular continued medical education would help improve clinical outcomes and better utilization of existing resources.

By regular audits, promoting child friendly practices and monitoring the progress of improvements, the system would evolve and we would be able to make it a better experience for children and their parents.

Recommendations:

- Awareness of clinical outcomes regardless of method used to allay anxiety in children.
- Empowering team members to identify and discuss the need for distraction technique
- Use of distraction techniques more effectively to achieve better outcomes.
- Use as a quality indicator for pediatric cases, if feasible.

CONCLUSION

The efficacy of preinduction positive distraction technique of parental presence and toy car, showed limited benefit in terms of cry, restrain and anxiety levels.

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Controlling Post-Partum Hemorrhage Using A Novel Technique of Multiple Sponge-Holding-Forceps Applied Along Cervical Canal

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ABSTRACT

Introduction: Postpartum hemorrhage (PPH) is not only one of the leading causes of maternal mortality but also the most feared complication amongst obstetricians. Despite various predictive factors, PPH can occur in low risk pregnancies without predictive factors. Application of sponge holding forceps vaginally can invariably stop bleeding, leading to prevention of serious consequences.
Aims & Objectives: To determine the proportion of success of PPH control using sponge holders applied vaginally to the cervix after failure of medical treatment and before going for hysterectomy, in immediate post partum period.
Place and duration of study: This study was a cross-sectional study conducted at the labour room, Indus Hospital Raiwind for duration of one year from June 2020 to June 2021.
Material & Methods: Clinical and demographic features were recorded on a pre-designed proforma. Parity and gravidity was determined. Amount of bleeding was estimated and recorded. Additionally, the final result of patients was reported, as well as if they required surgical intervention. Data was analyzed using SPSS 24.p value ≤0.05 was considered significant.
Results: The mean (SD) age in our study was 26.28 (4.11) years. In this study majority of the patients were primipara 65% (n=26) and 35% (n=14) were multipara. On the basis of gravidity, more patients 37.5% (n=15) were primigravida while multigravida and grand multigravida patients were 27.5% (n=11) and 35% (n=14) respectively. The mean (SD) blood loss before the procedure was 720 (200) ml while after the procedure it was 90 (130) ml (p=0.02). This procedure was successful in all the patients and there was no need of further surgical intervention.
Conclusion: Our study concludes that use of sponge holding forceps as cervical clamp around cervix is one of the effective, economic and safe procedure for PPH patients.

Key words: Cervix; Efficacy; Postpartum hemorrhage; Sponge holding forceps

INTRODUCTION

Postpartum hemorrhage (PPH) is not only one of the leading causes of maternal mortality but also the most feared complication amongst obstetricians. Despite various predictive factors, PPH can occur in low risk pregnancies due to unknown etiology. Application of sponge holding forceps vaginally can invariably stop bleeding, leading to prevention of serious consequences.

Postpartum hemorrhage is defined as blood loss of more than 500ml after normal vaginal delivery or 1000ml after c-section. Postpartum hemorrhage results in 25% of maternal deaths occurring each year. PPH results in maternal deaths of about 1 per 1000 deliveries in low resource countries and 1 in 100,000 deliveries in developed countries.[1,2] The preponderance of these fatalities 88 % occur within four hours after birth, suggesting that they are the result of third-stage labour events. Too little, too late is a frequent motif in PPH. Due to the episodic character of PPH and the fact that it is virtually always unexpected, birth attendants are unprepared to cope with it on a routine and repeated basis. About 14 million women around the world go into PPH every year with approximately 26 women every minute. The prevalence of PPH in Pakistan is 1.6%.[3] All pregnancies are at risk of PPH even if no predisposing factor is found. Most of the maternal deaths occur within first few hours of delivery. The major problems encountered in developing countries is late diagnosis of PPH, limited availability of pharmacological agents, absence of skilled staff to manage PPH, and availability of blood required to manage patients in case of failure of medical treatment.[4]

The most common cause of primary PPH is uterine atony, others include genital trauma, retained

placenta or adherent placenta, uterine rupture, maternal bleeding disorder.[5,6]

The active management of third stage of labour is practiced widely to control bleeding. It includes giving prophylactic uterotonics before delivery of baby, early cord clamping and controlled cord traction.[5] Now transamine has also being recently added as active management of third stage of labour.[7-11] PPH has historically been the largest cause of global maternal death, accounting for over 34% of the 275,000 maternal fatalities globally in 2015 and as high as 17.62 % of Chinese maternal fatalities in 2018, according to China's 2018 National Maternal and Child Health Annual Report.[12,13] The World Health Organization's (WHO) most recent prescription for preventing PPH is 10IU of oxytocin for all deliveries, and uterotonics such as carbetocin, ergometrine, and misoprostol may aid in successful uterine contraction[14] But, desensitization to oxytocin could diminish efficacy, and uterotonics has medication contraindications and adverse effects such as water intoxication, nausea, vomiting, and elevated blood pressure.[15] Additionally, in limited resources countries, the absence of uterotonics, blood products, or interventional treatment may significantly raise the likelihood of maternal mortality.[12] After these measures if still bleeding is not controlled, and any treatable cause is ruled out then next step is use of different types of tamponades to control bleeding. Though these tapenades are found effective in 90 percent of cases.[16] In case of failure surgical measures are resorted to promptly as substantial blood loss has already been endured.[17] As many deaths related to PPH occur in low resource areas and by unskilled staff, some quick and easy methods have to be introduced to save the lives of many.

Objective of this study was to describe a simple and effective technique for avoiding excessive blood loss in PPH in those patients where medical therapy has failed. This method provides different advantages such as it can be used safely in any setting with or without facility, can be used to prevent as well as treat hemorrhage, very cheap, easily accessible, easy to use, can save from surgical intervention and preserve fertility and most importantly it can be used as temporary measure to shift the patient from periphery to nearby hospital.

Therefore this study was carried out to determine the proportion of success of PPH control using sponge holders around the cervix after failure of medical treatment (Oxytocin, methylergonovine, Misoprostol (Cytotec),† a prostaglandin E_1 analogue[18] and before going for hysterectomy, in immediate post partum period at the Indus Hospital Raiwind.

MATERIAL AND METHODS

This study was a cross-sectional study conducted at the labour room, Indus Hospital Raiwind. The duration for this study was one year from June 2020 to June 2021. All patients of postpartum hemorrhage who were given medical treatment (Oxytocin, methylergonovine, Misoprostol (Cytotec),† a prostaglandin E_1 analogue[18] but not responding to medical treatment and are hemodynamically stable were selected. A consent form was signed from all the included patients for para-cervical clamps. The inclusion criteria for our study was patients with failed medical treatment for postpartum hemorrhage that were vitally stable, atonic uterus or patients referred from peripheries with heavy bleeding, women who want to preserve reproductive potential, women giving informed written consent for all surgical intervention including paracervical clamp, postpartum obstetrical hysterectomy while the criteria for exclusion was patients who were rapidly deteriorating vitally or in a stage of progressive/refractory shock, suspected uterine rupture, confirmed perineal tears, Placenta accreta percreta picked before or during surgery.[19] Patients routinely undergo cervical clamping as a desperate measure before hysterectomy in case of continuous vaginal bleeding. All patients suffering from PPH were identified in the labour room or coming in emergency room by obstetric residents. Data from such patients was recorded after informed consent. Clinical and demographic features were recorded on a pre-designed performa. Medications given during the management were recorded and, time of application of clamps and time of removal was noted. Amount of bleeding was estimated and recorded. PPH calculated by “a pictorial reference guide to aid visual estimation of blood loss at obstetric haemorrhage”.[20] Additionally, the final result of patients was reported, as well as if they required surgical intervention. Any woman who has given birth two or more times is referred to be "multipara." A grand multipara is a woman who has given birth five or more times.[21] This novel methodology includes the use of two speculums applied vaginally and 4 sponge forceps applied to cervix. The principle for this procedure is temporary occlusion of uterine arteries and its branches which represent the source of 90% of blood flowing to the uterus. The main procedure for this technique is as follow:[22]

1. Patient is placed in lithotomy position
2. Cervix is explored and any cervical tear identified if bleeding stitched
3. Anterior and posterior lip of cervix is grasped with help of sponge forceps

4. To occlude the right uterine artery, the cervix is pulled to the left, and a sponge forceps/paracervical clamp is applied to tissue within the lateral fornix as high and close to uterus as possible in the hopes of occluding the uterine artery within the tissue bundles, while avoiding the ureter.
5. Forceps/clamps to be locked only by one lock
6. The procedure is repeated on opposite side
7. 2-4 sponge holding forceps can be used according to need. If bleeding stops with the two sponge holders applied laterally then Sponge holding forceps applied to anterior and posterior lip of cervix is removed, otherwise can be kept in place.
8. The amount of bleeding decreases. The patient is constantly monitored. If the patient's hemodynamic status deteriorates or if bleeding persists, the patient is transferred to the operating room for additional treatment.
9. The vagina is packed with a roll guaze such that the forceps stay in place and away from vaginal wall to prevent any injury to vaginal mucosa. Forceps are kept for 6-8 hours and then removed.
10. With these clamps in place the blood flow to uterus through uterine arteries will be stopped.
11. Vaginal tears sutured at the end.

Statistical analysis:

Data was analyzed using SPSS 24. Continuous variables like age, were reported as Mean (SD). Categorical variables like parity, gravidity success of clamps was represented as frequencies and percentages. Associated factors of success of clamp such as duration of bleeding before and after clamping was done by using chi-square tests. P-value of <0.05 was considered significant.

RESULTS

This study was conducted at the labour room, Indus Hospital Raiwind for duration of one year from June 2020 to June 2021. A total of 40 patients were included in this study.

In the age wise distribution, majority of the patients 45% (n=18) were in age group of 21-30 followed by age group ≤20 25% (n=10), age group 31-40 20% (n=8) and age group ≥41 10% (n=4) (Fig-1). The mean (SD) age in our study was 26.28 (4.11) years. According to the gestational age majority 72.5% (n=29) of the patients have gestational age of ≥38 while 27.5% (n=11) patients have gestational age of ≤37 (Table-1). In this study majority of the patients 65% (n=26) were primipara and 35% (n=14) were multipara. (Table-2) On the basis of gravidity, more patients 37.5% (n=15) were primigravida while multigravida and grand multigravida patients were 27.5% (n=11) and 35% (n=14) respectively (Table-3). In our study majority of the mode of delivery 90% (n=36) were spontaneous vaginal deliveries while the lower segment Caesarean section cases were 10% (n=4). Two were primigravida and lower segment Caesarean section was done due to fetal distress while two were multigravida and lower segment Caesarean section was done due to previous Caesarean section (Fig-2) Majority of the labours 55% (n=22) in our study were spontaneous. The cases of induced labour were 40% (n=16) while 5% (n=2) labour were elective (Fig-3). This procedure shows efficient haemostatic effect. The mean (SD) blood loss before the procedure was 720 (200) ml while after the procedure it was 90 (130) ml. This was significant statistically (p=0.02) (Table-4). In our study blood transfusion was needed in 25% (n=14) patients while it was not needed in 75% (n=26) patients. (Fig-4) This procedure was successful in all the patients and there was no need of further surgical intervention due to careful selection of patients.

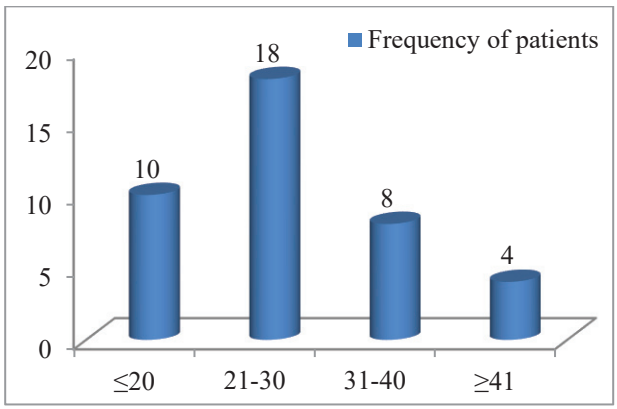

Fig-1: Age wise distribution of patients

Gestational age	Frequency	Percentage
≤37	11	27.5%
≥38	29	72.5%

Table-1: Distribution of patients on the basis of gestational age

Parity	No of patients	Percentage
primipara	26	65%
multipara	14	35%

Table-2: Distribution of patients on the basis of Parity

Gravidity	No of patients	Percentage
Primigravidity	15	37.5%
Multigravidity	11	27.5%
Grand multigravidity	14	35%

Table-3: Distribution of patients on the basis of Gravidity

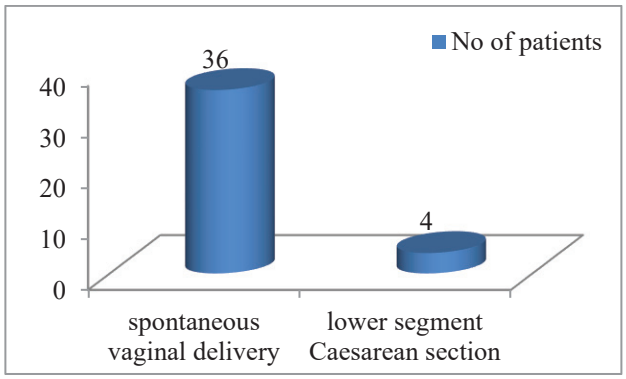

Fig-2: Distribution of patients on the basis of mode of delivery

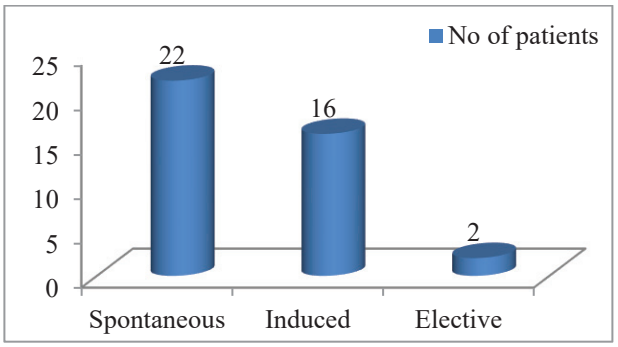

Fig-3: Distribution of patients on the basis of Labour

Blood loss	Mean (SD)	p
Before procedure	720(200) ml	0.02
After procedure	90(130) ml	

Table-4: Mean blood loss before and after procedure of clamping

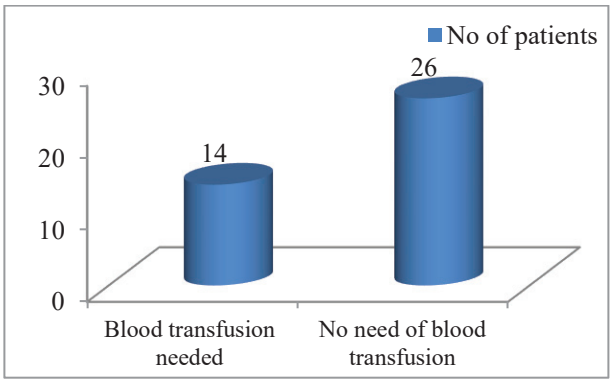

Fig-4: Distribution of patients on the basis of need for blood transfusion

DISCUSSION

Postpartum haemorrhage (PPH) is the major cause of maternal death globally, with a prevalence of 6%.[23] PPH is responsible for more than 30% of all maternal fatalities in Africa and Asia.[24] PPH is thought to raise the risk of morbidity by 50 times and has a 5 times greater disease rate than death rate. It has been suggested that early treatment of PPH is critical.[25] Despite the fact that the majority of patients may be managed with medicine like prostaglandins, oxytocin, and uterine massage while in around 10% of women with PPH need substantial surgical operations, including hysterectomy, which has the unfortunate side effect of preventing future fertility. Before turning to the final remedy for PPH, hysterectomy, it is crucial to have conservative management strategies in place when medical therapy fails.

