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Editorial

Pregnant Women presented with Placenta Accreta Spectrum Disorders: Bangladesh Perspective

Mahbuba

Professor and Head, Department of Gynaecology & Obstetrics, Monno Medical College and Hospital, Manikganj, Bangladesh.

Placenta accreta spectrum (PAS)also known as morbidly adherent placenta refers to the range of pathologic adherence of the placenta with its degree of invasion to the myometrium; placenta accreta when placenta invades the decidual layers, placenta increta when it invades the myometrium and placenta percreta when it invades up to the uterine serosa and adjacent organs[1]. Among them placenta percreta is most difficult to treat. It is associated with severe life-threatening hemorrhage often requires hysterectomy resulting increased maternal morbidity as well as mortality, loss of future fertility and adverse maternal outcome. The rates of PAS are increasing. Observational studies from 1970 and 1980 s describes the prevalence of placenta accreta as between 1 in 2510 and 1 in 4017 compared with a rate of 1 in 533 from 1982 to 2002[2]. The incidence of PAS (placenta accreta spectrum) has gradually been increasing with the increase rate of caesarean section throughout the world as placenta accreta found in (79%), placenta increta (14%) and placenta percreta (7%)[2,3]. For woman with placenta praevia, the risk of placenta accreta is 3%,11%,61% and 67% for the first, second, third and 4th fifth or more caesarean sections respectively[3].

The most favoured hypothesis regarding the aetiology of placenta accreta spectrum is any defect in the endo-myometrial interface leads to a failure of normal decidualization like in the uterine scar, which allow abnormal deep placental anchoring villi and trophoblast infiltration[4]. Among the types placenta percreta is the dangerous one in the form of transmural extension of trophoblast to the surrounding organs such as urinary bladder and rectum results complexity in management. The diagnosis of PAS disorders requires clinical assessment of high-risk cases during regular antenatal visit with supportive investigations and finally evidences of gross placental invasion at the time of surgery. Histological diagnosis of peripartum hysterectomy confirms the diagnosis. Antenatal diagnosis and patient assessment can be done by grey scale ultrasonography (TVS/TAS) with or without colour doppler sonography and MRI. Ultrasonography signs of PAS vary with gestational age, thickness of placental bed, number of prior uterine scar, depth of invasion and lateral extension of villous tissue[5]. The grey scale abnormality that are associated with placenta accreta spectrum includes multiple vascular lacunae within the placenta, loss of normal hypoechoic zone between the placenta and myometrium, decreased retro placental myometrial thickness (less than 1 mm), abnormalities of uterine serosa-bladder interface and extension of placenta myometrium ,serosa and urinary bladder[6,7]. The use of colour Doppler imaging shows turbulent lacunar blood flow, increased sub placental vascularity, gaps in myometrial blood flow and vessels bridging placenta and to the uterine margin[6,7]. Magnetic resonance imaging can be helpful in difficult cases with an overall sensitivity 94.4% and specificity 84%[8]. The imaging shows dark intra placental bands on T_2 weighted imaging, abnormal bulging of placenta, abnormal and disorganized placental blood vessels[8].

Patient with PAS disorders often present with mild to moderate APH, anaemia and haematuria in case of bladder invasion. So high risk cases should be evaluated in special settings and delivery should be planned with adequate preparation. Placenta praevia with history of previous caesarean section or uterine scar should be evaluated carefully. It is preferable to do caesarean hysterectomy leaving the placenta in situ without any attempt of separation and this reduce the amount of intra operative haemorrhage and need of blood transfusion[9]. Placenta percreta with urinary bladder invasion needs obstetrician

Correspondence: Prof. Dr. Mahbuba, Professor & Head, Department of Gynae & Obs, Monno Medical College and Hospital, Manikganj, Bangladesh. Email: mahbubab38@gmail.com, Orcid: https://orcid.org/0009-0006-9433-7991, Contact no-01711468692.

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-surgeon team approach. Expectant management in selective cases for the purpose of uterine conservation with techniques like uterine devascularization, uterine artery balloon placement embolization or ligation, postdelivery methotrexate etc. associated with risk of haemorrhage, local necrosis septicemia and drug related toxicity. In Bangladesh like other Asian countries the caesarean section rate is increasing. It is done in almost all the Upazilla Health Complexes and in remote private sectors in our country. With the increasing trend of caesarean section, the risk of PAS disorders is found frequently in hospital settings although exact data is not available. Zesmin et al[9] in their study shows the outcome of placenta accreta management by peri partum hysterectomy.

Parvin and Hossain[10] also had a study on foetal outcome in PAS disorders. The consciousness of regular antenatal checkup of high-risk cases like post caesarean pregnancy is still very much lacking. Particular attention must be taken in case of anterior placenta praevia with history of previous caesarean section. Antenatal diagnosis by ultrasonogram specially colour doppler facility is not available in all settings. MRI is expensive also. So, patients having multiple caesarean sections and other high-risk cases often missed in diagnosis. Unplanned surgery or lack of expertise in management of the PAS cases may result in severe per-operative haemorrhage complicating surgery resulting maternal disability and often death. So, awareness building about the gravity of the condition is necessary among risk group. Risk assessment and referral to higher centers for antenatal diagnosis, planned surgery with expert team can reduce the morbidity and mortality associated with this catastrophic condition.

Obstetricians must be alert about the condition and should take timely and necessary measures. A management protocol is to be developed to follow the relevant sectors.

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Creating awareness and increased vigilance among the risk group so that delivery can be done in equipped hospitals with provision of blood transfusion, combined obstetrician-surgeon team facility, good anaesthesia, elective and timely caesarean section with intensive care support is necessary and lifesaving

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Original Article

Degree and Pattern of Hearing Loss among Community Level Patients attended at ENT Department of a Tertiary Care Hospital Outside Dhaka City in Bangladesh: A Retrospective Study

Md. Shaheeduzzaman[1], Sazzadul Haque[2], Md. Mahbubul Alam[3], Md. Aminul Islam[4], Dipankar Sharkar[5], Md. Ilius Mahamud[6]

[1]Professor & Head. Department of Otolaryngology & Head Neck Surgery, Monno Medical College, Manikganj, Bangladesh; [2]Associate Professor, Department of Otolaryngology & Head Neck Surgery, Monno Medical College, Manikganj, Bangladesh; [3]Registrar, Department of Otolaryngology & Head Neck Surgery, Monno Medical College and Hospital, Manikganj, Bangladesh; [4]Honorary Medical Officer, Department of Otolaryngology & Head Neck Surgery, Dhaka Medical College Hospital, Dhaka, Bangladesh; [5]Assistant Registrar, Department of Otolaryngology & Head Neck Surgery, Monno Medical College and Hospital, Manikganj, Bangladesh; [6]Medical Officer. Department of Otolaryngology & Head Neck Surgery, Monno Medical College and Hospital, Manikganj, Bangladesh.

Abstract

Background: Hearing loss is one of the major health hazards specially in under develop and developing countries including Bangladesh. Here it is significantly increasing trend affecting all age group. ***Objective:*** The purpose of the present study was to analyze the degree and pattern of hearing loss among patients attending ENT outpatient department. ***Methodology:*** This was a retrospective review of data retrieved from the record of Pure Tone Audiometry (PTA) results from Department of Otorhinolaryngology in Monno Medical College Hospital, Manikganj, Bangladesh between January 2017 to December 2024. Total 840 patients with hearing loss, who had undergone PTA were included in this study. The data were tabulated, analyzed and results were expressed in number and percentage. ***Results:*** Among 840 patients, 80 patients had normal hearing on both ears. So, a total of 760 patients had hearing loss. Among 760 patients, 428 (56.31%) cases were male and 332 (43.68%) were female. Most commonly affected age group was 21-30 years followed by 41-50 years age group. Out of 760 patients, 189 (24.85%) patients had unilateral hearing loss whereas 571 (75.15%) cases had bilateral hearing loss. Conductive hearing loss was the most common 663(43.6%), followed by mixed 381(25.1%) and sensorineural 127 (8.35%) hearing losses respectively. Conductive hearing loss was more common in younger age groups whereas mixed hearing loss was more common in older age groups. Mild hearing loss was seen in 604(39.7%) ears, moderate in 316 (20.8%), severe in 125(8.2%) and profound in 64 (4.2%). Regarding cause chronic suppurative otitis media was found most common cause which was 314 (41.3%) cases followed by otitis media with effusion in 136(17.9%) cases and presbyacusis in 86(11.3%) cases. ***Conclusion:*** In conclusion, the commonest type of hearing loss is conductive and the commonest degree of hearing loss is mild degree.

Keywords: : Hearing loss; pure tone audiometry; conductive hearing loss; sensorineural hearing loss; mixed hearing loss

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Correspondence: Prof. Dr. Md. Shaheeduzzaman, Professor & Head, Department of Otolaryngology & Head Neck Surgery, Monno Medical College and Hospital, Manikganj, Bangladesh; **Email:** shaheeduz@gmail.com, **ORCID:** https://orcid.org/0009-0003-8282-677X ,**Contact no:** 01715153599

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Introduction:

Hearing is a special sense and no doubt it is the blessing contact with each other by means of language. A person can learn to speak and express himself/herself through hearing. Hearing impairment and its effects is an invisible disability. Thus, its impairment of any degree will be certainly significant. It is in one sense our hearing is like that of an antenna (along with other special senses) if we compare our brain to a television set. Like that of our brain response depends on its input signals. So if anyone is unable to enjoy this privilege is really unfortunate. We have witnessed in an Oscar award winning movie 'Children of lesser God' where the actress was a 'deaf and dumb' and thereby expressed her agonies of being deprived of these blessings in her master class acting. Indeed, it was very touchy.