At this stage in the care of PPH, a range of conservative treatments have been developed and used, including the use of sponge forceps as a cervical clamp. The use of sponge forceps as cervical clamps are a new, effective, easy, and minimally invasive surgical procedure for preventing excessive blood loss in postpartum hemorrhage in individuals who have failed to respond to medicinal therapy.[26] It allows for early intervention, preventing hysterectomy and consumption coagulopathy, as well as the preservation of reproductive potential. It may be employed in any situation, whether it has or does not have a facility. It serves as both a preventative and a therapeutic measure. It is affordable, convenient, and simple to use. Both traumatic and atonic PPH may be treated. It might be used as a transient treatment to move the patient to the peripheral for further therapy.

The present study was conducted at the labour room, Indus Hospital Raiwind for duration of one year from June 2020 to June 2021. A total of 40 patients were included in this study.

In this study, the mean (SD) age was 26.28 (4.11) years. These findings were in line with the Shekavat et al. and Gungorduck et al. who reported that the mean age in their study was about 26 years while our study mean age was lower that mean age reported by Gai et al. and Movafegh et al. who observed the mean age as 29.71±4.18 years and (27.0±3.4 years) respectively.[7-10] In the age wise distribution, majority of the patients 45% (n=18) were in age group of 21-30 followed by age group ≤20 25% (n=10), age group 31-40 20% (n=8) and age group ≥41 10% (n=4). Similar results were shown by a previous study done by Al-Zirqi et al. who reported that the majority of the patients were from 20-30 age group.[27] According

to the gestational age majority 72.5% (n=29) of the patients have gestational age of ≥38 while 27.5% (n=11) patients have gestational age of ≤37. A previous study done by Munir SI et al. shows similar findings to our study and reported that the gestation of the majority of the patients was between 37-40 weeks.[28] Santhanam R et al. also reported a similar finding, stating that 93.44 % patients were in gestational age >37 weeks.[29] In this study majority of the patients 65% (n=26) were primipara and 35% (n=14) were multipara. These results were similar to the findings reported by Al-Zirqi et al.[27] Another earlier study done by Bhavana et al. at also reported consistent results with our study.[5] On the basis of gravidity, more patients 37.5% (n=15) were primigravida while multigravida and grand multigravida patients were 27.5% (n=11) and 35% (n=14) respectively. In our study majority of the mode of delivery 90% (n=36) were spontaneous vaginal deliveries while the lower segment Caesarean section cases were 10% (n=4). Although LSCS is a stronger risk factor for PPH, but in our study vaginal birth has a higher rate of PPH patients. This might be because a bigger proportion of women accepted to the institution delivered vaginally, increasing the absolute number. Gupta et al. did a comparable research and observed a similar result by reporting that the majority of cases, 19 out of 25 76 % were normal vaginal delivery, while 6 (%) were delivered through caesarean section.[30] This procedure shows efficient haemostatic effect. The mean (SD) blood loss before the procedure was 720(200) ml while after the procedure it was 90 (130) ml. (p=0.02). In our study blood transfusion was needed in 35% (n=14) patients while it was not needed in 75% (n=26) patients. This procedure was successful in all the patients and there was no need of further surgical intervention. These findings were comparable to another study who reported that bleeding can be effectively controlled by this in PPH patients without further surgical intervention.[31]

The strength of our study was that inclusion and exclusion criteria were followed strictly while the limitation of our study is small sample size and lack of long term follow up. Our study suggests conducting a randomized control trial with large sample size and long follow up to determine the efficacy of this novel procedure in more effective way.

CONCLUSION

Postpartum hemorrhage (PPH) is a significant factor of maternal death and serves morbidity in the shape of fertility loss in future, particularly in impoverished countries like Pakistan. This mortality associated with PPH and devastating morbidity of fertility loss may be reduced when appropriate conservative treatments are used before invasive surgery such as hysterectomy. Our study concludes that use of sponge holding forceps as cervical clamp around cervix is one of the effective, economic and safe procedure for PPH patients. This procedure is more effective in low ncome countries. Our study shows that there was no need of surgical intervention in all the patients therefore our study also concludes that the need of surgical intervention in PPH can be reduced by using this technique. Our study suggests conducting a randomized control trial with large sample size and long follow up to determine the efficacy of this novel procedure in more effective way.

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Colebrookea Oppositifolia Anti Arthritic Potential Vs Methotrexate in Pristane Induced Rat Arthritis

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ABSTRACT

Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease of joints with 0.24% global prevalence. Numerous pharmacological agents are available for the management of RA, but they are associated with many adverse effects. Alternative therapies for disease management are urgently needed. *Colebrookea oppositifolia (CO)* is an important herb with various traditional uses and pharmacological actions. In the present study, anti-arthritic effect of ethanolic extract of *Colebrookea oppositifolia* (EECO) leaves was evaluated in pristane induced arthritic rats (PIA) and compared to methotrexate by assessing body weight, clinical score of inflammation and histopathology.

Aims & Objectives: To study anti-arthritic effect of *C. oppositifolia* on a rat model of pristane induced arthritis.

Place and duration of study: This experimental study was conducted in Animal House of Post Graduate Medical Institute, Lahore from March to September 2019.

Material & Methods: Total forty female Sprague Dawley rats were categorized into five equal groups (n=8). Group A (normal control), group B (disease control). Group C and D belonged to low dose (250mg/kg) and high dose (500mg/kg) EECO treated groups respectively, while group E was methotrexate treated group. Arthritis was induced within two weeks by single intradermal injection of pristane on day 0 in groups B, C, D and E. At Day 15, treatment was initiated and at day 28 paw joint sections were taken for histopathology. Data input and analysis was done by using IBM SPSS version 24. p value ≤0.05 was considered significant.

Results: At week 4, significant increase (approximately 16%) in body weight was observed in all treated groups as compared to disease control. A significant reduction (more than 50%) in clinical score of arthritis was observed in all treated groups compared to diseased control group in which clinical score was 14.50±0.2. All extract and MTX treated groups showed significant improvement ($p<0.001$) in total histological score of arthritis (no rat was having severe disease) as compared to disease control group (75% of the rats were having severe disease).

Conclusion: This study supported anti-arthritic effect of EECO as illustrated by reduction in inflammatory and histopathological score.

Key words: Ethanolic extract of *Colebrookea oppositifolia* (EECO), *Colebrookea oppositifolia* (*C0*), anti-arthritic activity, pristane induced arthritic rats (PIA), histopathology.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic disorder predominantly affecting synovial joints, with global prevalence 0.24%.[1] RA being an autoimmune disease is associated with various immune cells (T cells, macrophages, fibroblasts and B cells) contributing to joint damage. The main histopathological changes in RA include abnormal proliferation of fibroblasts and synovial cells, resulting in thickening of synovial membrane, which progressively results in erosion of underlying structure of joint.[2] RA cannot be cured completely, however, recommended treatment approach is 'Treat to target approach', the objective of which is to achieve either remission or to lower disease activity.[3]The medical management of RA includes non-steroidal anti-inflammatory drugs (NSAIDS), steroids, disease modifying anti rheumatic drugs (DMARDs) and biological agents, depending on duration as well as severity of the disease process.[1] Among the DMARDs, methotrexate (MTX) acts as an anchor drug, as it is used alone or can be combined with other drugs.[4] However, its extensive use is associated with various detrimental side effects. Apart from the adverse effects, patient's expenditure on the management of the complications associated with treatment is also an issue of concern.[5]

Colebrookea oppositifolia (*C0*) is an important herb with various traditional uses and pharmacological actions.*C0* possess anti-inflammatory,[6] anti-oxidant,[7] cardio-protective,[8] anti-microbial,[9] and anti-ulcer[10] activities because of its rich flavonoids contents. The current study was conducted to evaluate the anti-arthritic activity of *C0* in pristane induced arthritic (PIA) rats.

MATERIAL AND METHODS

This experimental study was conducted in Animal House of Post Graduate Medical Institute, Lahore from March to September 2019, after approval by ethical committee of PGMI vide letter number 1137/EC/PGMI/2019.

Drugs and reagents: Pristane (Sigma), Ethanol (Merk) and Methotrexate (Howards) were purchased from local market.

Collection and Extraction of *C0*: Collection was done from Haripur, Hazara located in Khyber Pakhtunkhwa, province of Pakistan and authentication was done by Botany Department of GCU, Lahore vide voucher number Herb.Bot.3636. Shade dried 100g coarsely powdered leaves were dipped in 80% ethanol at 1:10 ratio for 72 hours, followed by filtration and evaporation via a rotary evaporator, yielding 20g of concentrated greenish brown, sticky, semisolid extract, which was freeze dried afterwards.[11]percentage yield of extract was 20%. EECO was readily soluble in distilled water forming homogenous solution.

Animals: Total 40 female Sprague Dawley rats at 6-8 weeks of age (120-150g) were taken and acclimatized according to the standard laboratory conditions for 7 days with free access to water and regular rat diet.

Grouping Experimental animals were randomly divided into five equal groups (A-E); with eight animals per group.

Establishment of pristane induced arthritis: Arthritis was induced within 2 weeks in all animals of group B, C, D and E by single intradermal injection of 0.5ml pristane at the tail base on day 0.[12]

Group	Intradermal injection at day 0	Oral treatment from 2-4 weeks (14-28 days)
A	0.5ml normal saline	Distilled Water 1ml
B	0.5ml pristane	Distilled Water 1ml
C	0.5ml pristane	250 mg/kg EECO
D	0.5ml pristane	500 mg/kg EECO
E	0.5ml pristane	1 mg/kg MTX

Table-1: Experimental groups for induction and treatment of rats.

Evaluation of body weight and clinical score of inflammation:

Each animal was weighed on day 0, followed by weekly measurement for next 4 weeks.

Clinical scoring of joint inflammation was performed on alternate days on all four limbs.

Arthritic score ranged from 0 to 4. Absence of swelling or redness =0, Swelling and/or redness in single joint (digit or paw) =1, in two joints=2, in more than two joints=3 and 4 =whole paw and digits involved.[13]

Histopathological examination of ankle joints:

At the end of study, on 4th week all animals were euthanized. Tissue specimen was examined under microscope after hematoxylin-eosin staining.

Histopathological scoring[14] was done by the following criteria:

Scoring for infiltration by mononuclear cells: 0=absent, 1= mild, 2= moderate, 3= severe.

Scoring for hyperplastic synovial cell infiltration: 0=absent, 1=mild (involvement of 1-3 layers), 2=moderate (involvement of 4-6 layers), 3=severe (involvement of 7 or more layers).

Scoring for villous hyperplasia: 0=absent, 1=few, short and scattered hyperplastic villi, 2= marked finger liked villi, 3=marked and diffused hyperplastic villi.

Scoring for pannus formation: 1=absent 2=mild pannus formation, 3=moderate synoviocytes proliferation and cartilage or bone invasion, 4=severe synoviocytes and inflammatory cell invasion into the cartilage or bone.

Total scoring for arthritis: Total scoring for arthritis was done by sum of above mentioned four histopathological parameters. 0=absent, 1-3=mild, 4-6 =moderate, more than 6= severe arthritis.

Statistical analysis:

Data input and analysis was done by using IBM SPSS version 24. As the data was normally distributed (tested by Shapiro Wilk test) ANOVA and post hoc Tukey's tests were used to test significance among all groups. Statistically significance was considered when p value was ≤ 0.05.

RESULTS

Body Weight

Comparison among groups at week 2, have shown significant lowering ($p \leq 0.001$) in mean weight in all disease induced groups in comparison to group A. Non-significant difference existed amongst all other groups at the end of week 2.

At week 4, mean body weight of groups A, C, D and E was significantly high ($p \leq 0.001$) in comparison to group B.

Week/ Group	week 0	week 2	week 4
A	122.2±0.6	140.0±1.5	153.2±1.8
B	123.5±0.	114.8±1.0	109.6±1.0
C	122.3±1.0	115.1±1.0	131.5±0.9
D	123.8±1.3	115.3±1.0	120.7±2.1
E	124.3±1.5	115.3±1.5	130.2±1.2

Table-2: Effect on body weight of rats (g) by *C0* and methotrexate in PIA (n=8).

Clinical Score

At week 2, significant difference ($p \leq 0.001$) was observed in mean clinical scoring of disease induced groups in comparison to group A and there was non-significant difference among all other groups. While, at week 4, significant difference ($p \leq 0.001$) existed in groups A, C, D, and E in comparison to group B. Similarly, significant difference also existed in groups A, B, C and D as compared to group E.

Week/Group	week 2	week 4
A	0.00	0.00
B	13.75±0.4	14.50±0.2
C	13.87±0.3	8.87±0.2
D	13.87±0.3	7.37±0.3
E	13.75±0.3	3.87±0.2

Table-3: Effect on clinical scoring of rats by *CO* and methotrexate in PIA (n=8).

Histopathological Scoring

Pair wise comparison for total arthritic score indicated that significant difference($p<0.01$) was present in total scoring of groups A, C, D &E in comparison to group B.

Total Histopathological Scoring	A	B	C	D	E
	%	%	%	%	%
Normal	100	0	0	0	0
Mild	0	12.5	50	100	87.5
Moderate	0	12.5	50	0	12.5
Severe	0	75	0	0	0

Table-4: Effect on histopathological scoring of rats by *CO* and methotrexate in PIA (n=8).

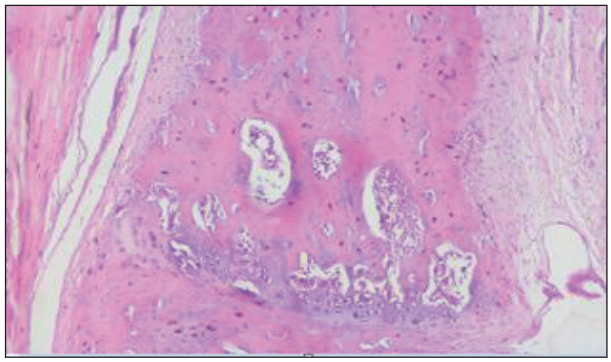

Fig-1: Group-A (Normal Control). Joint of normal healthy rats.

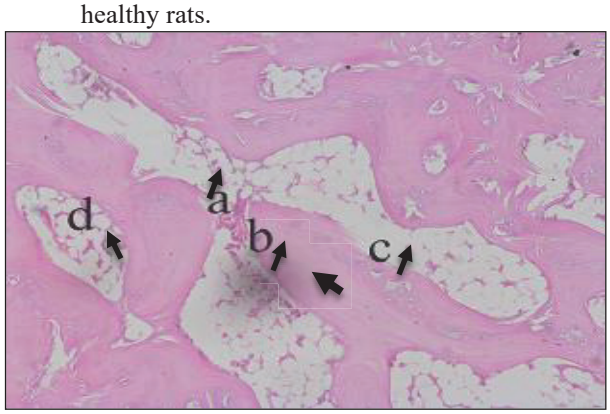

Fig-2: Group B (Disease Control), **a** & **c** shows bone erosion and pannus formation, **b** & **d** shows inflammatory cell infiltration.

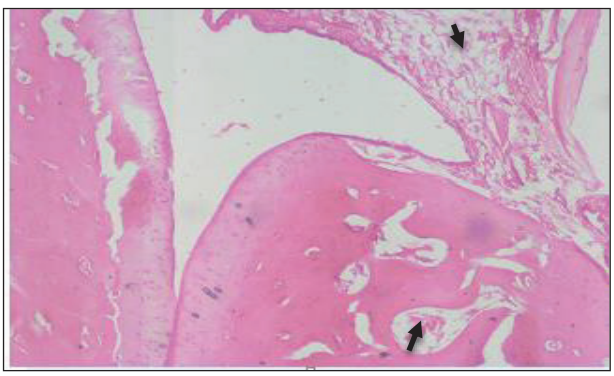

Fig-3: Group C (Low dose EECO). Treatment with low dose extract shows less infiltration by inflammatory cells.

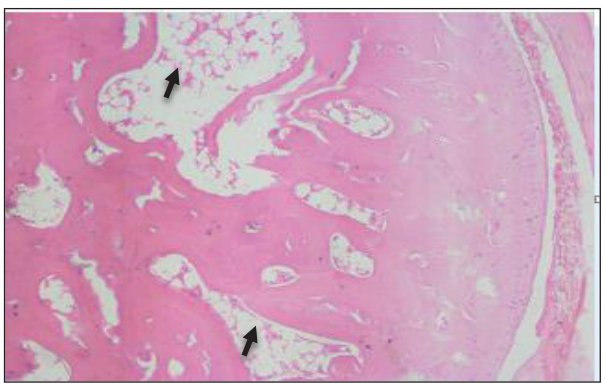

Fig-4: Group D (High dose EECO). High dose treatment of extract recovery of joints.

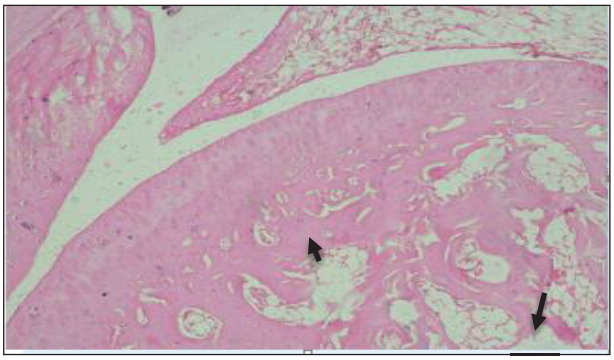

Fig-5: Group E (MTX treated). Reduced inflammatory cells infiltration and decreased synovial and villous hyperplasia.

Recovery of joint with reduced inflammatory cells infiltration and decreased synovial and villous hyperplasia in groups C, D and E.

DISCUSSION

Arthritis was induced by single pristane injection and its confirmation was done by observing signs of inflammation (swelling or redness) in all four limbs and by histopathological scoring.

Body weight declined significantly ($p<0.001$) in all disease induced groups till week 2 with almost 6% decline in body weight in contrast to normal control. While, at week 4 it significantly increased ($p<0.001$) in all extract and methotrexate treated groups approximately 16% in comparison to disease control group. Comparison of increase in body weight of rats between MTX treated group, low dose EECO extract treated group and high dose EECO extract treated group has shown a similar gain in body weight of rats. Similar pattern of restoration of body weight in arthritic animals has also been observed in previously conducted studies.[14,15]

At week 2, the clinical score for arthritis increased significantly ($p<0.001$) in all disease induced groups (group B, C, D and E) compared to group A, and at week 4, it significantly decreased in all extract and methotrexate treated groups in comparison to disease control group showing anti-arthritic activity of MTX and *C. oppositifolia*. These results are similar to previous studies, where significant reduction in clinical scoring of arthritic rats was observed by the use of medicinal plants having phytochemicals (aglyconic and glycosylated flavonoids, phenolic compounds and glycosides like acteoside) similar to that of *C. oppositifolia*.[16,17,18]

Significant reduction ($p<0.01$) in total histopathological scoring was noted in all treated groups in comparison to disease control group where decreased cell infiltration and reduced synovial hyperplasia was observed owing to anti-arthritic activity of MTX and *C. oppositifolia*. These results of histopathology are similar to previously conducted studies of plants having a glycolic and glycosylated flavonoids where reduction in cell infiltration and synovial hyperplasia has led to their anti-arthritic activity.[19,20]

Leaves of *CO* are locally used as decoction in northern areas of Pakistan for the treatment of rheumatism.[21] But no scientific data was available regarding the effectiveness of *CO* in the treatment of RA. This research was conducted for evaluating the effect of *CO* in arthritic rats. Recently performed study has shown that EECO has anti-arthritic potential owning to its rich flavonoids contents. This anti-arthritic action is due to its cytotoxic activity and inhibition of pro-inflammatory transcription factors NF-KB (nuclear factor kappa-light-chain-enhancer of activated B cells) and AP-1 (Activator protein 1).[6]

CONCLUSION

Results of the conducted study have shown that *CO* has anti-arthritic effects in PIA in rats, which is comparable to that of methotrexate. Further studies are required in order to identify various active principles of *CO* that have potential anti-arthritic potential.

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Pseudobasophilia: A Helpful Screening Tool in Diagnosis of Dengue

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ABSTRACT

Introduction: Pakistan is dealing with an epidemic situation of dengue. Serological testing for its diagnosis is not available everywhere across the country. So, in the current scenario, basophilia flagged by automated hematology analyzer can be a helpful screening tool in early diagnosis and prognosis of dengue in a resource limited country like ours.
Aims & Objectives: To assess the utilization of basophilia flag as a screening tool in early diagnosis of dengue by studying the frequency of basophilia flag and it's prognostic significance by correlating absolute basophil count with severity of thrombocytopenia.
Place and duration of study: It was a cross sectional study and conducted at Chughtai Institute of Pathology from August 2021 to October 2021.
Material & Methods: Total 1007 patients who had NS1 positive confirmed dengue infection were included in the study and EDTA blood samples were run on Mindray BC-6800 six-part hematology analyzer. Basophilia flag was noted, its frequency was calculated and expressed as percentage. Also, correlation of absolute basophil count with platelet count was calculated. Statistical analysis was performed using SPSS 23.0, p value <0.05 was taken as significant.
Results: WBC flag showing "basophilia" was seen in 136 patients (13.6%) and a significant correlation was seen between raised absolute basophil count and thrombocytopenia using Pearson test.
Conclusion: Pseudobasophilia is an important screening tool in diagnosing dengue patients and as significant prognostic marker as increased absolute basophilic count correlates with severity of thrombocytopenia.

Key words: Dengue, NS1 antigen, Pseudobasophilia, Automated hematology analyzer.

INTRODUCTION

Dengue is one of the most common causes of fever in Pakistan.[1] There has been a history of outbreaks of dengue fever in country since 1994 when first confirmed outbreak of dengue fever occurred. The next one happened in 2010 and recently we suffered from this epidemic in 2021.[2] Dengue is caused by the bite of female Aedes mosquito. The clinical symptoms include nausea, vomiting, high grade fever, joint pains (hence the name bone breaking fever) and skin rashes.[3]

Basic testing for dengue includes Complete blood count (CBC) which reveals changes in its parameters including Hemoglobin (Hb), Hematocrit (Hct), Platelet count, Mean platelet volume (MPV), Total white cell count (TLC), differential count including neutrophils, lymphocytes, monocytes, eosinophils and basophils (percentages as well as absolute counts). Differential white cell count can be helpful in diagnosis and prognosis of Dengue in resource limited areas.[4] Certain white cell flags generated by automated hematology analyzers can be a helpful screening tool in diagnosing and assessing the severity of dengue fever.[5]

Basophilia is defined as an increase in the number of basophils. It is commonly seen in allergic and acute inflammatory conditions and is also documented in myeloproliferative neoplasms such as chronic myeloid leukemia.[6] Hematology analyzers count basophils by electrical impedance and flow cytometric methods. Pseudobasophilia is a hematology analyzer phenomenon that is flagged by cell population other than basophils in conditions like leukemia, lymphoma, myeloma and infectious mononucleosis.[7,8] In a dengue-endemic country like Pakistan, pseudobasophilia is a common finding due to the presence of atypical lymphocytes.[9]

MATERIAL AND METHODS

A cross sectional study was conducted at Chughtai institute of Pathology from August 2021 to October

2021.Approval was obtained from the ethical and research committee of the institute. Total 1000 patients, both males and females of all age groups, who had NS1 positive confirmed dengue infection were included in the study. Two ml of peripheral blood sample was taken from each patient in EDTA tube following standard procedures. Samples were run on Mindray BC-6800 six part hematology analyzer. Basophilia flag was noted and correlated with peripheral smear examination. Suspected dengue patients who were NS1 negative were excluded from this study. Statistical analysis was performed using SPSS 23.0. Frequencies were calculated and expressed as percentages. Correlation of absolute basophil count and severity of thrombocytopenia was observed using Pearson test. P value <0.05 was taken as significant.

Statistical analysis:

Statistical analysis was performed using SPSS 23.0. Frequencies were calculated and expressed as percentages. Correlation of absolute basophil count and severity of thrombocytopenia was observed using Pearson test. P value <0.05 was taken as significant.

RESULTS

817 male, 190 female (total 1007) dengue patients were included in the study with mean age 40.77±15.58 years (Table-1). CBC showed mean Hb 15.75±6.5 g/dl, Hct 46.9±5.3 %, TLC 5.8±3.1 x 10^9/l, platelet count 27.5±26.04 x 10^9/l, Absolut Basophil Count (ABC) 0.08±0.23 (Table-2). A significant correlation (p = <0.004) was observed between increased Absolute Basophil Count (ABC) and fall in platelet count using Pearson test. (Table-3). Our study showed 136 dengue samples with flagging "basophilia" but when peripheral smears were prepared from these samples, there was no increase in basophils (hence the term pseudobasophilia). However, reactive lymphocytes with basophilic cytoplasm were seen on the smears which were falsely counted as basophils by automated hematology analyzer.

Characteristics	Values
Mean age (years)	40.77±15.58
Male	817 (81.1%)
Female	190 (18.9%)

Table-1: Mean age and gender frequency of patients

	Hb (g/dl)	HCT (%)	TLC ($\times10^9$/L)	Platelets ($\times10^9$/L)	ABC
Mean	15.75	46.92	5.83	27.51	0.0889
SD	6.527	5.347	3.178	26.048	0.235

Table-2: Hematological parameters

Hb: hemoglobin, HCT: hematocrit, TLC: total leukocyte count, ABC: absolute basophil count

		Platelets	ABC
Platelets	Pearson Correlation	1	-.119**
	Sig. (2-tailed)		.000
	N	1007	1007
ABC	Pearson Correlation	-.119**	1
	Sig. (2-tailed)	.000	
	N	1007	1007
**Correlation is significant at the 0.01 level (2-tailed).			

Table-3: Correlation between ABC (absolute basophil count) and thrombocytopenia

DISCUSSION

CBCs of 1007 dengue patients who were confirmed NS1 positive were analyzed which showed significant findings like rise in Hb, Hct, ABC and decrease in white cell count and platelet count. Along with these parameters, "basophilia" flags generated by automated hematology analyzer Mindray BC6800 were analyzed.

A higher hemoglobin and hematocrit level in dengue is due to plasma leakage caused by increased vascular permeability with the highest values seen on day 7.[10] A study by Martina et al, showed that plasma leakage is due to the cross reactivity of inflammatory cytokines and anti-NS1 antibodies with the surface proteins on the endothelial cells which result in the apoptosis of these endothelial cells.[11]

Basophilia is generally seen in conditions like infections, inflammation and also in myeloid neoplasms.[12] However in dengue patients basophilia flag suggests presence of atypical or reactive lymphocytes owing to the infective process. Hence, the instrument gives falsely raised basophil count; that's why the term pseudobasophilia is used. To confirm this finding, peripheral smear should be prepared and examined carefully. In dengue patients, basophilia flag gives a hint of infection which can be further confirmed on peripheral smear where basophilic reactive lymphocytes can be appreciated.[13] Our study showed 136 dengue samples with flagging "basophilia" but when peripheral smears were prepared from these samples, there was no increase in basophils (hence the term pseudobasophilia). However, reactive lymphocytes with basophilic cytoplasm were seen on the smears which were falsely counted as basophils by automated hematology analyzer.

In another study from India, basophilia > 2% was seen in 52.9% of dengue patients.[14] In a study from Thailand, basophil count was not found to be elevated.[10] In another study in a different endemic area, dengue was responsible for 91.2% of cases with pseudobasophilia and thrombocytopenia on the Sysmex XE-2100. However, peripheral smear examination of basophilia flags revealed reactive/atypical lymphocytes.[15] This wide variation with the basophil counts could be due to the day of fever when the sample was collected, the duration of sample standing time, and the reagents used. Studies have also shown that there is a poor concordance between analyzers regarding the basophil count.[8,16] Pseudobasophilia in dengue is an underreported phenomenon which was observed more frequently in cases with "atypical lymphocytes" and "blasts" flags.[17] Our study also shows similar results with high frequency of basophilia along with atypical lymphocytes flag on Mindray BC6800. Increased ABC also showed correlation with severity of thrombocytopenia i.e with increasing absolute basophil count there was a marked decrease in platelet count.

CONCLUSION

Pseudobasophilia can be used as a helpful tool in the diagnosis of dengue in a resource restricted country like Pakistan where serological confirmation of the disease is costly and not easily available across the country. Also early detection of severe thrombocytopenia by using basophil flag in dengue patients can prevent them from bleeding complications.

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Is *Coriandrum sativum* Hypolipidemic in Alloxan Induced Diabetic Rats?