Anyhow hearing impairment is one of the common health hazards globally and Bangladesh is a part of it. It is a hidden handicap initially but very soon become visible when it hampers speech leading to become a 'deaf n dumb' and thus detaching him from the society. Hearing impairment is a common problem that affects people of all age groups. It affects more than 1.33 billion people globally[1]. Hearing impairment at any stage of life can compromise individual's quality of life[2]. Hearing impairment may lead to negative consequences like poor general health, poor academic performance, and lack of confidence, higher unemployment, social isolation and an increased risk of depression[3]. The burden of hearing loss is higher in developing countries[4].

Hearing loss can affect one or both ears. It can be classified as conductive, sensor neural and mixed type[5]. Conductive hearing loss is due to the defect in the sound conducting mechanism of the ear. Sensory neural hearing loss occurs due to the abnormality in the cochlea along with cochlear nerve, neural pathway, or the auditory cortex. Here two distinct subdivisions are cochlear (sensory) and retro cochlear (neural). It is also called perceptive type hearing loss. Mixed hearing loss has components of both conductive and sensory neural hearing losses. World Health Organization (WHO) has developed the grading system to assess the degree of hearing impairment[6].

Pure tone audiometry (PTA) is a tool used for the diagnosis of hearing loss. It is performed by the audiologists as per the recommendation from the otorhinolaryngologists[7,8]. PTA gives information regarding the degree, type, configuration of hearing loss and helps in further management planning[9].

This study carried with the motive to analyze the degree and pattern of hearing loss among patients reporting ENT department in Monno Medical College which is 70 km away from Dhaka city. Study was done totally on the basis of pure from our creator. The sense of hearing enables to establish Tone Audiometry finding. Total 840 patients with hearing impairment complain undergone pure tone audiometry and after analysis the results were expressed in number and percentage.

Methodology

Study Settings and Population: The retrospective cross-sectional study was conducted in the ENT Department at Monno Medical College Hospital, Manikganj, Bangladesh from January 2017 to December 2024 for period of 8 years. Patients were living in the rural area. This area is 70 km away from the capital of Bangladesh. All the patients complain of hearing loss were selected from OPD irrespective of age, sex and religion. For the collection of data, we used a pretested data sheet, prior to interview verbal consent was taken and the purpose of the study was elaborate clearly.

Sample Collection Procedure: The clinical diagnosis was established by history, detailed clinical examination including otoscopic examination after taking a verbal informed consent from patient or legal guardian and all findings were recorded. All hearing test were done in acoustically treated room (20 dB ambient noise) of Monno Medical College Hospital by well- trained audiometrician. Pure tone average (PTA) was done on averaging the hearing threshold at 0.5, 1 and 2kHz with reference to ISO:R 389-1970. For the collection of data, we used a pretested data sheet.

Statistical Analysis: Statistical analysis was performed with SPSS software, versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data that were normally distributed were summarized in terms of the mean, standard deviation, median, minimum, maximum and number of observations. Categorical or discrete data were summarized in terms of frequency counts and percentages. When values are missing, the denominator was stated. Chi-square test was used for comparison of categorical variables. Every effort was made to obtain missing data. A two-sided P value of less than 0.05 was considered to indicate statistical significance. All the data were checked and verified thoroughly. The data obtained from the study were compiled and standard calculator as well as computer software were used.

Ethical Clearance: All procedures of the present study were carried out in accordance with the principles for

human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the institutional research ethics. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and analyzed using the coding system.

Results

Total of 840 patients with a complaint of hearing loss, who underwent PTA, were selected for the study. Among them 80 patients had normal hearing on both ears. So, a total of 760 patients had hearing loss on either one or both ears. Among them, most commonly affected age group was 21-30 years followed by 41-50 years group. Among 760 patients, 428 (56.31%) were male and 332 (43.68%) were female, and the male to female ratio was 1.28:1(Table 1).

Table 1: Age and sex distribution of the respondents

Variables	Hearing Loss	Normal Hearing	Total
Age Group			
0 to 10 Years	57	7	64
11 to 20 Years	98	23	221
21 to 30 Years	147	16	163
31 to 40 Years	120	25	145
41 to 50 Years	134	7	141
51 to 60 Years	106	1	107
61 to 70 Years	71	1	72
71 to 80 Years	22	0	22
More Than 80 Years	5	0	5
Gender			
Male	428		473
Female	332		367
Total	760		840

Out of 760 patients, 82 cases (10.78%) had hearing loss in right ear only, 107 (14.07%) cases had hearing loss in left ear only, whereas 571 (75.15%) cases had bilateral hearing loss (Table 2).

Table 2: Involvement of ear in disease process (n = 760)

Ear Involved	Frequency	Percent
Only Right Ear	82	10.8
Only Left Ear	107	14.1
Both Ear	571	75.1
Total	760	100.0

In our study conductive hearing loss was the most common 663 (43.61%), followed by mixed 381(25.06%) and sensorineural 127(8.35%) hearing losses respectively. In right ear 332 (43.7%) was conductive, 192(25.3%) cases were mixed and 52(6.84%) was sensorineural. whereas, in left ear, 331(43.55%) was conductive, 189(24.86%) was mixed and 75(9.86%) was sensorineural. (Table 3).

Table 3: Type of hearing loss among the patients (n=760)

Type of Hearing Loss	Right Ear	Left Ear	Total
Normal	184 (24.2%)	165 (21.71%)	349 (22.96%)
Conductive	332 (43.7%)	331 (43.55%)	663 (43.61%)
Sensorineural	52 (6.8%)	75 (9.86%)	127 (8.35%)
Mixed	192 (25.3%)	189 (24.46%)	381 (25.06%)
Total	760 (100%)	760 (100%)	1520 (100%)

On correlating the age groups with the type of hearing loss, conductive hearing loss was more common in younger age groups whereas mixed hearing loss was more common in older age groups. On evaluating degree of hearing loss, mild hearing loss was seen in 604 ears (39.73%), moderate in 316 (20.78%), severe in 125 (8.22%) and profound in 64 (4.21%) (Table 4).

Table 4: Type of hearing loss according the age group of patients (n = 760)

Age Group	Type of Hearing Loss		
	Conductive	Sensorineural	Mixed
0 to 10 Years	47	4	9
11 to 20 Years	80	11	7
21-30 Years	118	10	19
31 to 40 Years	85	7	28
41 to 50 Years	68	12	54
51 to 60 Years	41	14	51
61 to 70 Years	26	6	39
71 to 80 Years	4	8	10
More Than 80 Years	0	1	4

Regarding degree of hearing loss most common was mild 604 (39.73%) followed by moderate 316 (20.78%), sever 125 (8.22%), profound 64 (4.21%), moderately severe 62 (4.07%). (Table 5).

In this series regarding causes of hearing loss, most common cause was Chronic Suppurative Otitis Media 314(41.30%) followed by otitis media with effusion 136(17.89%), presbyacusis 86 (11.31%), SSNHL 51(6.71%), otosclerosis 49(6.40%), congenital hearing loss 36(4.73%), NIHL 24 (3.15%), ET Tube dysfunction 23 (3.02%) (Table 6).

Table 5: Degree of Hearing Loss among the Patients (n = 760)

Degree of Hearing Loss	Right Ear	Left Ear	Total
Normal (<25 dB)	184 (24.21%)	165 (21.71%)	389 (22.96%)
Mild (26–40 dB)	283 (37.23%)	321 (42.23%)	604 (39.73%)
Moderate (41–55 dB)	151 (19.46%)	156 (21.71%)	316 (20.78%)
Moderately Severe (56–70 dB)	37 (4.86%)	25 (3.28%)	62 (4.07%)
Sever (71-90 dB)	67 (8.81%)	58 (7.63%)	125 (8.22%)
Profound (>90 dB)	38 (5.00%)	26 (3.42%)	64 (4.21%)

Table 6: Causes of Hearing Loss Among the Patients (n = 760)

Causes of Hearing Loss	Frequency	Percent
Chronic Suppurative Otitis Media	314	41.30
Otitis Media with Effusion	136	17.89
Presbycusis	86	11.31
SSNHL	51	6.71
Otosclerosis	49	6.40
Congenital hearing loss	36	4.73
NIHL	24	3.15
ET Tube dysfunction	23	3.02
Traumatic rupture of Tympanic Membrane	14	1.84
Meniere's disease	10	1.31
Drug induce hearing loss	8	1.05
Ossicular discontinuity	7	0.92
Microtia and External Auditory Canal atresia	2	0.26
Total	**760**	**100**

Discussion

Hearing loss is a significant public health hazard in countries like Bangladesh. The prevalence of adult hearing impairment substantially higher in middle- and low-income countries than high-income countries[6]. WHO estimates 38,000 deaf children are born every year in South East Asian Region[7-8]. The pattern of hearing loss may vary from community to community, place to place, one geographic region to other and from hospital to hospital. Knowledge of pattern of hearing loss can help health personnel to make the proper diagnosis and treatment as per requirement. Such study helps in timely detection of the disease and treatment, ultimately will help in reducing morbidity and improve quality of life.

Hearing loss has a significant financial and socio economic burden in low and middle-income countries[4]. Hearing loss has a negative impact on the individual, which may result into poor general health, poor academic performance, higher risk of unemployment and depression[3]. Hearing loss also increases the financial burden to the society.

The pattern of hearing loss may vary between different geographic regions and between different hospitals. Knowledge of pattern of hearing loss can help health personnel to make the proper diagnosis and provide best treatment to the patients. Such study helps in early identification of the hearing problems and their management, ultimately helping to reduce morbidity and improve the quality of life[7].

In this study, pure tone audiograms of 840 patients who presented to ENT outpatient department with the complaint of hearing loss were analyzed. Among 840 patients, 80 patients had normal hearing on both ears. So, a total of 760 patients had hearing loss on either one or both ears. In this study, hearing loss was highest in 21 to 30 years age group and it was 147(19.34%). The next order was seen in age group of 41-50 and it was 134(17.63%), followed by 50-60 years which was 106 (13.94%). Lowest incidence was seen in age group of above 80 years which was 5(0.65%). These results are different from the study by Browning et al[11] which showed that the hearing loss was highest in 61 to 80 years age group (45.3%) followed by 41 to 60 years age group (17.4%). This difference might be due to lack of awareness about hearing impairment and poor access to health care services especially among elderly in the developing country like Bangladesh.