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ABSTRACT

Introduction: Diabetes mellitus is a widely recognized metabolic condition. Due to various side effects of currently available medicines, studies have reported the use of many plants in various areas for the traditional management of diabetes.
Aims & Objectives: To determine the effects of *Coriandum sativum* seeds on lipid profile in diabetic rats.
Place and duration of study: This experimental research was conducted at Physiology Department, Services Institute of Medical Sciences (SIMS), Lahore from August 2013 to January, 2014.
Material & Methods: Ninety rats were randomly distributed into three groups of (n=30) each. Group A was healthy control, group B was disease control and group C was diabetic experimental which was treated with *Coriandrum sativum* with a single dosage of 250 mg/kg of body weight/day through a gavage tube for 28 days. On next day, 4-5ml blood was collected through heart from each rat. The markers evaluated were high density lipoprotein (HDL), triglycerides, total cholesterol, low density lipoprotein (LDL) and very low density lipoprotein (VLDL). Data was analyzed by using SPSS version16 , p value <0.05 was considered significant.
Results: The experimental group showed highly significant (p=0.000) lower levels of serum LDL (25.47±3.90mg/dl), serum cholesterol (100.33±2.81 mg/dl), serum triglyceride (96.97±4.79 mg/dl) and serum VLDL (19.40±0.97 mg/dl) as compared to healthy control and disease control group. The level of serum high density lipoprotein revealed (55.27±3.69 mg/dl) highly significant rise (p=0.000).
Conclusion: Oral administration of *Coriandrum sativum* has revealed the lipid lowering impacts in alloxan induced diabetic rats.

Key words: *Coriandrum sativum,* diabetes mellitus, alloxan, serum triglycerides.

INTRODUCTION

Diabetes mellitus is the most widely recognized metabolic condition which is linked with deranged lipid metabolism and lipoproteins. Over 90% of patients with type 2 DM had one or more types of dyslipidemia[1]. Type 2 diabetes (T2D) is an eminent element of danger for coronary artery disease. Prompt detection and treatment of hyperlipidemia in diabetic patients cuts the risk for cardiovascular and cerebrovascular diseases. Lifestyle modification such as diet and regular exercise are very significant in improving diabetic dyslipidemia.[2]

Due to various side effects of currently available medicines, the consumption of different herbs as medication has been reported in the traditional treatment of many disorders. World Health Organization (WHO) revealed that 80% populace of underdeveloped nations such as Africa and Asia has been using herbal medicine for primary care.[3] Studies have reported the use of many plants in various areas of the world for the traditional management of diabetes.

Coriandrum sativum L. is probably originated from Eastern Mediterranean belongs to apiaceae, umbelliferae family, otherwise called coriander, cilantro, Arab parsley, Chinese parsley and dhania. It is usually utilized in cooking.[4]

The phytochemical screening of different *Coriandrum sativum* components has revealed a substantial amount of phyto constituents, such as, essential oil, terpenoids, reducing sugar, alkaloids, flavonoids, fatty acids, and sterols. *Coriandrum sativum* is generally consumed as remedy, such as, in

the management of gut problems, respiratory diseases, anxiety, insomnia, headache and dizziness.[5] Its all extracts have high total phenolic contents like caffeic acid, glycitin and pyrgallol[6].The hypolipidemic,[7] hypoglycemic,[8] antioxidant,[6] anti-anxiolytic[9] and analgesic effects of coriander seeds have been investigated.

This study was conducted in order to investigate the effects of coriandrum sativum on lipid parameters in diabetic rats as varying data exists on this important subject.

MATERIAL AND METHODS

An experimental research was done at Physiology Department, Services Institute of Medical Sciences (SIMS), Lahore from August 2013 to January, 2014. This research project was approved by Research Evaluation Unit (CPSP/PHY/2011/060/003-E, 14 November, 2012). Ninety male albino rats (150-200gm weight) were selected according to following criteria.

Inclusion criteria: Healthy male albino rats.

Exclusion criteria: Rats which did not become diabetic after administration of alloxan.

Out of ninety rats, sixty rats selected and were given intraperitoneal injection of alloxan monohydrate (120mg/kg) to make them diabetic. Rats with blood glucose > 200 mg /dl were considered as diabetic and included in the experiment.[10] After being diabetic, these 60 rats were indiscriminately distributed into two groups (Group=B & C) of 30 rats each and the remainder serving as non- diabetic healthy control rats.

Group A: Healthy control, on normal pellet diet

Group B: Disease control, on normal pellet diet

Group C: Diabetic rats, treated with ethanolic extracts of seeds of C*oriandrum sativum*.

Treatment of rats of group C was started with ethanolic extract of seeds of *Coriandrum sativum* daily with single dosage of 250 mg/kg of body weight/day through a gavage tube for 28 days. On 29thday, a few ml of blood was collected through heart from each one. The Parameters measured were total cholesterol (through CHOD-PAP enzymatic colorimetric method), serum triglyceride (by GPO-PAP enzymatic colorimetric process), serum HDL-C (by Precipitation method) and serum LDL –C (by using Friedewald formula, LDL=TC- (HDL+TG/5).

Statistical Analysis:

Data was analyzed by using SPSS version16. One way ANOVA test were used to determine the statistical significance of difference of different markers (Serum HDL, triglycerides, cholesterol, LDL, VLDL) among the three groups. A p-value of ≤0.5 considered significant.

RESULTS

The difference of serum lipid profile parameters between the Healthy control, disease control and experimental groups was highly significant (p=0.000) (Fig-1).

By giving the 28 days supplementation of *Coriandrum sativum* to the rats of diabetic (experimental) group, all the lipid parameters including triglycerides, cholesterol, LDL and VLDL except serum HDL, showed highly significant lower levels (p=0.000) in comparison to healthy and disease control. Whereas serum HDL had a highly significant (p=0.000) higher levels in experimental group.

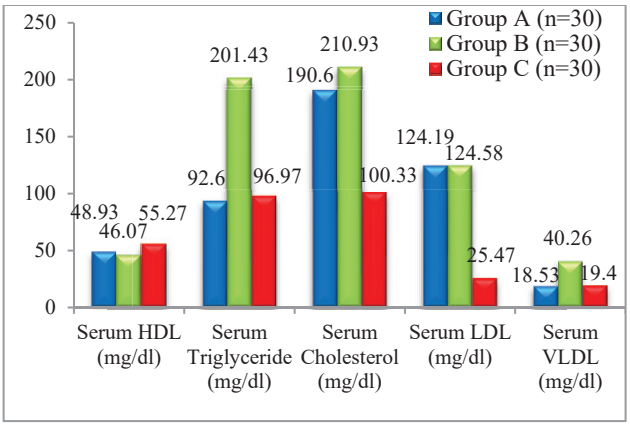

Fig-1: Comparison of serum lipid profile among healthy control, disease control and experimental groups (one way ANOVA)

Group A: Healthy control, on normal pellet diet

Group B: Disease control, on normal pellet diet

Group C: Diabetic rats, treated with ethanolic extracts of seeds of *Coriandrum sativum*

DISCUSSION

In our study the consequence of ethanolic concentrate of C*oriandrum sativum* was measured on rat lipid profile. The lipid profile (serum triglycerides, cholesterol, LDL, and VLDL) of experimental group decreased highly significantly in comparison to health and disease control. Whereas serum HDL had a highly significant (p=0.000) higher levels in experimental group. Our findings concurred with some researchers and contradicted others. Das et al conducted a study on Streptozotocin (STZ) induced diabetic rats. The hypolipidemic activity of *Coriandrum sativum* seed extract was compared to the standard drug metformin. They concluded that

oral administration of CS seed extracts significantly lowered total cholesterol (TC), LDL: HDL ratio, TC: HDL ratio, so, decreasing the cardiovascular risk.[7]

Our results were also similar to the findings of study of Vijaya Durga et al, who used a combination of aqueous extract of *Coriandrum sativum* and ginger to wister rats and reported a significant reduction in lipids parameters but rise in serum HDL[11]. Previously, hypolipidemic effects of methanolic, etheric,[4] aqueous,[11] ethanolic[12] extract of *Coriandrum sativum* fixed oils and essential oils have been reported.[5]

Contradictory results to our study were reported in a South Africa to investigate the effect of feeding dietary coriander seeds on growth performance, hepatic and visceral adipose tissue lipid storage and circulating metabolic substrates in healthy growing female rats. They divided the rats in two groups in which one group was fed with normal diet and the experimental group was supplemented with 500 mg kg-1 day-1 of whole, crushed coriander seeds for five weeks. They found no difference in the levels of blood triglycerides and plasma free fatty acids in both groups and concluded that dietary coriander seeds had no effect on growth performance, plasma lipids and blood glucose.[13]

A study was conducted in Iran to observe the lipid lowering effects of *Coriandrum sativum* extract and endurance training in diabetic rats. It concluded that endurance training increased the lipid lowering effects of *Coriandrum sativum* in diabetic rats.[14]

Zeb et al in 2018 concluded by comparing the effects of garlic and coriander that garlic has highest influenced on BMI, TC, LDL and HDL than coriander.[15]

CONCLUSION

Oral administration of *Coriandrum sativum* has the lipid lowering effects in rats which were made diabetic with alloxan. Coriandrum sativum possibly have substantial beneficial effects as a hypolipidemic drugs and may be recommended as a significant ingredient in nutrition.

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Comparative Study of Déjà-Vu and Associated Attributes Among Epileptics and Non-Epileptics

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ABSTRACT

Introduction: Déjà vu is a feeling of familiarity experienced when one undergoes certain events when in reality it is unknown. Déjà vu in epilepsy has shown to occur frequently, last somewhat longer and associated with fatigue, hyperactivity, exhaustion, headaches, blackouts or fear.
Aims & Objectives: To determine any difference in déjà vu and its parameters among epileptic and normal healthy individuals.
Place and duration of study: The study was conducted at Central Park Medical College Lahore, Pakistan December 2019 to March 2020.
Material & Methods: A cross-sectional study was conducted to determine the frequency of the phenomenon of déjà vu between epileptics and non-epileptics. The assessment was done by IDEA questionnaires comprising of 23 items assessing quantitative and qualitative aspects of déjà vu with some additional demographic questions such as age, gender, parental consanguinity, drugs, cause of epilepsy, and fitness status. Data was analyzed using SPSS version 23, p value <0.05 was considered significant.
Results: The group difference was present about perceiving of the feeling of recognition between epileptics (2.00 $\pm$ 2) and non-epileptics (2.00 $\pm$ 3) with a p-value of 0.037. No significant difference was observed on any other parameter.
Conclusion: The study shows no difference in the prevalence of déjà vu and its associated parameters among both groups. The results have limitations due to sample size, time and resources.

Key words: Epilepsy, Déjà vu, Neurologist, Psychiatrist, Memory

INTRODUCTION

Déjà vu is a feeling of familiarity experienced when one undergoes certain events when in reality it is unknown.[1] Recollection involves the exact nature of the recalled experience and a variety of neuro-cognitive processing depending on the situation in which it is produced.[2] People encounter this phenomenon Déjà vu describe the event as though the present moment has happened already or maybe they had already been a part of such a situation with or without voices. But the fact behind it is that there is an illusion of recognition.[3] Olfactory cortex and auditory cortex is also active during recollection alongside the flashbacks which include both visual and auditory illusions and hallucinations.[4,5]
Throughout the world 60%-80% of people experience the feeling of recognition at least once in their lifetime.[6,8] The cause of the Déjà vu is unknown but it occurs due to our unconscious memory being ahead of our conscious memory and this lack of coordination even as brief as a few milliseconds yield this effect.[9,10] It has been proved that it is related to the involvement of brain structures that have a role in memory such as the amygdala, the hippocampus, parahippocampal gyrus, perirhinal cortex, entorhinal cortices, temporal and the prefrontal cortex.[11,13] Déjà vu might be a result of the manifestation of neural imbalance which may be narrated as physiological as there are no harmful effects observed on the body.[14] Surprisingly, Déjà vu is also reported in the form of activity in some medial temporal regions which may accompany seizure activity.[6]
Déjà vu is thought to be an indication of many psychiatric disorders[15] e.g. schizophrenia etc. The neurological disorder epilepsy is a set of abnormalities of the central nervous system in which activity of the brain is affected causing seizures, amnesia, mood swings, anxiety, and fatigue. The patients are more prone to fatigue, delusions, and mental health issues.[13] Hallucinations, delusions and illusions are symptoms of localized or network-based neuronal spike.[16] Déjà vu in epilepsy has been shown to occur frequently which last longer, associated with prior fatigue, hyperactivity, exhaustion, headaches, blackouts and fear than physiological déjà vu.[14] A widely cited cause of Déjà vu experiences is an

epileptic seizure. That is therefore termed as psychic symptoms associated with complex partial seizures[17] in previous literature and to assess the frequency of this phenomenon and its consequences and comparison between epileptic and non-epileptic, a cross-sectional study is conducted in local populous.

MATERIAL AND METHODS

The study was conducted at Central Park Medical College Lahore, Pakistan in collaboration with the University of Hafr Al Batin, Kingdom of Saudi Arabia from December 2019 to March 2020. Informed written consents were obtained in advance from all the volunteers (both epileptics and non-epileptics) and ethical approval (CPMC/IRB-Number-1743) was obtained from the Institutional Review Board of Central Park Medical College, Lahore via formal request (CPMC/ME/2020-1066). A total of 133 volunteers participated in this research work who were divided into two groups; Group 1 epileptics (n=64) and Group 2 non-epileptics (n=69) based on pre diagnosed epilepsy whose age was between 15 years to 40 years. Epileptics whose epilepsy was due to neurosurgery or spinal injuries were excluded from the study. The questionnaires were employed in the English language as all the participants were well versed in English and the questionnaires were filled in the presence of a medical researcher to avoid any ambiguity. The questionnaire consisted of two sections; Section 1 of demographic and basic information about type and nature of epilepsy along with the basic information such as age, gender, parental consanguinity, drugs use and abuse, cause of their epilepsy, and fitness status, while Section 2 was comprised of Inventory Déjà Vu Experiences Assessment (IDEA)[14] to evaluate Déjà vu.

For the assessment of Déjà vu (DV) Inventory Déjà Vu Experiences Assessment (IDEA)[9] was employed to evaluate DV both qualitatively and quantitatively. IDEA is 23 items questionnaire which is sub-grouped into two sections: Section 1 comprised of 9 questions to quantify and assess the prevalence of Déjà vu while Section 2 comprised of 14 questions to evaluate the qualitative nature of Déjà vu. IDEA involves the study and evaluation of psychological experiences i.e., DV, serialization, paranormal quality, remembering dreams, travel frequency, daydreams. According to the IDEA, if subjects mark "Don't know," it will be considered as "never" with the frequency of zero and they would not continue to Section 2. On the whole, it assesses all the para and supranormal phenomena that may or may not lead to Déjà vu and all those 23 parameters of IDEA were based and assessed Likert scale ranging 0 to 10.

Statistical analysis:

Data was entered in SPSS ver. 23 (USA Chicago) and was assessed for errors and omissions. Descriptive and demographic data were presented in frequencies and percentages and were also presented in charts and graphs where data was described with Median $\pm$ IQR. Mean Whitney U test was employed to assess the group differences for the frequency and severity of Déjà vu between Group 1 and Group 2. Spearman correlation was employed to assess correlation for Déjà vu prevalence and associated factors. A p-value $<.05$ was considered significant.