In present study, maximum number of hearing loss was seen in 21-30 years followed by 41-50 years. This may be due to higher level exposure to risk factors among these age groups as constitute working class of people. Most of peoples in these age groups have increased awareness as well as easy access to hospital services compared to other age groups. Early visit to hospitals among these age group even after mild hearing impairment is common as slightest loss in hearing power may have negative impact on their work.

In present study, among 760 patients with hearing loss, 428 (56.31%) were male and 332 (43.68%) were female. The male to female ratio was1.28:1. The study performed by Uju[10] also found the higher prevalence of hearing loss in male as compared to female. Similar results have been shown by other studies[11,12]. The higher prevalence of disease in male has been attributed to their increased exposure to the outdoor activities and other risk factors as well as early and easy access to health care services compared to females.

In present study bilateral hearing loss was seen in 571 (75.15%) cases and 189 (24.85%) had unilateral involvement. These results are similar to the studies by Rabbani et al[8] and Varshney et al[13] which have shown that bilateral hearing loss more common and similar distribution of right and left ear involvement.

In present study, conductive hearing loss was the most common 663 (43.61%), followed by mixed 381(25.06%) and sensorineural 127(8.35%) hearing losses respectively. In right ear 332 (43.7%) was conductive, 192(25.3%) cases were mixed and 52(6.84%) was sensorineural. Similarly, in left ear, 331(43.55%) was conductive, 189(24.86%) was mixed and 75(9.86%) was sensorineural. These results are similar to the findings of studies by Louw et al[14], Shuaibu et al[15] and ichels et al[9]. In those study, Mild hearing loss was seen in 604 ears (39.73%), moderate in 316 (20.78%), severe in 125 (8.22%) and profound in 64 (4.21%). These findings are similar to the results of other studies[9,12,14,15].

Regarding Causes of hearing loss, most common cause was Chronic Suppurative Otitis Media 314(41.30%) followed by otitis media with effusion 136(17.89%), presbycusis 86 (11.31%), SSNHL 51(6.71%), otosclerosis 49(6.40%), congenital hearing loss 36(4.73%), NIHL 24 (3.15%), ET Tube dysfunction 23 (3.02%) & others.

Different series showed different most common etiology for hearing loss. One survey showed 24% cause of hearing loss due to genetic factor, while other survey showed otitis media with effusion was the most common (30.7%) cause followed by presbycusis (22.7%)[16,17]. In this series chronic suppurative otitis media was found most (41.30%) common cause followed by otitis media with effusion (17.89%), presbyacusis (11.31%). Pure tone audiometry is a simple and accurate and cheap conventional method for the diagnosis of hearing impairment.

The main limitation of this study is that it is a retrospective study and correlation of the hearing loss with ear pathology was not done. Another limitation is the relatively smaller sample size. Studies with larger sample size are required to have better result.

Conclusion

Majority patients of hearing impairment belong to the working age group. Hence early identification with timely intervention can reduce the morbidity of deafness in this age group, which in turn helps to improve the productivity of the nation. Hearing impairment leads to social isolation in elderly persons as they become unable to participate in interaction. Childhood hearing impairment will undoubtedly play a role in education. In spite of, in Bangladesh context, the diagnosis is usually delayed until certain degree of hearing loss occurs. An early and adequate diagnosis has an important role in adapting sound amplification devices and rehabilitation procedures for auditory function in elderly. In pediatric age groups, cochlear implant is possible if deafness is identified in the early stage. This in turn helps to improve their language, social interaction and personal skills. Deafness prevention can be done only by mutual cooperation of both medical and nonmedical personnel. Improvement in health care delivery system and awareness programs can help in early diagnosis, treatment and rehabilitation of hearing impairment.

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Original Article

Antibacterial activities of Leaf extracts of Indian Bay leaf (Cinnamomum tamala) against Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa

Shompa Sharmin Rasna[1], Munira Afrin[2], Nazmun Nahar Nahida[3], Manira Khanam Nishi[4]

[1]Assistant Professor, Department of Pharmacology and Therapeutics, Monno Medical College, Manikganj, Bangladesh; [2]Professor & Head, Department of Pharmacology and Therapeutics, Monno Medical College, Manikganj, Bangladesh; [3]Associate Professor, Department of Forensic Medicine & Toxicology, Monno Medical College, Manikganj, Bangladesh; [4]Assistant Professor, Department of Pharmacology and Therapeutics, Shaheed Monsur Ali Medical College, Uttara, Dhaka, Bangladesh

Abstract

Background: : Natural herbal remedies have shown promising antimicrobial properties and fewer side effects than synthetic antimicrobial agents. ***Objective:*** The purpose of the present study was to investigate the antibacterial activities of Indian bay leaf extracts against Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa. ***Methodology:*** This experimental study was carried out from July 2019 to June 2020 in the Department of Pharmacology and Therapeutics with the collaboration of the Department of Microbiology at Mymensingh Medical College, Mymensingh, Bangladesh. The antibacterial activity was tested at different concentrations (20, 10, 5,2.5, 1.25 & 0.625 mg/ml) of both spice extracts by using the disc diffusion & broth dilution method. The extracts were prepared by using solvents aqueous & methanol. The test microorganisms were also tested for their activity against a standard antibiotic Gentamicin (80 mg) by broth dilution method. The result was compared with that of Aqueous and Methanolic extracts. ***Results:*** Salmonella typhi, Staphylococcus aureus, and Pseudomonas aeruginosa were all inhibited by ALE at doses of 15 mg/ml and higher. Pseudomonas aeruginosa was inhibited in the case of MLE at concentrations of 15 mg/ml and higher. At doses of 10 mg/ml, Staphylococcus aureus and Salmonella typhi began to exhibit noticeable activity. The Zone of Inhibition (ZOI) for ALE using the disc diffusion technique varied from 6 to 25 mm at various extract concentrations. Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa had minimum inhibitory concentrations (MIC) of 10, 15, and 15 mg/ml in ALE and 5, 5, and 7.5 mg/ml in MLE, respectively. Additionally, this finding was compared to that of the common antibiotic Gentamicin, whose MICs were lower than those of ALE and MLE. The current investigation revealed that leaf extracts, both aqueous and methanolic, exhibited antibacterial properties against Salmonella typhi, Pseudomonas aeruginosa, and Staphylococcus aureus. ***Conclusion:*** The study observed a definite antibacterial effect of both the aqueous and methanolic extract of leaves of Cinnamomum tamala against Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa. The physiologically active components of Cinnamomum tamala that give it its antibacterial properties need to be identified and isolated via more research.

Keywords: :Antibacterial activity; Cinamomum tamala; Staphylococcus aureus; Salmonella typhi; Pseudomonas aeruginosa; Zone of Inhibition; Minimum inhibitory concentration; Broth dilution and disc diffusion; Aqueous; methanolic extracts

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Correspondence: Dr. Shompa Sharmin Rasna, Assistant Professor, Department of Pharmacology and Therapeutics, Monno Medical College, Manikganj, Bangladesh. **Email**: shomparasna1207@gmail.com. **ORCID**: https://orcid.org/0009-0007-0645-7713l; **Contact no**: 01745491141

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Introduction

One of medicine's greatest triumphs of the twentieth century has been the development of antibiotic chemotherapy. This medicine is commonly used to treat a variety of microbiological illnesses, although Fleming cautioned that overuse of antibiotics may cause bacteria to become resistant. Worldwide public health is at increased risk from these drug-resistant types of microorganisms[1]. These days, because plant-based medications are inexpensive and have few adverse effects, research is being done to determine their effectiveness in traditional medicine. Approximately 80.0% of people worldwide still primarily use herbal treatments, according to the WHO[2]. The plant C. tamala, sometimes called Indian bay leaf, is a member of the Lauraceae family and genus Cinnamomum. It goes by several other names in India, including Tejpat and Tejpatta. There are up to 350 species of this plant in the world. The plant C. tamala is an evergreen plant of modest size. There are a lot of leaves on its grey-brown trunk, and its bark is delicate and wrinkled[3].

Cinnamomum tamala dried leaves are used to add taste to various culinary preparations. Aromatic essential oils found in plant bark and leaves include phenolic chemicals that have several medicinal benefits against diabetes, arteriosclerosis, Alzheimer's disease, and arthritis[4]. The most often used portion of Cinnamomum Tamala is the leaf. It has a variety of components, but the main ones are the essential oils, which include curcumenol (2.3%), β-caryophyllene (6.6%), sabinene (4.8%), germacrene D (4.6%), and furanogermenone (59.5%)[4]. It contains other ingredients including eugenol and cinnamon aldehyde, which are found in the barks and give them their scent[5]. The oil's diuretic, carminative, and anti-flatulent qualities make it useful in medicine[6]. The extract fractions of Cinnamomum Tamala contain phenol, alkaloids, and terpenoids. The spice plant has phytochemicals that are used to develop antibacterial agents[7].

Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa are important pathogens for causing hospital acquired infection. Strong antibacterial action against gram-positive and gram-negative bacteria as well as fungi is demonstrated by C. tamala. Strong antibacterial action was demonstrated by oil and its constituents against Helicobacter pylori, Pseudomonas aeruginosa, Bacillus subtilis, Aspergillus fumigatus, and methicillin-resistant Staphylococcus aureus. Essential oil has shown growth-inhibiting properties against Escherichia coli and Mycobacterium tuberculosis[8].

Cinnamomum tamala leaf methanolic extract also significantly lowers blood glucose, blood glycosylated hemoglobin, LPO, serum AST, and ALT levels while significantly raising antioxidant enzymes like superoxide dismutase. It could be applied as a diabetic adjunct treatment[9]. Asthma, colic, blood dysentery, diarrhea, constipation, flatulence, indigestion, jaundice, hyperacidity, anorexia, dysmenorrhea, leucorrhea, postpartum hemorrhage, high fever, skin conditions, sore throat, sexual weakness, and tuberculosis have all been treated with this plant for a long time[10].