RESULTS

A total of 133 participants participated in the study who were segregated into two groups; Group 1 epileptics (n= 64) and Group 2 non-epileptics (n=69) with the overall mean age of 23.00 ± 5.98 with the age range of 15 to 40 years. In Group 1 the mean age was 25.86 ± 6.305 and in Group 2 the mean age was 19.95 ± 2.368 years. Among epileptics cause of epilepsy was assessed and depicted in Fig-1. Out of 64 epileptic patients, 16 have congenital epilepsy while 28 acquired it later in the life and among 8 patients the cause was idiopathic and rest of the 12 patients had other causes and reasons of onset of epilepsy. In Group 1 (epileptics), there were 26 male and 38 females while in group 2 (non-epileptics) there was 40 males and 29 females. On application of Spearman correlation, a positive correlation of female gender and epilepsy was observed with an R-value of 0.173 and p-value of 0.046 suggesting a higher prevalence of epilepsy in females than males. A significant mean difference was observed for the family history of epilepsy between Groups 1 and 2 with a mean difference of -1.02 with a p-value of .001 suggestive of family history may contribute to the onset of epilepsy.

On application of Mann Whitney U test between epileptics (Group 1) and non-epileptics (Group 2) no significant difference was observed on major parameters of Déjà vu as explained in Table-1. Only difference was observed with the question about traveling between group 1 (2.00 ± 1) and group 2 (3.00 ± 1) with a z value of -.584 and p-value of .006 indicating epileptics usually travel more that lead to contributing factor in the onset of Déjà vu among epileptics. Group difference was also noticed regarding perception of feeling of recognition between Group 1 (2.00 ± 2) and Group 2 (2.00 ± 3)

with a z value of -2.082 and p-value of .037 and besides these no significant difference was observed.

Déjà Vu Parameters	Median ± IQR		P-value
	Group 1 (Epileptics)	Group 2 (Non-Epileptics)	
Have you ever felt that having experienced sensation in same way or experienced the same thing before?	3.00 ± 1	3.00 ± 1	.901
Have you ever felt that everything in your life seemed to be going unreal?	3.00 ± 1	2.00 ± 2	.440
Have you ever felt like you had never met anything before and in fact you had experienced it before?	2.00 ± 2	2.00 ± 2	.707
Has it once happened to you that you had experienced something that had happened before in a dream?	3.00 ± 4	3.00 ± 2	.945
Have you ever felt that something didn't happen to you but to someone you are watching at?	2.00 ± 3	2.00 ± 2	.850
Do you experiment any paranormal activities?	2.00 ± 2	2.00 ± 2	.643
How often do you remember a dream that you would tell someone about?	3.00 ± 2	4.00 ± 2	.237
How many times a year do you travel from your neighborhood?	2.00 ± 1	3.00 ± 1	.006*
Has it ever happened to you that you were daydreaming?	4.00 ± 3	3.00 ± 3	.547
Have you ever had this feeling of "recognition"?	11.00 ± 11	11.00 ± 0	.368
Have you ever experienced feeling of recognition in same way before?	2.00 ± 1	2.00 ± 3	.554
When did you experience it last time?	5.00 ± 3	4.00 ± 3	.212
What is the duration of this feeling of "recognition"?	3.00 ± 5	3.00 ± 1	.093
Sensation of recognition is associated with particular experience, things or events?	3.00 ± 1	3.00 ± 1	.173
Have you ever had the feeling of being "recognized" at a certain time of the day?	1.00 ± 2	1.00 ± 3	.790
Can you predict what's going next when you experience this phenomenon?	2.00 ± 2	2.00 ± 3	.037*
When you were experiencing this, have you felt that you are watching yourself?	2.00 ± 3	2.00 ± 3	.247
When you perceive similarity of a current event to a known event, how much similar is it?	3.50 ± 3	3.00 ± 3	.781
Do you feel it's unreal when you experience the sense of 'recognition'?	2.00 ± 2	2.00 ± 3	.734
In general, how does this feeling of "recognition" affect you?	5.00 ± 7	6.00 ± 7	.320
What do you think is the meaning of these "known" feelings?	5.00 ± 5	4.00 ± 4	.531
What you feel before having this feeling of being "known"?	6.00 ± 9	6.00 ± 4	.625
Have you experience the sense of being 'known' in these situations?	3.00 ± 2	4.00 ± 3	.696

Table-1: Indicating Group Differences in Déjà Vu on the appliance of Mann Whitney U test.

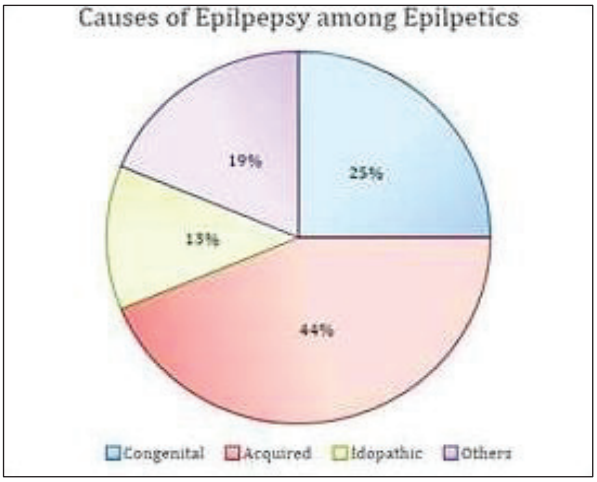

Fig-1: Elaborating the causes of Epilepsy among Epileptic Volunteers.

DISCUSSION

Analysis of research literature on the experience of déjà vu showed that the only difference between the epileptic and non-epileptic groups was the frequency of experiencing déjà vu. Our analysis showed no significant difference between the prevalence of déjà vu among them, which shows a contradiction with previous literature. According to Adachi et al.[7] the frequency in epileptic patients was relatively low whereas Warren et al.[14] explain that the difference is only because of the frequency of déjà vu in both the groups.

By the Qualitative analysis, we found out the parameter showing the association of Déjà vu with traveling is significantly high in Epileptic than in the control group, that is similar to recent Labate et al.[6] study which also concluded the frequency of travelling was mostly correlated to DV in Epilepsy suggesting that the network comprise of visual-memory may be involved in epileptic DV.[18] Hence Pakistan is a developing country, people have to travel from rural to urban areas for the proper medications and availability of necessities of life so if epileptic patients have to travel a lot for their medical needs,[19] they will surely experience the déjà vu more frequently.

Quantitative analysis of people experiencing déjà vu are mostly females but most of the recent studies contradicts this and stated that Déjà vu is not a gender-based problem rather it depends on age.[20] So, due to lack of any previous authentication we deduced from our research that most females experience déjà vu due to their emotionally imaginative and empathic nature or might be it is due to the ethnic differences among the population of our studies with recent studies,[21] whereas we cannot be sure if it is a gender-based problem due to the less

male patients in our research. So this will remain an open question that Deja vu is a gender based phenomenon or not.

Despite the facts about the frequency of déjà vu we also found out the ratio of different causes leading to epilepsy (Fig-1) in which head and spinal injuries with trauma were highest ratio parameters causing epilepsy in patients in Pakistan which might be due to the worse conditions of emergency and intensive care units in the country.[22] Another major cause of epilepsy in Pakistan is congenital due to genetic predisposition in the family history which is likely to happen due to the high rate of consanguineous marriages in Pakistan which is around 70% of total population.[23] Another reason for epilepsy is the patient's lack of awareness and education about mental health problems as people do not know about their symptoms and conditions because of their superstitious beliefs and societal reputation.[24]

Our study was limited by the small sample size due to restrictions of COVID19, thus, these results may not be generalized to the whole population. Apart from the pandemic, we could also not expand our study due to problems related to lack of resources and logistics.

CONCLUSION

The frequency of déjà vu among both epileptic and non-epileptic is the same. Déjà vu is mostly experienced by females and people who travel a lot.

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Nigella sativa Seeds Protective Ability in Pyrazinamide Induced Hyperuricemia in Mice

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ABSTRACT

Introduction: Hyperuricemia results in an increased level of blood uric acid, a prerequisite of gout. Commonly prescribed agents for the treatment of hyperuricemia include allopurinol, febuxostat, and probenecid. Multiple adverse effects like hypersensitivity, gastrointestinal upsets and hepatotoxicity limit their use.
Aims & Objectives: To evaluate the serum uric acid lowering effects of *Nigella sativa* seeds on pyrazinamide induced hyperuricemia in mice.
Place and duration of study: This study was carried out at research facility for animals of NIH, Islamabad and Pharmacology Department of Islamic International Medical College, Rawalpindi from April to June, 2017.
Material & Methods: Sixty-eight male mice (Swiss Albino) were separated into four groups. Group A mice were labelled as negative control and mice in this group were given chow & glucose water. Group B mice received 500mg/kg Pyrazinamide (PZA) added in glucose water once daily. Group C mice were given low dose *Nigella sativa* seeds powder in a dose of 500 mg/kg suspended in the glucose water accompanied by PZA in a dose of 500mg/kg. The mice in group D received high dose of *Nigella Sativa* seeds powder 1000 mg/kg suspended in glucose water along with 500mg/kg of PZA. All the doses of Pyrazinamide and *Nigella sativa* seeds suspension were given orally for six weeks. Blood sample was collected three times from each group. On day 0, sample from two mice from each group was taken for baseline uric acid levels and of five mice from all groups in mid of study to check uric acid levels. On 42nd day, the blood from remaining 10 mice in each group was taken to check the serum uric acid levels. Analysis of data was done using Graph Pad Prism Version 8, p value <0.05 was considered significant.
Results: Acute pyrazinamide administration caused a rise in uric acid levels in group B as compared to group A (from 5.94±1.94 to 28.03±15.52 mg/dl). The *Nigella sativa* seed powder extract suspended in glucose water in a dose of 500mg/kg and 1000mg/kg reduced the rise in uric acid levels in pyrazinamide treated group C and D (10.47±3.32 mg/dl & 7.53±1.78 mg/dl).
Conclusion: *Nigella sativa* possesses antihyperuricemic effect and showed a significant reduction in serum uric acid levels in a dose of 500mg and 1000 mg/kg.

Key words: *Nigella sativa,* serum uric acid (SUA), gout.

INTRODUCTION

The term hyperuricemia refers to clinical disorder in which there is an abnormal increase in the uric acid inside blood. In epidemiologic investigations, the cut of level of blood uric acid level in women is 6 mg/dl & about 7 mg/dl in men, Whereas an accepted reference level of 6.8 to 7.0 mg/dl depicts a theoretically soluble concentration of uric acid inside biological fluids.[1] The estrogen lowers uric acid level which protects premenopausal females from gout, that's why it makes hyperuricemia more common in males than in females.[2]

Hyperuricemia is a common problem due to several reasons including drugs. Many medications raise uric acid concentrations including diuretics, antitubercular drugs, calcinuerin inhibitors, anti-neoplastic and immunosuppressants. Hyperuricemia secondary to drugs can result from a decrease in the excretion or an increase in the production of uric acid.[3]

Uric acid is a heterocyclic organic compound with low solubility in water and plasma, whereas albumin is its main transporter.[4] It is an oxidative end derivative of purines. Xanthine oxidase catalyzes oxidation of hypoxanthine into xanthine and then

xanthine into the uric acid. Kidneys are the chief organs responsible for uric acid homeostasis. The amount of uric acid excretion is regulated by kidney via glomerular secretion and reabsorption.

Th net increase in either the production or decreased in the excretion of uric acid results in hyperuricemia. It is more prevalent in individuals who consume seafood, alcohol, and sweet beverages. Furthermore, drugs like pyrazinamide, ethambutol,[5] chlorothiazide, Ethacrynic acid, and salicylate can also elevate serum uric acid. Hyperuricemia is a common presentation in patients taking pyrazinamide with an incidence varying between 43.3 to 86.3%. Pyrazinamide induced hyperuricemia can result in acute gouty arthritis as well as moderate arthralgia. The hyperuricemic effect of pyrazinamide is attributed to its active metabolite pyrazinoic acid which decreases renal clearance of uric acid. It is reported to have trans-stimulatory effect on URAT1 causing reabsorption of uric acid from the luminal side into tubular cells.[6]

The deposition of urate crystals in the joint cavities results in gouty arthritis. The protein (animal source) is metabolized into oxalate & urate. The uric acid is responsible for the nucleation of calcium oxalate salts and that's why elevated levels of uric acid cause of formation of renal stones.[7]

Uric acid induces glomerular injury, tubule interstitial fibrosis and is responsible for causing metabolic syndrome.[8] Commonly prescribed drugs for the treatment of hyperuricemia are inhibitors of enzyme xanthine oxidase e.g., allopurinol & febuxostat whereas probenecid and benzbromarone reduce serum uric acid via their uricosuric mechanisms. Allopurinol causes diarrhea and severe cutaneous reaction[9] while febuxostat induces arthralgia and deranged liver enzymes.

The relative lack of drugs having antihyperuricemic effects, research work is in undergoing on classical medicinal plants. Flavonoids present in plants extracts possess xanthine oxidase inhibitory activity which is of great interest from biological research point. The xanthine oxidase enzyme inhibition is evident by plants such as *Cinnamomum cassia, Artemisia vulgaris, Onion* extracts*, H. lantanaefolia, Caesalpinia sappan, Lycopus europaeus* and *Allium Cepa*.[11]

Nigella sativa has been used for the treatment of different clinical disorders. The seeds and oil of *Nigella sativa* are frequently prescribed for treatment of many diseases such as acute and chronic cough, fever, extreme nasal congestion, chronic asthma, long standing diabetes mellitus, hypertension, severe eczema and inflammation, dizziness and gastrointestinal issues.[12] *Nigella sativa* has many pharmacological functions including antioxidant, anti-inflammatory, anticancer, antimicrobial, hypoglycemic, hypolipidemic, hepato-protective and spasmolytic.[13]

The therapeutic effects of *N. sativa* are considered to be due to the ingredient thymoquinone, a major active phytochemical present in oil.[14] Other constituents of *N. sativa* seed are natural carbohydrates and proteins along with the essential and fixed oil, alkaloids, sterols and saponins, crude fiber and organic acids, vitamins & minerals.[15]

The aim of this study is to observe the serum uric acid lowering effect of *Nigella sativa* seed powder suspension on pyrazinamide induced hyperuricemia in mice.

MATERIAL AND METHODS

A randomized controlled study was conducted for 3months (from April to June, 2017). The approval was given by Ethical Committee of faculty of Health and Medical Sciences, Riphah International University; Letter. No. Ripah/IRC/15/0116, Dated: April16, 2015. This study has been carried out at the animal house of NIH, Islamabad and Pharmacology Department of Islamic International Medical College, Rawalpindi. Blood sample evaluation was done at multi disciplinary research Lab of same college. Sixty-eight mice were divided into 4 groups containing seventeen in each group via non-probability technique.

Animals used: White male albino mice of 2 months age, having 25-35 grams weight were taken for study. Mice were kept under standard required conditions i.e., humidity 40-60%, temperature 20 ±20 C, & 12 hour light and dark cycle along with water and food *ad libitum.* Mice were acclimatized for 1 week before any intervention.

Chemicals:

Research grade salt of PZA was obtained from Pfizer Pharmaceuticals. *Nigella sativa* seeds were bought and certified by National Agriculture Research Centre (NARC), Islamabad. Electrical grinder was used to convert *Nigella sativa* seeds into fine powder. Glucose water was then added into powder to form Suspension. 1 gram *NS (Nigella sativa)* powder was added to 5ml of glucose water.