It has a very promising role in having diverse activities. Therefore, the present study was aimed to evaluate the antibacterial effects of Aqueous and Methanolic extract of Leaves of Indian bay leaf against some food-borne pathogens.

Methodology

Study Settings and Population: From July 2019 to June 2020, this experimental study was conducted in the Department of Pharmacology & Therapeutics in cooperation with the Department of Microbiology at Mymensingh Medical College, Mymensingh, Bangladesh.

Study Procedure:

Tested Bacterial Strains: Bacterial strains, Staphylococcus aureus ATCC 25923, Salmonella typhi ATCC 14028 and Pseudomonas aeruginosa (ATCC 27853) were used in the study. The Department of Microbiology at Mymensingh Medical College in Mymensingh provided the pure cultures of these.

Maintenance of bacterial culture and inoculum preparation: On nutrient agar slants and plates, pure cultures were routinely maintained and replenished. Following a 24-hour incubation period at 37°C, the cultures were streaked on sterile nutrient agar plates and then refrigerated at 4°C. To prevent contamination, bacterial cultures were replaced every one to two weeks. The pure bacterial culture was grown in nutrient broth at 37°C for the whole night to create the inoculum.

Plant Material: The fresh tender leaves were purchased from a rural area of Mymensingh.

Preparation of Aqueous Leaf Extract: The leaves were washed and dried in the shade at room temperature for six to seven days. Finally, dried materials were pulverized into fine powdered substances by a grinder. 50 gm of powder of Indian bay leaves were weighed with the electric balance and transferred into one conical flask. Then 500 ml distilled water in the flask was added. The solution was kept at room temperature for at least 24 hr. The aqueous extract was then

filtered by using a muslin cloth. The filtrate was again filtered using Whatman no.1 filter paper under strict aseptic conditions. The resulting filtrate was collected in previously tared sterilized Petri plates and dried in a rotary flash evaporator at 450 C for proper dehydration. After the complete removal of the solvent, the Petri plates were weighed and then the net weight of the dried extract was determined and used. 1gm dried extract was then dissolved in 50ml sterilized distilled water.

For the preparation of the aqueous stock solution, 1gm of ALE was dissolved in 50 ml of D/W to get a concentration of 0.02 gm/ml i.e. 20mg/ml which was labeled as stock solution. From the above stock solution, different concentrations such as 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, and 0.625mg/ml were prepared with appropriate volumes of D/W.

Table 1:Preparation of the ALE solutions of different concentrations

Amount of Solution (ml) taken from Stock Solution	Amount of Distilled Water (ml)	Concentration (milligram /ml)
1	31	0.625
1	15	1.25
1	7	2.5
1	3	5
1	1	10
1	00	20

Preparation of Methanolic Leaf Extract: The leaves were washed and dried in the shade at room temperature for six to seven days. Finally, dried materials were pulverized into fine powdered substance by a grinder. 50 gm of powder of Indian bay leaves were weighed with the electric balance and transferred into one conical flask. Then 500 ml 100% Methanol in the flask was added. The solution was kept at room temperature for at least 24 hr. The methanolic extract was then filtered by using a muslin cloth. The filtrate was again filtered using Whatman no.1 filter paper under strict aseptic conditions. The resulting filtrate was collected in previously tared sterilized petriplates and dried in a rotary flash evaporator at 45℃ for proper dehydration. After the complete removal of the solvent, the petriplates were weighed and then the net weight of dried extract was determined and used. 1gm dried extract was then dissolved in 50ml sterilized distilled water. For the preparation of the methanolic stock solution, 1gm of MLE was dissolved in 50 ml of D/W to get a concentration of 0.02 gm/ml i.e. 20mg/ml which was labeled as stock solution. From the above stock solution, different concentrations such as 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, and 0.625mg/ml were prepared with appropriate volumes of methanol.

Table 2: Preparation of the MLE Solutions of Different Concentration

Amount of solution (ml) taken from a stock solution	Amount of Distilled Water (ml)	Concentration (milligram /ml)
1	31	0.625
1	15	1.25
1	7	2.5
1	3	5
1	1	10
1	00	20

Antibacterial Sensitivity Testing Using Disc Diffusion Method: Antibacterial sensitivity test was performed by Kirby-Bauer disc diffusion technique. Filter paper disc of 6mm diameter using Whatman No[1]. filter paper was prepared and sterilized. After matching with 0.5 McFarland standards for each isolate, a sterile cotton swab was dipped into bacterial suspension and streaked in three directions on the surface of Mueller Hinton Agar plates and then left for 5-10 minutes at room temperature. The blank discs were aseptically placed over the Mueller Hinton agar plates seeded with the test microorganisms. Then with the help of micropipette 10µl 20mg/ml, 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, and 0.625mg/ml concentrations of Aqueous & methanolic leaf Extracts were transferred to different disc aseptically. while 10µL of distilled water & 100% methanol were added in a sterile filter paper disc as a negative control in both extracts. Plates were incubated at 37°C for 24 hours. After 24 hours the results were recorded. The antibacterial activity results were expressed in terms of the diameter of the zone of inhibition <9mm zone was considered inactive; 9-12mm was partially active; while 13-18mm was active and >18mm was very active as described in Gupta et al[11].

Determination of Minimum Inhibitory Concentration (MIC) of *Cinnamomum tamala* Leaves Extract Against Test Bacteria by Broth Dilution Method

Preparation of ALE Stock and Working Solutions: As described before, 1gm Aqueous extracts powder was dissolved in 50 ml D/W in which 1 ml of solution contained .02 gm or 20 mg of ALE powder and it was the stock solution used to prepare ALE working solutions. Sets I, II, III, IV, V, VI, and VII respectively were made in different test tubes by mixing the measured amount of ALE stock solution with the measured amount of nutrient broth

medium. The concentrations of these sets were 15mg/ml, 10mg/ml, 7.5mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, and 0.625mg/ml ALE respectively. Set-VIII (Control-1) was . made with ALE stock solution. Set-IX (Control-2) was made with a nutrient broth medium. Set-X (Control-3) was made with nutrient broth medium in test tubes.

Preparation of MLE Stock and Working Solutions: As described before, stock solution 20 mg/ml. Different sets of working solutions & controls were prepared as described before.

Inoculation of Bacterial Suspension to Working Solutions of ALE & MLE in Test Tubes: After matching the turbidity of bacterial suspension with 0.5 McFarland standards, 20µl of bacterial suspension of *Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa* were separately added to each concentration of working solutions of ALE & MLE in separate test tubes. These inoculums were also added to the Control-1 and 2, but not to Control-3. These were Incubated at 370 C for 18-24 hours.

Examination of Growth of Test Organisms in Different Concentrations of ALE & MLE: After 18 to 24 hours of incubation, the growth of test organisms in different preparations of ALE & MLE were examined and compared against that of controls by matching their turbidity. The clear preparations were considered as "No growth" of bacteria and turbid ones, as "Growth of bacteria". The MIC was reported as the lowest concentration of ALE & MLE required to prevent the visible growth of test organisms.**Testing Antimicrobial Activity of a Standard Antibiotic:** The test microorganisms P. aeruginosa were also tested for their activity against the antibiotic Gentamicin (inj. 80mg) by broth dilution method.

Statistical analysis: Findings were recorded and analyzed. Collected data were checked and edited first and processed with the help of the software Statistical Package for Social Sciences (SPSS) version 21 and analyzed. Statistical analyses were done by using appropriate statistical tools. Statistical significance was assessed at the 0.05 level for all analyses.

Ethical Clearance: Ethical Clearance: Institutional Review Board (IRB) clearance, Memo no. MMC/IRB/2020/240, Dated 11/02/2020. This is to certify that the thesis protocol entitles Antibacterial activities of Indian bay leaf (Cinnamomum tamala) leaves extracts against *Staphylococcus aureus, Salmonella typhi,* and *Pseudomonas aeruginosa* submitted by Dr. Shompa Sharmin Rasna as a student of M. Phil. (Pharmacology) part- Final has been reviewed and approved by the Institutional Review Board (IRB) of Mymensingh Medical College.

Table 3 : Preparation of the MLE Solutions of Different Concentration

No. of Sets	ALE Solution (ml)	Nutrient broth medium (ml)	Total (ml)	Concentration of ALE (mg/ml)	Test Organism (µl)
Set- I	7.5	2.5	10	15	20
Set- II	5	5	10	10	20
Set- III	3.75	6.25	10	7.5	20
Set- IV	2.5	7.5	10	5	20
Set- V	1.25	8.75	10	2.5	20
Set- VI	0.625	9.375	10	1.25	20
Set- VII	0.3125	9.6875	10	0.625	20
Set- VIII C-1	10	0	10	20	20
Set-IX C-2	-	10	10	-	20
Set-X C-3	-	10	10	-	-

Table 4: Composition and Different Concentrations of Working MLE Solutions and the Controls

No. of Sets	MLE stock Solution (ml)	Nutrient broth medium (ml)	Total (ml)	Concentration of MLE (mg/ml)	Test Organism (µl)
Set- I	7.5	2.5	10	15	20
Set- II	5	5	10	10	20
Set- III	3.75	6.25	10	7.5	20
Set- IV	2.5	7.5	10	5	20
Set- V	1.25	8.75	10	2.5	20
Set- VI	0.625	9.375	10	1.25	20
Set- VII	0.3125	9.6875	10	0.625	20
Set- VIII C-1	10	0	10	20	20
Set-IX C-2	-	10	10	-	20
Set-X C-3	-	10	10	-	-

Table 5: Composition and Different Concentrations of Working Gentamicin Solutions and the Controls

No. of Sets	Gentamicin Stock Solution-2 (ml)	Nutrient Broth Medium (ml)	Total (ml)	Concentration of Gentamicin (µg/ml)	Test Organism (µl)
Set-I	2	8	10	2	20
Set-II	1.5	8.5	10	1.5	20
Set-III	1	9	10	1	20
Set-IV	0.75	9.25	10	0.75	20
Set-V	0.5	9.5	10	0.5	20
Set-VI	0.25	9.75	10	0.25	20
Set-VII (C-1)	-	10	10	-	20
Set-VIII (C-2)	-	10	10	-	-

Results

Indian bay leaves were shown to be efficient against the test bacterial strains in this investigation. When using the disc diffusion technique, the zones of inhibition for ALE Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa were 18 mm, 21 mm, and 25 mm respectively, at doses of 20 mg/ml. The greatest zone of inhibition against Pseudomonas aeruginosa (25 mm) was seen at a dosage of 20 mg/ml. Salmonella typhi and Staphylococcus aureus also began to exhibit distinct activity at 15 mg/ml conc, while Pseudomonas aeruginosa did the same. At 20 mg/ml concentrations, the zone of inhibition for MLE Staphylococcus aureus, Salmonella typhi, and Pseudomonas aeruginosa was 21 mm, 25 mm, and 26 mm, respectively against the aforementioned microorganisms. Pseudomonas aeruginosa showed the largest zone of inhibition at a dosage of 20 mg/ml (26 mm).

Pseudomonas aeruginosa begins to exhibit clear activity at concentrations of 20 mg/ml, whereas Salmonella typhi and Staphylococcus aureus begin to show clear activity at 10 mg/ml conc. There was no zone against any bacterium on the negative control disc, which contained solely D/W and methanol. Pseudomonas aeruginosa was determined to represent the test organism most vulnerable to ALE and MLE. As seen in the image, the outcomes of the broth dilution method for extracting leaves were also contrasted with those of the common antibiotic Gentamicin. The MICs of the aqueous extract for Salmonella typhi (15 mg/ml), Pseudomonas aeruginosa (15 mg/ml) and Staphylococcus aureus (10 mg/ml) were determined using the broth dilution method. Pseudomonas aeruginosa 7.5 mg/ml, Salmonella typhi 5 mg/ml and Staphylococcus aureus 5 mg/ml are the MICs of the Indian bay leaf methanolic extract for the test organisms. Gentamicin had minimum inhibitory concentrations (MICs) of 1µg/ml, 1µg/ml, and 1.5µg/ml against Salmonella typhi, P. aeruginosa and S. aureus respectively According to CLSI (2016), standard sensitive MICs of Gentamicin for test bacteria were ≤4µg/ml, consistent with my study results[12].

Table 6: Antibacterial Activity of Different Concentrations of ALE Measured in Zone of Inhibition

Concentrations of ALE solutions in milligram	Zone of Inhibition (ZOI) in mm		
	Staphylococcus aureus	*Salmonella typhi*	*Pseudomonas aeruginosa*
20	18	21	25
10	08	08	08
5	07	07	07
2.5	06	06	06
1.25	06	06	06
0.625	06	06	06
Control	06	06	06

Table 7: Antibacterial Activity of Different Concentrations of MLE Measured in Zone of Inhibition

Concentrations of MLE solutions in miligram/ml	Zone of Inhibition (ZOI) in mm		
	Staphylococcus aureus	*Salmonella typhi*	*Pseudomonas aeruginosa*
20	21	25	26
10	15	21	09
05	10	08	07
2.5	07	07	6.5
1.25	06	06	06
0.625	06	06	06
Control	06	06	06

Figure II: Determination of MIC of ALE against test organisms

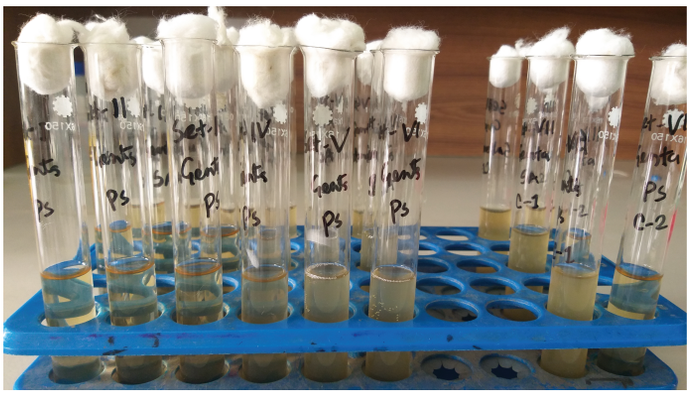

Figure III: MIC of Gentamicin

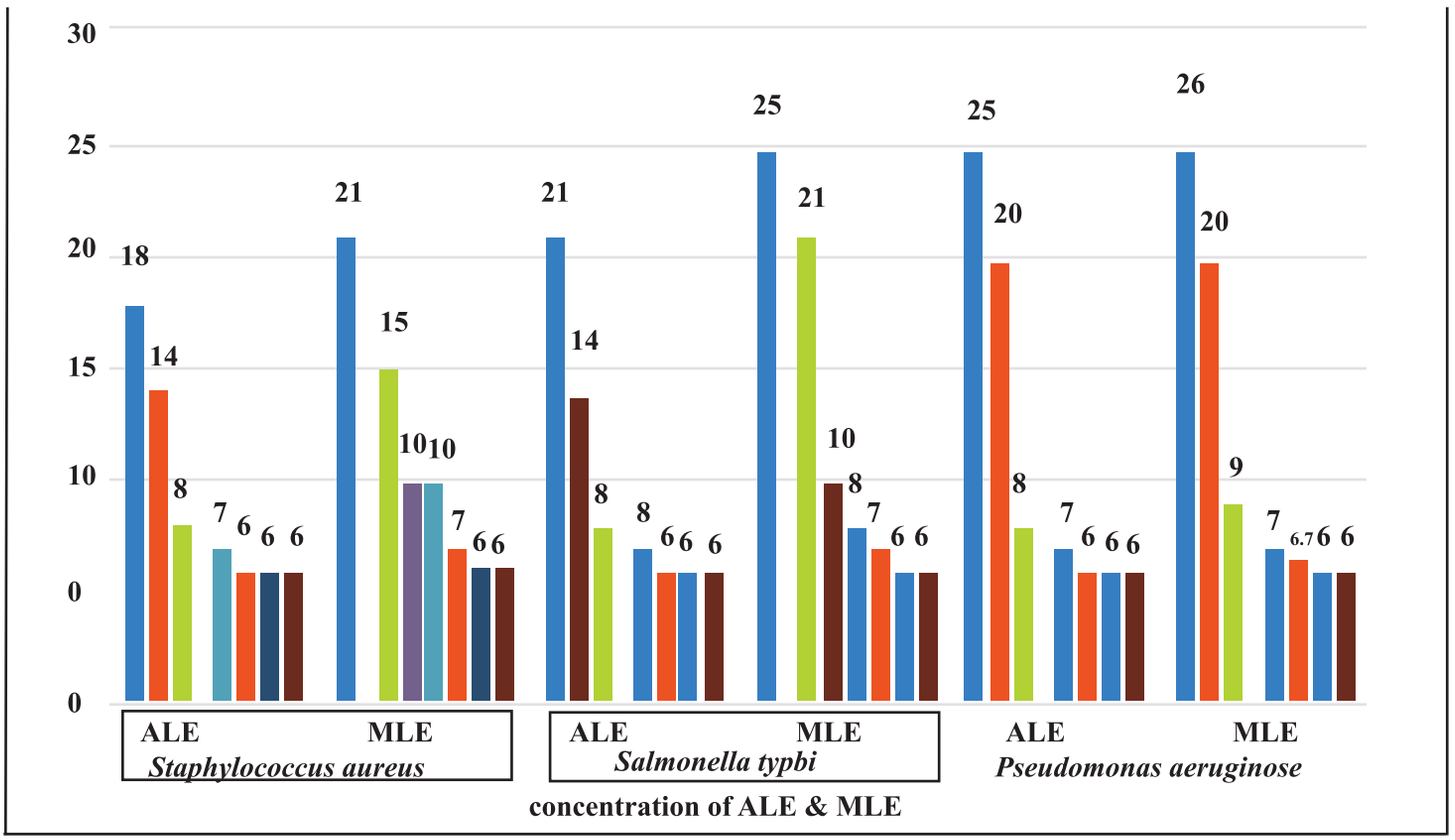

Figure I: Multiple bar diagram showing comparison of Zone of Inhibition (ZOI) between ALE & MLE

Table 8: MIC of ALE against *Staphylococcus aureus*, S*almonella typhi,* and *Pseudomonas aeruginosa*

No. of sets	Concentrations (ALE) mg/ml	*S. aureus*	*S. typhi*	*P. aeruginosa*
Set-I	15	No growth	No growth	No Growth
Set-II	10	No growth	Growth	Growth
Set-III	7.5	Growth	Growth	Growth
Set-IV	05	Growth	Growth	Growth
Set-V	2.5	Growth	Growth	Growth
Set-VI	1.25	Growth	Growth	Growth
Set-VII	0.625	Growth	Growth	Growth
	Controls			
Set-VIII C-1	20	No growth	No growth	No growth
Set-IX C-2	NB media + Bacteria	Growth	Growth	Growth
Set-X C-3	NB media + No Bacteria	No growth	No growth	No growth

Table 9: MIC of MLE against *Staphylococcus aureus*, *Pseudomonas aeruginos*, and *Salmonella typhi.*

Number of Sets	Concentrations (MLE) mg/ml	*S. aureus*	*S.typhi*	*P. aeruginosa*
Set-I	15	No growth	No growth	No growth
Set-II	10	No growth	No growth	No growth
Set-III	7.5	No growth	No growth	No growth
Set-IV	5	No growth	No growth	Growth
Set-V	2.5	Growth	Growth	Growth
Set-VI	1.25	Growth	Growth	Growth
Set-VII	0.625	Growth	Growth	Growth
	Controls			
Set-VII C-1	20	No growth	No growth	No growth
Set-IX C-2	NB media +Bacteria	Growth	Growth	Growth
Set-X C-3	NB media +No Bacteria	No growth	No growth	No growth