Preparation of pyrazinamide dosage form:

The 500mg PZA was mixed and suspended into 5ml of water containing glucose to get a homogenized solution. The PZA dose was calculated as per the body weight of mice.

Animal Groups:

Following 4 groups were formed. Chow and drugs were administered through oral gavage for 6weeks:

Group A: Animal in control group were given chow & glucose water

Group B: Mice of group B received 500mg/kg PZA added in glucose water per oral OD.[16]

Group C: Group C mice were given low dose *Nigella sativa* seeds powder in a dose of 500 mg/kg of *Nigella sativa* seeds powder dissolved in the glucose water along with 500mg/kg of Pyrazinamide (PZA)

Group D: This group was given a high dose of *Nigella sativa* seeds powder in a dose of 1000 mg/kg of *Nigella sativa* seeds powder dissolved in the glucose water along with 500mg/kg of Pyrazinamide (PZA).[16]

Blood samples collection:

Blood sample was collected three times from each group. On day 0, sample from two mice from each group was taken for baseline uric acid levels & of five mice from all groups on day 21of study for evaluating the changes. On 42nd day, remaining mice (10 in each group) were anaesthetized with chloroform and cardiac puncture was done to take a blood sample that was subjected later to the serum uric acid analysis Alinity Uric Acid Reagent Kit by Abbott was used for this purpose.

Statistical analysis:

Analysis of data was done using Graph Pad Prism Version 8. Quantitative data was given as Mean ± SD. The multiple comparisons among groups were done via the *Post hoc* Tukey test. The *p*-value of <0.05 was taken as research significant.

RESULTS

The mean uric acid levels ± standard deviation of all the groups on day 0, 21 and 42 are given in Table-1. The comparison (means values of all research groups) via ANOVA yielded a significant difference of means between groups with *p* value of <0.001 (Fig-1).

Serum Uric Acid levels						
Groups	Day 0		Day 21		Day 42	
	Mean	SD	Mean	SD	Mean	SD
Group A Negative Control	5.94	1.94	4.85	2.33	7.06	2.4
Group B Disease Control	28.03	15.52	7.4	0.84	13.16	6.03
Group C Experimental 500 mg/kg	10.4	3.32	4.2	1.76	8.98	4.39
Group D Experimental 1000mg/kg	7.5	1.78	2.55	0.77	5.04	2.39

Table-1: Serum uric acid values on day 0 (n=2), day 21 (n=5) and day 42 (n=10)

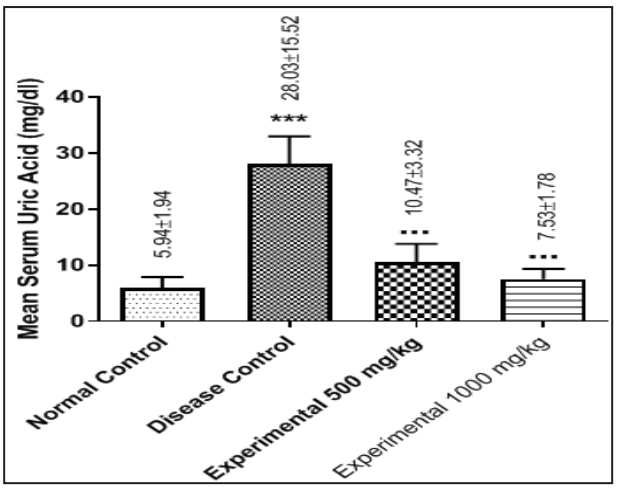

Fig-1: Effect of Pyrazinamide and Pyrazinamide plus 2 different doses of Nigella sativa seeds powder on uric acid levels (Mean ±SD) in mice (n=10) on day 42.
***=*p*-value <0.001 (vs Group A, the Normal Control)
… = *p*-value <0.001 (vs Group B, the Disease Control)

The serum uric acid level difference in between groups was evaluated via the *post hoc* Tukey's test. The disease control group had higher levels of serum uric acid as compared to the normal control, whereas the experimental groups had markedly significant lower levels of serum uric acid as compared to the disease control group (Table-1).

Both the experimental groups had high serum uric acid levels as compared to normal control which was statistically non-significant. The difference among 2 doses of Nigella sativa seed powder was statistically non-significant.

DISCUSSION

Hyperuricemia is present in up to 18% of the general population and is predominant in males than in females.[17] The uric acid is final product of the purine nucleotide catabolism[18] and hyperuricemia occurs from either increase in production or decrease in excretion of uric acid. Allopurinol remains to be the dominant xanthine oxidase inhibitor available to treat hyperuricemia, but has limited utilization due to related adverse effects.[19] Other uricosuric agents like probenecid & sulfinpyrazone are nephrotoxic. The benzbromarone use is associated with severe fulminant liver toxicity.[20] Thus, the quest for uric acid lowering agent is highly necessary.

Phenolic compounds in fruit of *Nigella sativa* are present in dried fruit and it is very good source of flavonoids which possesses excellent antioxidant properties.[21]

Pyrazinamide treatment caused a twofold increase in the levels of serum/blood uric acid. On the 42nd day, serum concentration of uric acid significantly

declined (p-value < 0.001) in both experimental groups as compared to the positive control. Similar studies representing the serum uric acid lowering effect of *Nigella sativa* are not available, that's why research studies performed on other medicinal herbs were considered as reference to compare the results of current study e.g., the result of current research are comparable to the work accomplished by Haideri et al[22] and the researchers determined that the onion juice reduced uric acid concentration significantly with a $p$value < 0.001 in hyperuricemic rats both in time & dose-dependent manner. Similarly, the current results are in consensus with the effects of the crude flavonoid compound of *Zingiber officinale*[23] and the essential oils of the leaves of the *Cinnamomum Osmopholieum*[24] which significantly suppressed the high uric acid concentration in the hyperuricemic rats in the dose dependent manner only ($p$value < 0.001).

The proposed mechanism of hyperuricemia caused by pyrazinamide is due to its strong urateretention ability.[25] Pyrazinamide also increases serum uric acid by the trans stimulatory effect on URAT1causing the reabsorption of uric acid from luminal side to tubular cells.[26] Pyrazinamide also inhibits the OAT2 (a protein present in basolateral membrane of PCT (proximal tubule cell) and is responsible for the secretory transport of urate).[27] OAT2 is a possible target of antiuricosuric effects of the pyrazinamide as well as the URAT1.

Nigella sativa contain some important compounds that help to improve renal health as phenolic compounds, flavonoids, minerals, and vitamins diminish uric acid levels and keep a safe kidney from damage, the mechanism underlying this effect is probably their molecular structure. These antioxidants act as direct superoxide scavengers & xanthine oxidase inhibitors, resulting in the suppression of Reactive Oxygen Species (ROS) and uric acid formation.[28] Thus, *Nigella sativa* reduces the uric acid formation and maintains normal levels of uric acid whenever PZA interferes the uric acid levels.

The greatest strength of this study is that it is natural and cost-effective way to keep uric acid levels in normal range in patients taking PZA. The limiting factors of this study is the lack of parallel comparison with the similar studies. Additionally, no control group of known antihyperuricemic drug was taken into account to compare the significance of antihyperuricemic effect of Nigella sativa

In this study, two different doses of *Nigella sativa* were selected to demonstrate its hypouricemic effect. The study results revealed that *Nigella sativa* seed powder significantly lowered serum uric acid level in a dose of 500mg/kg and 1000mg/ kg.

CONCLUSION

Nigella sativa seed powder has the potential to lower serum uric acid levels and it produced a significant anti hyperuricemic effect in a dose of 500mg/kg and 1000mg/kg.

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Therapeutic Effect of Berberine Versus Methotrexate on Histopathology in a Rat Model of Pristane-Induced Arthritis

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ABSTRACT

Introduction: Treatment options for rheumatoid arthritis have potentially fatal adverse effects and failure to achieve complete cure. Alternative medicines are, therefore, being researched for this purpose. Berberine is one of such compounds with high antioxidant activity that may prove beneficial in this disease.
Aims & Objectives: To compare effects of berberine with methotrexate on pristane induced arthritis in rats.
Place and duration of study: Post Graduate Medical Institute, Lahore; March to May 2014.
Material & Methods: Forty female Sprague Dawley rats were allotted to five groups including a berberine control. Arthritis developed in 14 days with a single intradermal injection of pristane in arthritis control and experimental groups. Starting Day 15, berberine and methotrexate were administered as single daily intraperitoneal injection for next 14 days. Arthritis resolution was assessed by measuring body weight, clinical score of arthritis on day 0, 14 and 28 and joint histopathology terminally. Data was analyzed using SPSS version 20, p value <0.05 was considered significant.
Results: Arthritis induction reduced body weight in pristane administered groups (142.87 ± 3.56, 146.25 ± 7.49, 112.37 ± 6.23, 114.50 ±3.85, 113.62 ± 7.72 g in Group I, II, III, IV and V respectively) at day 14. Berberine and methotrexate treatment restored body weight in comparison to continuous loss in arthritis control animals on day 28 (155.87 ± 3.72, 162.00 ± 7.96, 105.25 ± 8.04, 133.75 ± 4.89, 133.12 ± 9.24 g in Group I, II, III, IV and V respectively). Berberine and methotrexate both reduced joint inflammation (clinical score 15±1.51, 6.75±1.48, 3.25±1.48 in arthritis control, berberine and methotrexate treated groups). Methotrexate was, however, more effective in reducing clinical arthritis score than berberine on day 28 (p value <0.001). Histopathological changes were reversed similarly by both drugs.
Conclusion: Berberine is effective in treating rheumatoid arthritis though less than methotrexate.

Key words: Rheumatoid arthritis, Joint inflammation, Berberine, Methotrexate

INTRODUCTION

Rheumatoid Arthritis (RA) is a common autoimmune disease of chronic nature that affects many body systems especially synovial tissues, cartilage and bone. It is a highly debilitating disease causing disability, early deaths and is responsible for adverse socioeconomic outcomes.[1]

The major histopathological changes in rheumatoid arthritis include synovial hyperplasia, with inflammatory cells including lymphocytes and macrophages along with fibroblasts, all collectively known as a pannus. The pannus causes destruction of the underlying cartilage and bone by invasion and erosion. The synovial cavity is filled with inflammatory exudate comprising mainly of plasma containing neutrophils.[2]

Upon diagnosis of a patient with RA the main aim of the management is either to achieve complete remission or decrease the disease activity so as to minimize the joint damage, debility and systemic involvement of RA.[3]

Pharmacotherapy options include NSAIDs, corticosteroids and biological & non-biological DMARDs – disease modifying anti-rheumatic drugs. Methotrexate is a widely used first line non-biological DMARD given alone or in combination. It inhibits DNA synthesis and replication by inhibiting dihydrofolate reductase. For the doses used in treatment of RA, it is thought to inhibit enzymes engaged in purine synthesis, leading to adenosine accumulation; thus inhibiting activation of T cells.[4]

It is being used for RA since 1970 and it has proved to be quite effective in providing clinical improvement in at least 50% of RA patients using it. However, it has adverse effects, few of which may be serious, including bone marrow suppression and hepatotoxicity.[5]

Alternative medicines are now also being used to treat various diseases including RA. Among them, medicinal herbs are being used by a large number of people as they are thought to have less adverse effects. Berberine is a major chemical constituent of *Berberis lycium Royle (BLR),* a member of *Berberidiaceae* family of medicinal herbs. BLR, which is widely present in Gilgit, Balistan, Kashmir and Swat has found to have anti-diabetic, anti-hyperlipidemic, hepatoprotective, anti-bacterial, anti-cancer and most important in relevance to RA, anti-inflammatory effects.[6]

Various studies have been carried out to elicit the anti-inflammatory mechanisms of berberine. These include the effects like reduced pro-inflammatory cytokines level including interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE_2), nitric oxide (NO) and preventing expression of mRNA for COX-2 (cyclooxygenase-2).[7]

At the molecular level, there is a current and convincing proof for involvement of Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway in pathogenesis and inflammation development related to autoimmune diseases like RA and other autoimmune diseases. Berberine was found to bind to Janus kinase 3 (JAK-3) and inhibits its phosphorylation in an animal model of arthritis.[8]

Therefore, with this scientific basis for the hypothesis that berberine has significant anti-inflammatory activity, present study was carried out for studying histopathological effects in a pristane-induced arthritis rat model and compare them with that of methotrexate, taken as standard treatment.

MATERIAL AND METHODS

This experimental study was performed at PGMI (Post Graduate Medical Institute), Lahore, Pakistan during year 2014 after approval from Ethical Committee of PGMI (No: 8723, Dated: 15-06-2012). Adult female Sprague Dawley, 7-8 week age rats were obtained from University of Veterinary and Animal Sciences. They were kept and acclimatized in animal house at PGMI, Lahore for 7 days. Duration of intervention after acclimatization was 28 days.

Study Design

Animal Grouping: Forty rats weighing between 100-140 grams were randomly divided into five groups labelled from I to V with 8 rats in each group (Table-1).

Group	*Group Name*	*Arthritis induction*	*Treatment Day 15 onwards*
I	Normal Control	No	1 ml/kg normal saline i.p. daily
II	Berberine Control	No	2.5 mg/ml/kg Berberine i.p. daily
III	Arthritis Control	Yes	1 ml/kg normal saline i.p. daily
IV	Berberine Treated Group	Yes	2.5 mg/ml/kg Berberine i.p. daily
V	Methotrexate Treated Group	Yes	0.5 mg/ml/kg methotrexate i.p.daily

i.p. (intraperitoneal)

Table 1: Grouping of rats showing induction of arthritis and experimental interventions (n=8)

Induction of arthritis: Half ml of Pristane (Sigma, USA) was injected intradermally near rat's tail base to animals in arthritis control (group III), berberine treated (group IV) and methotrexate treated (group V) on day 0. Arthritis was induced within 14 days.[9] The progress and severity of arthritis was scored from 0-16 by grossly examining all four limbs of every animal on day 0, 14 and 28.

Administration of berberine and methotrexate: Administration of berberine was started on day 15, after two weeks of arthritis induction by pristane, as a single daily intraperitoneal injection in dose of 2.5 mg/kg to rats in berberine control (group II) and berberine treated (group IV) groups.[10] It was continued for next 14 days. Methotrexate was administered to rats in methotrexate treated group in a similar manner (group V) using 0.5 mg/kg dose.[11] Fresh solution of both drugs was prepared daily.

Parameters

Body weight: Body weight of all animals was measured on day 0, 14 and 28.