Table 10: Comparison between MICs of ALE, MLE, and Gentamicin against *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Salmonella typhi*

MIC of	Test Organisms		
	Staphylococcus aureus	*Pseudomonas aeruginosa*	*Salmonella typhi*
ALE	10 mg/ml	15 mg/ml	15 mg/ml
MLE	5 mg/ml	7.5 mg/ml	5 mg/ml
Gentamicin	1 µg/ml	1 µg/ml	1.5 µg/ml

Discussion

In the present study, the in vitro antibacterial activity of ALE and MLE was quantitatively evaluated based on the zone of inhibition by disc diffusion method, and the MIC was assessed by broth dilution technique. Different concentrations of the extract exhibited varying degrees of inhibitory effect. Several studies have been conducted to evaluate the antibacterial properties of Cinnamomum tamala

Waseem et al[13] analyzed the antimicrobial activity of Cinnamomum tamala leaves against *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi,* and other different bacteria by agar well diffusion method using different solvents such as aqueous, hexane, isobutanol, crude extract. In the aqueous extract, ZOI for *Staphylococcus aureus* was 14 mm and 15 mm, and for P. aeruginosa 17 mm and 19 mm at 6µl and 12µl concentration respectively. There was no effect observed in aqueous leaf extract against Salmonella typhi. This finding against *S. aureus* was somewhat similar to the present study in which ZOI against *S. aureus* was 14mm at 15 mg/ml. *Pseudomonas aeruginosa* showed 20 mm ZOI at 15 mg/ml of the extract. There was little difference in result which may be due to extraction procedure or different organism strain. Shete and Chitanand[14] examined the antimicrobial activity of some commonly used Indian spices against pathogenic organisms including *Staphylococcus aureus, Salmonella typhi,* and *Pseudomonas aeruginosa* using aqueous, ethanolic, and methanolic extract by well diffusion methods. There was no effect observed against *Pseudomonas aeruginosa.* In the aqueous extract for *S. aureus* ZOI was 12 mm and for *S. typhi* was 12 mm. In the present study for *S. aureus* and *S. typhi* was 14 mm at 15 mg/ml concentration of extract. This a bit difference in result may be due to extraction procedures or different organism strains.

In methanolic extract, ZOI for *Staphylococcus aureus* was 12 mm, for *Pseudomonas aeruginosa* was 15 mm and for *Salmonella typhi* was 14 mm. In the present study, ZOI for *Staphylococcus aureus* was 15 mm at 10 mg/ml, *Pseudomonas aeruginosa* had 20 mm at 15 mg/ml and *Salmonella typhi* showed 21mm at 10 mg/ml concentration of extract. The difference in results may be attributed to the difference in the concentration of extract or the difference of strains of the organism by the aforementioned researchers.

Another study was carried out by Sukumar et al[15] to assess of bioactivity of cinnamomum tamala against *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and other bacteria by broth dilution method using aqueous and methanolic extract. In the aqueous extract, the MIC for *Staphylococcus aureus* was 10 mg/ml, for *Salmonella typhi* MIC was 13 mg/ml and the MIC for *Pseudomonas aeruginosa* was 13 mg/ml. In the present study the MIC for S*taphylococcus aureus* was 10 mg/ml, for *Salmonella typhi* was 15 mg/ml, and for *Pseudomonas aeruginosa* was 15 mg/ml which is similar to that study.

Sukumar et al[16] examined the antibacterial activities of Cinnamomum tamala against *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and other bacteria by broth dilution method using aqueous and methanolic extract. In the methanolic extract, the MIC for *Staphylococcus aureus* was 2.25 mg/ml, for *S. typhi* MIC was 9 mg/ml and the MIC for *Pseudomonas aeruginosa* was 9 mg/ml. In the present study, the MIC for *S. aureus* was 5 mg/ml, *Salmonella typhi* was 5 mg/ml and *Pseudomonas aeruginosa* was 7.5 mg/ml. This finding of the present study is somewhat similar to that study.

Another study was carried out by Sarita et al[17] to assess the antibacterial activity of some medicinal plants such as Cinnamomum tamala against human pathogenic bacteria including *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*. In methanolic extract MIC for *Staphylococcus aureus* was 12.5 mg/ml whereas there was no effect against *Salmonella typhi* and *Pseudomonas aeruginosa* in the study which did not coincide with the present study where the MIC for S*taphylococcus aureus* was 5 mg/ml for *Salmonella typhi* was 5 mg/ml and for *Pseudomonas aeruginosa* was 7.5 mg/ml. This dissimilarity may be attributed to the difference in the procedure for preparation of extract or difference of strains of organisms by the aforementioned researchers.

Conclusion

Extracts from Indian bay leaf have the potential to be developed as a medicinal agent in preventing bacteria-related disorders. Additional research is needed to identify and isolate the biologically active components in Indian bay leaves that are responsible for this antibacterial action. The practice of employing medicinal plants like Indian bay leaf as supplementary or alternative medicine in underdeveloped nations may lower not only the clinical burden of drug resistance development but also the side effects and cost of the treatment when compared to manufactured drugs.

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Original Article

Clinical Presentation of Somatic Complaints among Generalized Anxiety Disorder Patients in Manikganj District of Bangladesh

Md Jahangir Hossain[1], Chayon Kumar Das[2], Kamrul Islam[3], Mohammad Asraful Siddike Pathan[4]

[1]Professor, Department of Psychiatry, Monno Medical College and Hospital, Manikganj, Bangladesh; [2]Clinical Psychologist, Department of Psychiatry, Monno Medical College and Hospital, Manikganj, Bangladesh; [3]Associate Professor, Department of Dermatology and Venerology, Monno Medical College and Hospital, Manikganj, Bangladesh; [4]Associate Professor, Department of Psychiatry, Community Based Medical College, Mymensingh, Bangladesh

Abstract

Background: :Generalized Anxiety Disorder (GAD) is such a psychiatric disorder where some specific somatic symptoms are prerequisite to confirm the diagnosis. ***Objective:*** This study was aimed at assessing somatic complaints reported by Generalized Anxiety Disorder (GAD) patients during treatment seeking in Bangladesh. ***Methodology:*** This cross-sectional survey design using purposive sampling technique was carried out in two psychiatric settings of Manikganj district from January, 2024 to December 2024. Data were collected by a psychiatrist who confirmed the diagnosis of GAD following the diagnostic criteria of Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5). Only adult GAD patients who provided consent to participate were included in this study. Ethics to conduct study with human participants were maintained strictly. ***Results:*** 228 participants with age range of 18-75 participated in this study in which most of the participants were female, married, and from rural areas. Somatic complaints reported by the participants while consulting with a psychiatrist can be categorized in three types: (i) head related complaints (ii) gastrointestinal complaints and (iii) palpitations. Head related complaints were Hotness in the head (84.65%), headache (69.73%), burning sensation in the head (60.96%), heaviness in the head (25.44%), uneasiness in the head (20.61%) and vertigo (19.74%). Reported gastrointestinal complaints by the participants were- lack of appetite (35.96%), gas formation (26.32%), constipation (16.23%) and vomiting (13.16%). Palpitations were also reported by 18.42% participants. **Conclusion:** Somatic complaints identified in this study will guide physicians and mental health professionals not only to ensure early diagnosis but also to provide appropriate treatment towards GAD patients of Bangladesh.

Keywords: Generalized anxiety disorder; GAD; somatic symptoms; cultural dimension
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Correspondence: Dr. Md Jahangir Hossain, Professor, Department of Psychiatry, Monno Medical College and Hospital, Manikganj, Bangladesh; Email: jhpsy2014@gmail.com, Orcid: https://orcid.org/0009-0006-6048-028X. Contact no: 01917281728

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Introduction

Anxiety disorders are a significant concern for a low-resourced country like Bangladesh where varied prevalence has been identified across different populations. Last nationwide national mental health survey of Bangladesh reported a prevalence of 4.7% and 4.5% for anxiety disorders among adults and children respectively[1].

High prevalence of anxiety was found among Bangladeshi school going students[2], university students[3], health care workers[4] slum dwellers[5] garment workers[6] in different studies. In addition, age, gender, family history of mental illness, lifestyle factors, and psychosocial factors were identified as risk factors of anxiety disorder in Bangladesh[7].

The term "Generalized Anxiety Disorder" was first coined during the initiation of third edition of Diagnostic and Statistical Manual (DSM-III)[8]. In previous editions of Diagnostic Statistical Manual (DSM), anxiety disorders were grouped under the general category of "Neurosis or Neurotic Disorders". So, The DSM-III marked a significant shift through introducing Generalized Anxiety Disorder (GAD) as a separate diagnosis distinct from other anxiety disorders (e. g. panic disorder, phobia). GAD was defined as excessive anxiety and worry (apprehensive expectation) about a number of events or activities. This anxiety and worry should be occurring more days than not for at least 6 months.

Diagnostic criteria of current version of DSM, DSM-5, emphasized cognitive symptoms of difficulty to control the worry[9]. Concomitantly, at least three of the following symptoms needs to be present for adults to be diagnosed as GAD patients- restlessness (feeling keyed up or on), being easily fatigued, difficulty concentrating (mind going blank), irritability, muscle tension and sleep disturbance[9]. So, to confirm the diagnosis of GAD, cognitive, emotional and physical complaints are required.

Bangladesh is a country with high stigma related to psychiatric disorders[10,11]. As a result, psychological symptoms are often misunderstood and underrepresented. Somatization of psychological symptoms is very common in Bangladesh[12]. Patients' tendency to present somatic complaints when encountering psychological issues if not explored is evident. As a result, the referral pathway of patients to psychiatric treatment in Bangladesh is bothersome[13]. In addition, western psychiatric diagnostic concepts in a non-western country like Bangladesh needs considering cultural dimension. So, considering cultural aspects and high levels of stigma related to psychiatric disorders in Bangladesh this study aimed at identifying somatic complaints reported by GAD patients during psychiatric treatment seeking in Bangladesh.

Methodology

Study Settings and Population: A cross-sectional survey design was implemented to conduct the study in two psychiatric settings of Manikganj. Data were collected from GAD diagnosed psychiatric patients through purposive sampling technique from January, 2024 to December, 2024. Diagnosis was confirmed using DSM-5 by a psychiatrist. Apart from diagnosis, only adult participants who provided consent were included in this study.

Sample Collection Procedure: Data were collected through face- to- face interviews using semi-structured questionnaires. The questionnaire of this study started with explanatory statements of the research and consent form. A personal information form was used to collect demographic information of the participants. Age, sex, residence, marital status, and duration of mental illness in months were collected as demographic information. Somatic symptoms were noted during psychiatric consultation of the participants.

Statistical Analysis: Statistical analyses were performed with SPSS software, versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Categorical or discrete data were summarized in terms of frequency counts and percentages. When values are missing, the denominator was stated. Every effort was made to obtain missing data.

Ethical Clearance: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the Institutional research ethics. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and analyzed using the coding system.

Results

Details of Participants: A total of 228 participants participated in this study (Table 1). The average age of the participants was found to be 39.22 with a range of 18 to 75. Most (173, 75.88%) of the participants were female and others (55, 24.12%) were male. 197 (86.40%) participants were married whereas 9 (3.95%) participants were unmarried. Among other 22 participants, 14 (6,14%), 4 (1.76%), and 4 (1.76%) were widowed, separated and divorced respectively. Most (133, 58.33%) of the participants were residents of rural areas. The average duration of mental illness was found to be 20.93 months ranging from 6 to 120 months.

Somatic Complaints among Participants: Somatic complaints related to "head" were the most common. Hotness in the head was reported by 84.65% of the GAD patients. In addition, headache and burning sensation in the head were mentioned by 69.73% and 60.96% participants respectively. 25.44% respondents mentioned heaviness in the head and 20.61% reported uneasiness feeling in the head.

19.74% reported vertigo. Apart from head related somatic complaints, gastrointestinal complaints were also common. Among gastrointestinal complaints, lack of appetite (82, 35.96%), gas formation (60, 26.32%), constipation (37, 16.23%) and vomiting (30, 13.16%) were reported by the participants. 18.42% participants also complained of experiencing palpitation (Table 2).

Table 1. Demographic Characteristics of the Participants

Demographic Characteristics	Frequency	Percent
Age Group		
18 to 30 Years	73	32.0
31 to 45 Years	95	41.7
46 to 60 Years	44	19.3
≥ 61 Years	16	7.0
Gender		
Male	55	24.1
Female	173	75.9
Marital status		
Married	197	86.4
Unmarried	9	3.9
Others	22	9.7
Residence		
Rural	133	58.3
Urban	95	41.7
Duration of mental illness		
6 to 60 months	214	93.9
60 to 120 months	14	6.1
Total	228	100.0

Table 2. Reported Somatic Complaints by the Participants

Somatic Complaints	Frequency	Percent
Headache	159	69.73
Hotness in the head	193	84.65
Heaviness in the head	58	25.44
Burning sensation in the head	139	60.96
Vertigo	45	19.74
Uneasiness feeling in the head	47	20.61
Vomiting	30	13.16
Gas formation	60	26.32
Palpitation	42	18.42
Lack of appetite	82	35.96
Constipation	37	16.23

Discussion

This study was conducted with a view to identifying somatic complaints among GAD patients during psychiatric treatment seeking. According to DSM-5, GAD is a disorder in which somatic symptoms (e.g., restlessness, fatigue, muscle tension) are needed along with other symptoms to confirm the diagnosis. Lack of mental health literacy and stigma can lead to representing physical symptoms only. Such presentation of physical symptoms and inability to report psychological symptoms can lead to misdiagnosis of GAD patients which will only increase sufferings of patients. So, identifying somatic complaints among GAD patients is critical for both general physicians and mental health professionals which will ensure appropriate diagnosis, referral and treatment.

Studies have been conducted in different settings of Bangladesh to reveal psychological problems among Bangladeshi patients with different physical diseases[14,15]. These studies identified different psychological comorbidities among patients with physical health issues. But investigation of physical symptoms among psychiatric patients is rare in Bangladesh, though somatization is common. A study participating youths of Bangladesh identified reporting of different somatic complaints16. Another study which intended to uncover cultural dimension of depression in Bangladesh revealed mentioning of somatic symptoms initially[12].

Results of this study revealed that head related, gastrointestinal and palpitation were identified as somatic complaints by GAD patients of Bangladesh. Previously, anxiety and headache were found frequently together[17]. In addition, hotness in the head,[16] burning sensa tion in the head,[18] heaviness in the head,[19] uneasiness in the head[19] and vertigo[20] were also evident with anxiety. In case of gastrological symptoms, global prevalence of 14.5% generalized anxiety was found in gastroenterology and hepatology outpatients[21]. In a study of National Gastroliver Institute and Hospital of Bangladesh, positive co-relation between anxiety and dyspepsia was found[22] which is an indication of gastrological complaints among patients with GAD. Palpitation, which was also reported by participants of this study, was also one of the common symptoms of anxiety[23]. Though results revealed in this study showed congruence with previous findings of anxiety disorders from different countries, it was not found to be identical with the somatic symptoms of DSM-5. This difference indicates cultural dimension somatic symptoms of GAD in Bangladesh.

Limitations of this study also need to be considered. Firstly, diagnosis of GAD was not conducted using any diagnostic tool like Structured Clinical Interview for DSM-5 (SCID-5). Participants were diagnosed based on the interview conducted by an experienced psychiatrist. Secondly, as the study was conducted in Manikganj, representativeness of the sample as the Bangladeshi population is questionable. Lastly, this study was conducted during treatment seeking of the participants. A community-based explorative study with the use of a valid screening tool is recommended in future studies.

Conclusion

This cross-sectional study in Manikganj district identified distinct somatic complaints of GAD patients in Bangladesh. Moreover, somatic complaints identified in this study differ from physical symptoms of GAD mentioned in DSM-5 which indicated cultural dimension in somatic symptoms of GAD patients in Bangladesh. This study urged the need for further exploration of GAD symptoms in Bangladesh. Physicians and mental health professionals should also consider these identified symptoms of GAD patients in Bangladesh while providing treatment, diagnosing, and referring patients to lessen the burden of disease.

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Original Article

Combined Antinociceptive Effects of Alpha-Tocopherol and Diclofenac in Acetic Acid-Induced Writhing Test

Tasneema Juaira[1], Mohammad Asaduzzaman Patwary[2], Habiba Begum[3], Munira Afrin[4], Mohammad Nahid Salman[5], Puspita Basak[6]

[1]Associate Professor, Department of Physiology, Monno Medical College, Manikganj, Bangladesh; [2]Junior Consultant, Department of Pediatric Nephrology, Square Hospitals Ltd, Dhaka, Bangladesh; [3]Associate Professor, Department of Microbiology, Delta Medical College, Dhaka, Bangladesh; [4]Professor, Department of Pharmacology, Monno Medical College, Manikganj, Bangladesh; [5]Associate Professor, Department of Physiology, Ad-din Medical College, Khulna, Bangladesh; [6]Assistant Professor, Department of Physiology, Monno Medical College, Manikganj, Bangladesh

Abstract

Background: The search for effective analgesic combinations with improved efficacy and fewer side effects is an ongoing challenge in pain management. **Objective:** The purpose of the present study was to determine the combined analgesic efficacy of alpha-tocopherol, a potent antioxidant, and diclofenac, a widely used nonsteroidal anti-inflammatory drug (NSAID), using the acetic acid-induced writhing test in rats. **Methodology:** This prospective experimental study was conducted in the Department of Physiology at Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh from January to December 2013. 15 male Long Evans rats, weighing 180 to 250 grams, were collected from Bangladesh Institute of Research and Rehabilitation for Diabetic Endocrine and Metabolic Disorders (BIRDEM), Shahbag, Dhaka, Bangladesh for this purpose. On the basis of vitamin and drug administrations, the rats were divided into three 3 groups (5 rats in each). Group A received normal saline, group B received diclofenac sodium, group C received combination of diclofenac sodium with α-tocopherol. All the groups received single dose and equal volume through intraperitoneal route 1 hour before the test. Just one hour after administrations, rats of each group were subjected to the acetic acid induced writhing test. After the completion of experiments, all the rats were sacrificed immediately to decrease their sufferings. **Results:** This study suggests that combining αT and DS in a single dose may be more beneficial than administering DS alone in reducing nociceptive and inflammatory pain. **Conclusion:** The results indicate a synergistic interaction between the two compounds, suggesting a potential role for alpha-tocopherol as an adjuvant in pain therapy.

Keywords: Pain; analgesic α-tocopherol; writhing test
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Correspondence: Dr. Tasneema Juaira, Associate Professor, Department of Physiology, Monno Medical College, Monno City, Gilondo, Manikganj, Bangladesh; Email: sumontasneem143@gmail.com, ORCID: https://orcid.org/0009-0008-5326-8613; Contact No.: +8801718012137.

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Introduction

Pain is a complex physiological response, often associated with inflammation and oxidative stress. It is an unpleasant emotional and sensory experience[1]. The most frequent reason for visiting a doctor is pain and suffering, which is consequently widespread. In a variety of medical conditions, pain is a common presenting sign and is linked to acute tissue damage, disease, or intervention[2,3]. Vital physiological sensation nociceptive pain is brought on by stimulation of peripheral nerve fibers. Nociceptors have different thresholds or sensitivities. Which acts as a crucial tissue damage early warning system. Nociceptive pain can

be mechanical, thermal, chemical, or electrical[3-4]. Diclofenac, a cyclooxygenase (COX) inhibitor, is effective in managing inflammatory pain but is associated with gastrointestinal and cardiovascular side effects[5,6]. α-tocopherol is a fat-soluble vitamin and widely known to be one of the reactive oxygen species (ROS) scavengers and a drug that has been shown to reduce the pain responses induced by various causes in animal pain models and also in human it has shown promise as an antioxidant with anti-inflammatory properties[7-9].