Clinical score of arthritis: Joint inflammation in all animals was assessed through clinical score of arthritis. It was calculated for one limb as 0 (no swelling or tenderness at all), 1 (involving one joint), 2 (involving two joints), 3 (involving more than two joints) and 4 (severe arthritis involving entire paw). The result for four limbs of one animal was added to get cumulative score of that animal.[12]

Histopathology: Ankle joints of all rats were amputated after their sacrifice at the end of study. Hematoxylin and eosin-stained slides were prepared after formalin fixation and decalcification by keeping them for 4-5 days in formic acid-formalin solution. Histopathological scoring was done in two paws of each rat separately by studying parameters given in Table-2 and adding them to get cumulative score for that animal.[13]

Parameter	Score	Description
Infiltration of synovium with mononuclear cells	0	No infiltration
	1	Mild
	2	Moderate
	3	Severe
Synovial Cell hyperplasia	0	1 – 3cell layers
	1	4 – 6 cell layers
	2	7 or above cell layers
Villous hyperplasia	0	Absent
	1	Short, few and scattered
	2	Finger like and marked
	3	Diffuse but marked
Pannus Formation	0	Absent
	1	Synoviocyte invasion - mild
	2	Synoviocyte and inflammatory cells- moderate
	3	Synoviocyte and inflammatory cells - Severe

Table-2: Histopathological parameters studied in H&E stained rat ankle slides with their scoring and grading. Each parameter was scored in two paws of an animal to obtain cumulative score for grading.

Statistical analysis:

Data was analyzed using SPSS version 20. Normality was checked by Shapiro Wilk test. Mean±SD, one-way ANOVA and post hoc Tukey and paired t-test were applied to quantitative variables, i.e., body weight and clinical score of arthritis. Frequency percentages were calculated for qualitative data obtained from joint histopathology. Kruskal Wallis ANOVA and Mann Whitney U tests were used to determine overall and group wise differences in histopathology.p value <0.05 was considered significant.

RESULTS

Body Weight (Table-3, Fig-1)

Body weight of normal control (Group I) and berberine control group (Group II) rats persistently increased throughout the study. Arthritis induction in arthritis control (Group III), berberine treated (Group IV) and methotrexate treated (Group V) group significantly reduced their body weight than normal control (Group I) and berberine group (Group V) till day 14 (p value < 0.001). Treatment with berberine and methotrexate in Group IV and V, however, restored their body weight above respective baseline at end of the study. It did not approach the normal control value but on Day 28, it was significantly higher than arthritis control (Group III) (p value <0.001) which showed continuous decline in body weight till end of the study. The difference between berberine (Group IV) and methotrexate (Group V) treated groups themselves was non-significant on Day 28.

Group	Day 0	Day 14	Day 28
Normal Control	119.87 ± 4.54	142.87 ± 3.56$^{\#}$	155.87 ± 3.72$^{\#}$
Berberine Control	120.63 ± 7.32	146.25 ± 7.49$^{\#}$	162.00 ± 7.96$^{\#}$
Arthritis Control	118.50 ± 6.78	112.37 ± 6.23*	105.25 ± 8.04*
Berberine Treated	122.00 ± 5.04	114.50 ±3.85*	133.75 ± 4.89$^{*\#}$
Methotrexate Treated	121.00 ± 7.85	113.62 ± 7.72*	133.12 ± 9.24$^{* \#}$

Table-3: Body weight (g) of pristane induced arthritic rats (n=8) shown as mean±S.D. – Effect of Berberine and methotrexate.

*p value ≤ 0.001 versus normal control

$^{\#}$p value ≤ 0.001 versus arthritis control

Clinical Score of Arthritis (Table-4)

No inflammation (clinical score 0) developed in normal control (Group I) and berberine control group (Group II) throughout the study. Arthritis was induced in arthritis control (Group III) and experimental groups (Groups IV and V) with almost similar clinical score on Day 14. Berberine and methotrexate treatment after Day 14 reduced the inflammation and its clinical score in berberine (Group IV) and methotrexate treated (Group V) groups respectively on Day 28. The score in arthritis control group (Group III), in comparison, remained unchanged after induction of arthritis between Day 14 and 28. Though berberine markedly reduced the inflammation of joints in Group IV than arthritis control (Group III) as measured on Day 28 (p value < 0.001), it was still significantly higher (p value < 0.001) than methotrexate treated group (Group V) which had the least value of clinical score on Day 28.

Group	Day 0	Day 14	Day 28
Normal Control	0	0	0
Berberine Control	0	0	0
Arthritis Control	0	15 ± 1.51	15 ± 1.51$^{\#}$
Berberine Treated	0	14.50 ± 1.77	6.75 ± 1.48$^{* \#}$
Methotrexate Treated	0	15.25 ± 1.48	3.25 ± 1.48*

Table-4: Clinical score of inflammation of pristane induced arthritic rats (n=8) shown as mean ± S.D. – Effect of Berberine and methotrexate.

$^{*}p$ value ≤ 0.001 versus arthritis control

$^{\#}p$ value ≤ 0.001 versus methotrexate treated group

Joint Histopathology (Table-5 & Fig-2*a-e*)

All parameters were normal in normal control (Group I) and berberine control group (Group II) while various grades of these derangements were present in all animals of arthritis control (Group III). Berberine and methotrexate treatment reversed these pathologies to normal in some animals and reduced

their overall severity in others in Group IV and V respectively (Fig 3*a-e*). The statistical difference for these groups versus normal control (Group I) and berberine control (Group II) groups, however, remained significant for most of the parameters.

Inflammation by mononuclear cells was significantly reduced than arthritis control (group III) in groups treated with berberine (p value < 0.001) and methotrexate (p value 0.001). This decrease was more significant in methotrexate treated group (Group V) than berberine treated group (Group IV) (p value 0.013) approaching a non-significant difference as compared with normal control (group I) having p value of 0.143.

Synovial hyperplasia was less affected by berberine treatment (p value 0.175 vs arthritis control, Group III) as compared to methotrexate which caused significant amelioration as compared to arthritis control (Group III) (p value 0.018). The difference between berberine and methotrexate treatment themselves was, however, non-significant.

Villous hyperplasia and pannus formation were also significantly less in both berberine treated (Group IV) and methotrexate treated (Group V) groups than arthritis control (Group III) (p values 0.007 and 0.019 for villous hyperplasia and 0.01 and 0.05 for pannus formation respectively). Severity of these two changes was, however, less in berberine treated group than methotrexate treated group, but the difference was statistically non-significant.

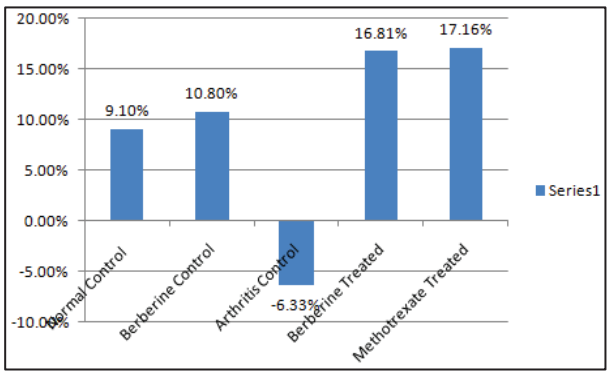

Fig-1: Body weight change (%) from day 14 to 28 in all study groups.

Parameter	Grading	Normal Control	Berberine Control	Arthritis Control	Berberine Treated	Methotrexate Treated
Infiltration of synovium with mononuclear cells	Normal	*100*	*100*	*0*	*12.5*	*75*
	Mild	*0*	*0*	*12.5*	*75*	*25*
	Moderate	*0*	*0*	*62.5*	*12.5*	*0*
	Severe	*0*	*0*	*25*	*0*	*0*
	p value	###	###	***	*** ### ^	###
Synovial Cell hyperplasia	Normal	*100*	*100*	*0*	*12.5*	*37.5*
	Mild	*0*	*0*	*62.5*	*75*	*62.5*
	Moderate	*0*	*0*	*37.5*	*12.5*	*0*
	Severe	*0*	*0*	*0*	*0*	*0*
	p value	###	###	***	***	** #
Villous hyperplasia	Normal	*100*	*100*	*0*	*25*	*37.5*
	Mild	*0*	*0*	*37.5*	*75*	*50*
	Moderate	*0*	*0*	*50*	*0*	*12.5*
	Severe	*0*	*0*	*12.5*	*0*	*0*
	p value	###	###	***	** ##	** #
Pannus Formation	Normal	*100*	*100*	*0*	*12.5*	*12.5*
	Mild	*0*	*0*	*12.5*	*62.5*	*37.5*
	Moderate	*0*	*0*	*62.5*	*25*	*50*
	Severe	*0*	*0*	*25*	*0*	*0*
	p value	###	###	***	*** #	*** #

Table-5: Percentages of four histological criteria in various groups of rats with pristane induced arthritis (n=8) and their statistical significances – Effect of Berberine and methotrexate.

$^{***}p$ value ≤ 0.001, $^{**}p$ value ≤ 0.01 versus normal control

$^{###}p$ value ≤ 0.001, $^{##}p$ value ≤ 0.01, $^{#}p$ value ≤ 0.05, $^{###}$versus arthritis control

$^{\wedge}p$ value ≤ 0.05 versus methotrexate treated group

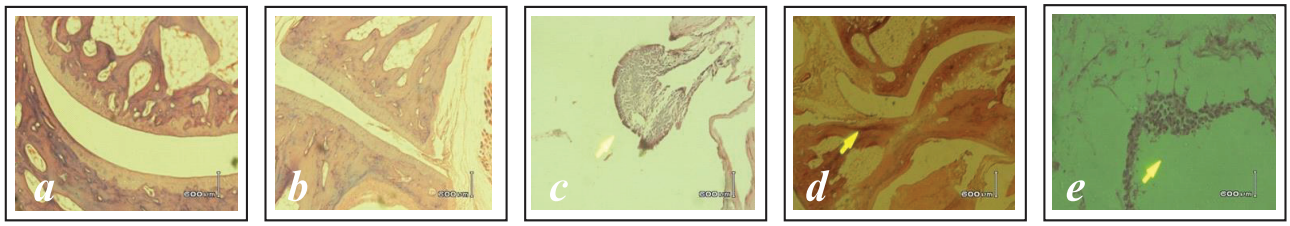

Fig-2a-e: Histopathological Findings

- **a.** Normal synovial joint anatomy in normal control group
- **b.** Normal synovial joint histology in berberine treated group
- **c.** Villous hyperplasia in arthritis control group
- **d.** Pannus formation in berberine treated group
- **e.** Synovial hyperplasia in methotrexate treated group

DISCUSSION

This study was designed to evaluate berberine's anti-inflammatory effect in the rat model, coupled with any physiological changes such as body weight in comparison with methotrexate .

Use of pristane is one of the several techniques available to induce arthritis in animal models.[14] Polyarthritis develops in about 14 days after a single intradermal injection of pristane and closely resembles in its clinical and histopathological findings to RA.

Arthritis induction decreased body weight of all animals in disease control, berberine and methotrexate groups while animals in normal control and berberine alone group got a steady and almost similar increase in body weight throughout the study. Other works have also reported decrease in body weight after arthritis induction with pristane.[15] Methotrexate and berberine both restored body weight in respective groups above baseline in comparison to disease control which lost weight continuously till the end of study. The percentage of restoration in weight (between day 14 and 28) was similar in both groups and was more than both control groups. This improvement in weight was most probably due to decrease in arthritis and not the effect of methotrexate or berberine themselves because methotrexate itself has a negative effect on weight in healthy animals[16] and animals in berberine control group of this study did not show any significant gain in body weight over normal control till the end of study. This finding is also supported by works done on methotrexate[17] and berberine[18] where resolution of arthritis has resulted in improvement of body weight.

Clinical score of arthritis was similar in arthritis induced groups on day 14. This was in accordance with previous works done with pristane,[14] signifying a successful induction of disease model in this study. Administration of methotrexate and berberine both reduced the score significantly in their respective groups as compared to disease control in which the scored remained high till end of the study. Methotrexate was, however, more effective than berberine as difference between these two was also significant on day 28. Experiments using berberine in other models of RA also affirm these results. Wang et. al., 2014 demonstrated improvement in clinical arthritis score by using berberine in a rat model of collagen induced arthritis.[18] Kim et. al., 2011 reported dose dependent decline in joint oedema by 25% and 47% with berberine as compared to placebo in carrageenan/kaolin induced mono arthritis in rats.[19]A relatively recent study demonstrated prevention of paw edema when berberine was administered to Freund's adjuvant induced arthritic Sprague Dawley rats from first day of induction.[20]

H & E stained slides of ankle joints were studied for infiltration by inflammatory cell, synovial hyperplasia, pannus formation and villous hyperplasia. While these parameters were deranged in all animals of disease control group, severity and percentage of animals developing these changes were significantly less than arthritis control in methotrexate and berberine treated groups. Methotrexate and berberine were almost equally effective in preventing these changes except for infiltration of inflammatory cells which was significantly less in methotrexate than berberine treated group. Reduction in these histopathological changes was also demonstrated in previous works on berberine incollagen-induced arthritis,[18] Carrageenan/kaolin-induced kneemonoarthritis[19] and adjuvant-induced rheumatoid arthritis.[21] All these studies used Sprague Dawley rats, the strain used in our study. Berberine not only ameliorated joint destruction in rats but also in arthritic mice.[22]

One facet in pathogenesis of RA is increased oxidative stress.[23] Different antioxidants have, therefore, been employed in an attempt to seek some effective treatment with less adverse effects.[24] Berberine is an alkaloid with strong antioxidant with

consequent anti-inflammatory properties, with multiple mechanisms underlying these anti-inflammatory actions being postulated.[25] Dinesh and Rasool, 2019 demonstrated in a rat model that berberine effectively reduces proliferation of Th17 cells and responses of synoviocytes to IL-21 thus effectively preventing inflammation in RA.[26] The resolution of arthritis obtained in this study may also be due to these anti-inflammatory properties of berberine.

This study used standard methodology for induction of arthritis and evaluation of clinical inflammation as well as joint histology but limited by absence of advanced parameters of underlying mechanism.

CONCLUSION

Berberine is effective in treating RA though less as compared to methotrexate. As doses of methotrexate used to treat RA carry a risk of fatal adverse effects and methotrexate or berberine alone do not confer complete amelioration of RA, combining berberine with methotrexate may enhance efficacy of treatment and reduce the dosage of methotrexate, too, resulting in fewer adversities. It may be used in combination with methotrexate to enhance efficacy and reduce required dose of methotrexate.

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Variations in Symptomatology of Migraine Among Local Population of Pakistan

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ABSTRACT

Introduction: Migraine is considered one of the most disabling neurological disorders worldwide. Migraine has recently been shown to effect population with a severe cascade of symptoms. With still a huge gap in understanding of pathogenesis of migraine, knowledge regarding migraine symptomatology is mandatory for effective diagnosis and treatment.
Aims & Objectives: To compare symptoms of migraine between two groups migraineurs (M) and migraineurs with high blood pressure (MBP).
Place and duration of study: The study was conducted at Shaikh Zayed Postgraduate Medical Institute, Department of Physiology and Lahore General Hospital, from January 2015 to June 2015.
Material & Methods: It was a cross-sectional comparative study. The study population was 35 diagnosed migraine patients (M) and 29 migraine patients with high blood pressure (MBP). Patients were questioned about various migraine symptoms based on the standard criteria for diagnosing migraine and the responses were recorded in written on a predesigned proforma. Collected data was studied and interpreted by using SPSS 23 , p value ≤ 0.05 was taken as significant.
Results: Symptoms of migraine were compared between two groups, migraineurs (M) and migraineurs with high blood pressure (MBP). The mean age of the patients was 25±7years in migraineurs (M) and 38±6 years in migraineurs with high blood pressure (MBP). The incidence of vomiting was significantly higher in patients who had both migraine and high BP, (p-value = 0.008). Collected data was studied and interpreted by using SPSS 23. The difference in the rest of the symptoms was nonsignificant between the two groups studied by using One Way Anova and T-test frequency and percentages. Chi-square test was applied using cross-tabulation to check the association of family history of migraine which was statistically non-significant.
Conclusion: Migraine is a female dominant disorder mostly affecting people in their 30's. There was no significant difference in major diagnostic migraine symptoms in both groups. However, among minor diagnostic symptoms, the frequency of vomiting was higher in patients with migraine with high BP.

Key words: Migraine, nausea, vomiting, photophobia, phonophobia

INTRODUCTION

Migraine is categorized as one of the most common and disabling diseases in the world interfering with the daily routine of the patient. It is known to be a primary neurovascular disorder with unclear Pathophysiology.[1,2] In the western world, 8% of the males and 25-30% of the females are migraine sufferers.[3]The principal nervous system structures that are proposed to play an important role in activating migraine pain are cranial blood vessels and trigeminovascular system and its connections with the parasympathetic outflow. The most common sites for pain in migraine are frontal and temporal regions but sometimes it presents as a referred pain as well in parietal, occipital, and upper cervical regions.[1]Migraine is currently diagnosed clinically as still no specific blood or radiological biomarker has been identified. A strong genetic component has been linked with a migraine that involves several gene-related contributing factors.[4] The mechanism and pathophysiology behind migraine are still unclear despite three proposed theories; 1: vascular, 2: neurological and 3: neurogenic theories. So migraine headache still largely remains underdiagnosed and misunderstood.[5]

Standard criteria used to diagnose migraine worldwide is the one defined by the International Headache Society, according to which migraine includes, an episodic attack of headache lasting 4-72 hours with two of the following major criteria; Unilateral headache, Throbbing headache, headache Aggravation by movements, Moderate/severe

intensity of pain, and with one of the following minor criteria; associated Nausea and/ or vomiting, photophobia and phonophobia.[6]

Migraine has emerged as a potentially severe headache in recent times, affecting masses of the general population. As its pathophysiology still has a number of loopholes, it remains underdiagnosed and no definite treatment is known for migraine.[7]

In this study, variations in the symptoms of migraine were compared between two groups; Migraineurs (M) and Migraineurs with high blood pressure (MBP) in a local population of Pakistan. The aim of the study was a better understanding of migraine symptoms in this part of the world that might be helpful in future diagnosis and treatment options for migraine.

MATERIAL AND METHODS

The study was conducted in Shaikh Zayed Post Graduate Medical Institute, Lahore and Lahore General Hospital, Neurosciences department from January 2015 to June 2015 (IRB approval letter # F.39/NHRC/Admn/IRB/136). It was a cross-sectional comparative study. A convenient, non-probability sampling technique was used to conduct the study. The study was conducted after taking approval from the Ethical Review Committee. Diagnosed patients of migraine (M) and migraine with high blood pressure (MBP) presenting in the outpatient department and hospital admission were included in the study after taking written informed consent. The study population included 35 diagnosed migraine patients (M) and 29 migraine patients with diagnosed Hypertension (MBP) on antihypertensive medication. The patient's blood pressure was measured by a mercury sphygmomanometer manually and recorded in millimeters of mercury. The study included both genders between the ages of 20 to 45 years. Patients with acute pulmonary embolism, pulmonary hypertension, sepsis, chronic obstructive pulmonary disease, hyperthyroidism, or renal failure were excluded. Detailed history and examination were done. The questioned parameters included marital status, employment status, and education Questions on migraine symptoms and family history of migraine were based on criteria for diagnosing migraine.[8] All the data was documented on a proforma.

Statistical analysis:

Collected data was studied and interpreted by using SPSS 23. Data for quantitative variables i.e., age, height, weight and BMI was described by using Mean ± Standard Deviation. Frequencies and percentages were calculated for qualitative variables like gender, nausea, vomiting, photophobia, and phonophobia. Comparison between groups was studied by using One Way Anova and T-test. Linear correlation was studied to compare the symptomatology of migraine in 2 groups. Chi-square test was applied using cross-tabulation to check the association of family history of migraine. P-value ≤ 0.05 was taken as significant.

RESULTS

The current study included 35 diagnosed migraine patients (M) and 29 migraine patients with diagnosed Hypertension (MBP). It was a cross-sectional comparative study in which symptoms of migraine were compared between two groups, migraineurs (M) and migraineurs with high blood pressure (MBP). The mean age of the patients was 25±7years in migraineurs (M) and 38±6 years in migraineurs with high blood pressure (MBP). The mean weight in the M group was 61±10 kg and 67±10 kg in the MBP group. The mean height of patients was 164±6 cm and 169±3 cm in M and MBP groups, respectively. The mean BMI of migraineurs (M) was 22±3 and 24±4 in migraineurs with high blood pressure (MBP). (Table-1, Fig-1)

Groups	Migraineurs(M) (Mean ± SD)	Migraineurs with Blood Pressure (MBP) (Mean ± SD)
Age(years)	24.88±6.90	38.27±6.25
Weight (kg)	61.31 ± 10.46	67.52 ± 10.53
Height (cm)	164.17 ± 6.10	169.02 ± 3.37
BMI	22.59 ± 3.56	23.63 ± 3.67

Table-1: Quantitative Variables (Mean ± SD)

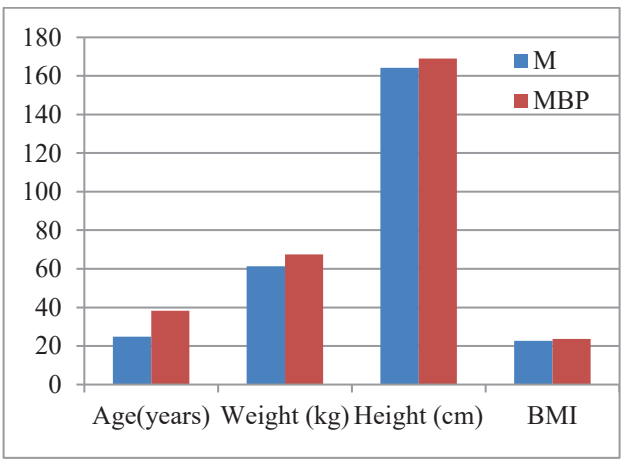

Fig-1: Graphical Representation of Quantitative Variables

The highest percentage of patients in the M group was up to 30 years (80%), while it was 31 years and above in the MBP group (41%). In the M group, most

of the patients were females (69%) and the same was true for the MBP group, with females being 70%. The majority of the patients in both M and MBP groups were married (77% and 97% respectively) and unemployed (71% and 76% respectively). The majority of the patients in the M group had done their graduation while the same percentage of patients was found to be illiterate in the MBP group (48%). (Table-2)

Groups		Migraineurs (M) n = 35 n (%)	Migraineurs with Blood Pressure (MBP) n = 29 n (%)
Age (years)	0-30	80%	17.2%
	31-40	17.1%	41.4%
	41 & above	2.9%	41.4%
Gender	Males	11 (31.4%)	7 (24.1%)
	Females	24 (68.6%)	22 (75.9)
Marital status	Married	27 (77.1%)	28(96.6%)
	Unmarried	8(22.9%)	1(3.4%)
Employment status	Employed	10(28.6%)	7(24.1%)
	Unemployed	25(71.4%)	22(75.9%)
Education	Illiterate	4(11.4%)	14(48.3%)
	Metric	2(5.7%)	4(13.8%)
	Intermediate	1(2.9%)	4(13.8%)
	Graduation	17(48.6%)	0(0.00%)
	Masters	6(17.1%)	5(17.2%)
	MBBS	5(14.3%)	2(6.9%)

Table-2: Stratification of Age, Gender, Marital Status, Employment Status, Education in both groups

Unilateral headache, a major diagnostic criterion for migraine according to modified International Headache Society criteria (IHS), was seen in 89% of the patients in the M group and 100% of the patients in MBP group. Throbbing headache, another major diagnostic criterion for migraine (modified IHS criteria), was a symptom found in 97% of the patients in both groups. Headache aggravation by the movement was seen in 76% and 72% of the patients in the M group and MBP group, respectively. Nausea was an accompanying minor symptom of migraine in 54% of the patients in the M group and 48% of the patients in the MBP group. The percentage of vomiting, another minor symptom of migraine, was 26% and 59% in the M group and MBP group, respectively. Photophobia and phonophobia, both minor symptoms of migraine, affected 83% and 80% of the patients respectively in the M group, and 69% and 93% of the patients respectively in the MBP group[6]. The frequency of migraine was observed to be more than 1 attack/month in 94% of the patients in the M group and 97% of the patients in the MBP group.

In the present study, the incidence of vomiting was significantly higher in patients who had both migraine and high BP, (p-value = 0.008). The difference in the rest of the symptoms was insignificant between the two groups. The frequency of migraine attack was found to be ≥1attack/month in both groups. Family history of migraine was statistically non-significant in both M and MBP groups. (P value=0.40) (Table-3)

Frequency of Migraine Symptomatology		Migraineurs (M) n = 35 n (%)	Migraineurs with Blood Pressure (MBP) n=29 n (%)	P-value (Chi-square)
Unilateral headache		31 (88.6%)	29 (100%)	0.06
Throbbing		34 (97.1%)	28 (96.6%)	0.89
Headache aggravation by movements		26 (76.5%)	21 (72.4%)	0.71
Nausea		19 (54.3%)	14 (48.3%)	0.63
Vomiting		9 (25.7%)	17 (58.6%)	0.008*
Photophobia		29 (82.9%)	20 (69.0%)	0.19
Phonophobia		28 (80%)	27 (93.1%)	0.13
F/H of Migraine		22 (62.9%)	23 (79.3%)	0.40
Frequency of migraine (attack/month)	<1	2 (5.7%)	1 (3.4%)	0.67
	≥1	33 (94.3%)	28 (96.6%)	

P-value ≤ 0.05

Table-3: Migraine Symptomatology in both groups

DISCUSSION

The current study aimed at finding out variations in symptoms of migraine in 2 groups of migraine patients, migraineurs (M group) fulfilling the diagnostic criteria for migraine according to modified IHS criteria and migraineurs with high BP (MBP group).

The mean prevalence age for migraine in the current study was found to be 30 years and above. A study conducted by Straube et al., in 2019 showed that the peak age for migraine was between 30-39 years.[9] Zahid et al, in 2014 studied the prevalence of migraine among students and patients in Khyber Pakhtunkhwa.[10] He found out the most of the migraine patients were above 30 years. A study by Takeskima et al, in 2019 also reported the mean age for migraineurs to be 30 years and above.[11] All these studies coincide with the results of the present study. However, a study by Ozge et al., in 2017[12] described the peak age for migraine in girls to be teens (14-17 years) and 10-12 years in boys, which is in contrast to the current study.[12]

A preponderance of female migraineurs in both the groups in the current study indicating that migraine is

a female dominant disorder. A study carried out by Buse et al, in 2013 showed a sex difference in the prevalence of migraine with a female to male ratio of 2:1 to 3:1.[13] Gul et al, conducted a study in January 2014 according to which 1 in 5 women and 1 in 16 men suffering from migraines.[14] Another study by Boley et al, in August 2015 indicated that migraine was three times more prevalent in females than in males.[15] Similar results regarding gender distribution were also documented by Gordon in 2015, according to which migraine is a neurovascular disorder affecting 17% of women and 6% of men.[16] A recent study by Guo et al, in 2019 also, demarcated migraine as predominantly a female disorder, and it was suggested that females probably being more anxious, develop migraine symptoms more frequently than males.[17] All these studies potentiate the findings of the present study indicating migraine as a female dominant disorder.

In our study majority of the patients in both groups (M and MBP) were married, 77% and 97% respectively. A study conducted by Buse et al, in 2019 documented that married couples suffered a lot of negative marital impacts that aggravated their migraine attacks. These included common and frequent arguments with spouses and also adverse behavior with children owing to severe migraine symptoms.[18] Another study by Buse et al, in 2016 highlighted the negative effects of migraine on family activities and relationships. The most severe adverse effects were seen in couples with chronic migraines.[19] These studies correlate with the results of the current study that indicates the majority of the patients are married suggesting that probably marriage adds to the stress of migraineurs.

Most of the patients in the present study in both the groups were unemployed, the percentage was 71% in the Migraine group (M) and 76% in Migraine with blood pressure group (MBP). A study conducted by Sullivan in 2014 had similar findings. According to the study, the risk of migraine was significantly evident in unemployed subjects and it was owed to the negative effects of migraine. Increased risk of migraine was seen with a lower level of schooling and education.[20] The majority of migraineurs in the current study had a low educational status, graduation in Migraine group (M) and illiterate in Migraine with blood pressure group (MBP), coinciding with the results of Sullivan.

In the present study, the incidence of vomiting was significantly higher in patients who had both migraine and high BP, (p-value 0.008). The difference in the rest of the symptoms of migraine was insignificant between the 2 groups. According to a study by Almohammadawi et al, in 2018, the most common symptoms of migraine included vomiting, nausea, and photophobia which helped in the diagnosis of migraine.[21] The results were similar to the current study which also showed vomiting as the most common symptom. A contrasting study by Laurell et al, in 2016 showed the highest co-occurrence of phonophobia and photophobia among the migraine sufferers.[22] According to a study conducted by Syed et al, in 2020, the most commonly associated symptoms of migraine included vertigo in 74.4% of the patients followed by nausea (67.9%).[23] Again, the results were in contrast to the present study.

In this study, the frequency of migraine attack was 1 attack/month. A study conducted by Shahzadi et al, in 2017 on the frequency of migraine in students of the University of Lahore, Lahore, indicated that the mean frequency of migraine attacks in a month ranged from 1-8 episodes.[24] The results were similar to the present study. According to Almohammadawi et al,[21] the mean frequency of migraine attack was 2 ± 4.63 days/month. The results were a bit different from the current study.

Family history of migraine was statistically non-significant in both groups in the present study, (p-value=0.40). A study by Frederich et al, in 2013, showed that migraine headaches were associated with a family history of migraine or headache.[25] In a study conducted by Peres et al, the most frequently reported family member who had a history of migraine or headache was the mother.[26] The results of both these studies contradict the present study that showed no positive correlation of family history of migraine in both groups.

CONCLUSION

This study concludes that migraine is a female dominant disorder with most of the affectees in their 30's. There was no significant difference in major diagnostic migraine symptoms in both groups. However, among minor diagnostic symptoms, the frequency of vomiting was higher in patients with migraines with high BP. Migraine is a medical condition that severely affects various daily activities and the quality of life of the patient exhibiting these symptoms. Therefore, the Pakistani population suffering from migraines should be encouraged to visit a physician for correct diagnosis and effective treatment to help improve their quality of life during the debilitating attacks.

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