To detect the severity of pain, its measurement is necessary This study aims to evaluate the antinociceptive effects of alpha-tocopherol in combination with diclofenac sodium using the acetic acid-induced writhing test in rats, a well-established model for peripheral nociception. This test is sensitive to opiates as well as non-opiate analgesics[10]. However, there is not enough information currently available to make any definitive judgments on this topic. It has not been reported that the analgesic effects of DS and α T together are comparable to that of individual administration of DS on nociceptive pain.

Methodology

Study Settings and Population: This prospective experimental study was conducted at Bangabandhu Sheikh Mujib Medical University (BSMMU) in the Pain Laboratory of the Department of Physiology between January 2013 to December 2013. Fifteen (15) apparently healthy adult male Long Evans rats weighing 180-250g were collected from Bangladesh Institute of Research and Rehabilitation for Diabetic Endocrine and Metabolic Disorders (BIRDEM), Shahbag, Dhaka, Bangladesh.

Study Procedure: It was 12/12 light/dark for the rats. The rats' thermo-neutral zone was regulated between 27 and 28°C. All rats had free access to a conventional laboratory diet and cooled boiled water. They were held there for seven (7) days before the trial to acclimate. To avoid circadian effects, all trials were conducted between 08:00 and 16:00 hours[11].

Allocation: On the basis of vitamin and drug administrations, all the rats were divided into three (3) groups (5 rats in each). The control group received normal saline at a dose of 5ml/kg body weight, one experimental group received diclofenac sodium at a dose of 10mg/kg body weight, and another experimental group received DS and αT at doses of 10mg/kg body weight and 500mg/kg body weight, respectively. 1 hour before the test, each group got a single dosage of the same volume (1ml) administered intraperitoneally. They were treated to an acetic acid induced writhing test just one (1) hour after injection. All the groups received single dose and equal volume through intraperitoneal route. Pain assessment was done by Writhing test[12,13].

Follow Up and Outcome Measures: On the day of experiment, the rats were placed in a plexiglas observation chamber (30× 30× 20 cm3) for acclimatization. Then all the rats were adminstrated by normal saline or DS or αT or combined dose of DS and αT intraperitonealy. One hour after administrations, 1ml of 2% acetic acid was injected intraperitoneally using a insulin syringe. Immediately after the injection, the latency time and the number of writhes were counted upto 60 minutes of observation period. Finally % analgesic activity was calculated by using following formula[14,15].

$$\% \text{ Analgesic activity} = \frac{\text{Mean writhing count (control group} - \text{treated group)}}{\text{Mean writhing count of control group}} \times 100$$

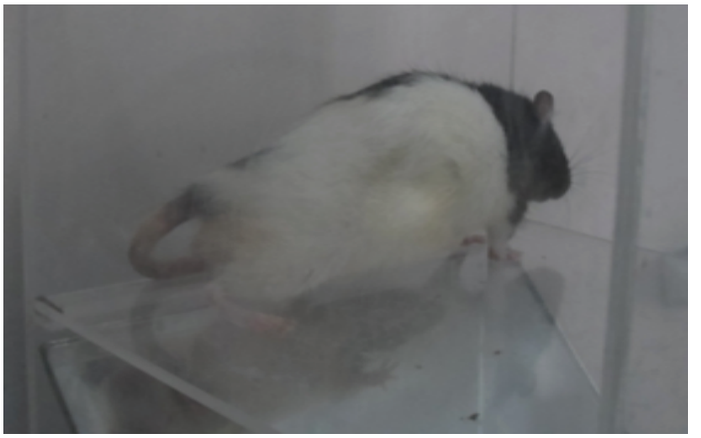

Figure I: Writhing test showing writhing response.

Latency Time: Period between acetic acid administration and the onset of the first writhing.

Writhing: Abdominal muscle contraction followed by hind limb extension.

Statistical Analysis: The results are expressed as mean standard error of the mean, and the data were evaluated statistically using ANOVA and the Bonferroni's post hoc test. The level of significance for interpreting the results was determined to be $p \leq 0.05$.

Ethical Clearance: All experiments and animal care were conducted under the guidelines established in the 'Manual for the Care and Use of Laboratory Animals' by the Animal Experimentation Ethics Committee (AEEC) of the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b 2002) and was approved by the BSMMU's institutional review board (IRB).

Results

Latency time in writhing test: The results were expressed as mean±SE and the data were statistically analyzed by Independent Student test. In the interpretation of results p 0.05 was accepted, as the level of significant. The mean±SE of duration of latency time in acetic acid induced writhing test after administration of vitamin and drug were 5.5 ± 0.63, 11.2 ± 0.34, and 14.6±0.43 minutes in group A, B, and C, respectively. The percent increments of this variable were 113.12 ± 21.03, 49.73± and 178.85 ± 30.46 in group B, and C, respectively in comparison to that of control. In this study, the differences of the mean values of among the groups were statistically significant (p≤0.05). Again, the mean values as well as percent increments were significantly higher (p≤0.05) in all the study groups than that of control. Moreover, the value of group C was significantly (p≤ 0.001) higher than that of group B.

Table 1: Mean Latency Time in Writhing Test in Different Groups of Rats (n=20)

Groups	n	Latency Time	
		Minutes	% Increment
A	5	5.5 ± 0.63 (4 to 7)	-
B	5	11.2 ± 0.34 (10 to 12)	113 ± 21.03 (64.29 to 175)
C	5	14.6 ± 0.43 (13 to 15.5)	178.85 ± 30.46 (114.29 to 275)

Data were expressed as mean±SE. Figures in parentheses indicate ranges. Percent (%) change was done in comparison to control (A); Statistical analysis was done by ANOVA followed by Bonferroni's Post Hoc test; Group A: with Normal Saline (control); Group B: with Diclofenac Sodium (10 mg/kg); Group C: with Diclofenac Sodium (10 mg/kg) + α-tocopherol (500 mg/kg); ***=significant (p≤ 0.001); **=significant (p≤0.01); n=number of rats.

Table 2: Comparison of Different Groups

Groups	P value
A vs B vs C	0.000***
A vs B	0.000***
A vs C	0.000***
B vs C	0.000***

Number of Writhes in Writhing Test: In this study, the mean±SE number of writhes in the writhing test were 71.8 ± 2.92, 33.6 ± 0.5, and 24.6 ± 1.07 Frequency / 60 minutes in group A, B, and C, respectively. The percentages of analgesic activity were 0, 53.2, and 65.73 % in group A, B, and C respectively. In addition, the percent reductions of this variable were -52.97 ± 1.57 %, -18.69 and -65.43 ± 2.4 % in group B, and C, respectively, in comparison to that of control. The differences of this mean value among the groups were statistically significant (p≤0.001). Again, the mean values as well as the percent reductions were significantly (p≤0.01) lower in all the study groups than that of the control. Moreover, the value of group B was significantly (p≤0.001) lower than that of group D.

Table 3: Mean Writhing Value in Different Groups of Rats (n=20)

Groups	n	Number of Writhes		
		Frequency /60 min	%Analgesic activity	% Reduction
A	5	71.8 ± 2.92 (67 to 83)	0	-
B	5	33.6 ± 0.5 (32 to 35)	53.2	- 52.97 ± 1.57 (-59.04 to-50.72)
C	5	24.6 ± 1.07 (22 to 28)	65.73	- 65.43 ± 2.4 (-71.08 to-58.21)

Data were expressed as mean±SE. Figures in parentheses indicate ranges. Percent (%) change was done in comparison to control (A); Statistical analysis was done by ANOVA followed by Bonferroni's Post Hoc test; Group A: with Normal Saline (control); Group B: with Diclofenac Sodium (10 mg/kg); Group C: with Diclofenac Sodium (10 mg/kg) + α-tocopherol (500 mg/kg); ***=significant (p≤0.001); **=significant (p≤ 0.01); n=number of rats.

Table 4: Comparison of Different Groups

Groups	P value
A vs B vs C	0.000***
A vs B	0.006**
A vs C	0.000***
B vs C	0.000***

Discussion

Pain is a complex, multidimensional perception. The severity of pain does not correlate with the degree of tissue damage and if not managed properly it affects a person's quality of life.16 Many studies have beenfound throughout the world to replace or at least to reduce the dose or duration

of drugs therapy, by intervening alternate pain medications. Good analgesic effect with this vitamin (500mg/kg) as a single dose in rats is found and it has been reported that toxic dietary level of this vitamin is more than 16g/kg[17]. Moreover, it has also been reported that, 10 mg/kg diclofenac might be the optimal dose to get effective response against nociceptive and inflammatory pain as well as inflammation in rat models[17-18].

Now a day's administration of combination of analgesics with antioxidants in pain treatment are applied to decrease the doses of analgesics and to prevent the negative impact of reactive oxygen species[19]. But still the information's regarding this matter is not enough to reach any final conclusion. Many experimental studies have shown analgesic and anti-inflammatory effects of αT. Significant reduction of the nociceptive pain was found after supplementation of this vitamin in rats[20-21]. Investigators from different countries were also reported these types of findings in rats and mice[22-24].

In this study, combined administration of αT and DS have shown more effectiveness in lowering pain and inflammation than individual intervention of DS, as evidenced by more decrements of all the study variables. Though the mechanisms involve for lowering the pain and inflammation cannot be explained exactly. This may be due to activation of different pain lowering pathway by αT and DS as both the mechanisms are involved and added together and causes more effectiveness[25].

Conclusion

The combination of alpha-tocopherol and diclofenac demonstrates significant synergistic antinociceptive effects in the acetic acid-induced writhing test. Further studies are needed to explore the molecular mechanisms underlying this interaction and its potential clinical applications in pain management.

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Original Article

Prevalence of Occupational Noise Induced Hearing Loss among Textile Industry Workers of Bangladesh.

Mohammad Sazzadul Haque[1], Mohammad Raqibul Alam[2], Mohammad Nasir Uddin[3], Kamrul Islam[4]

[1]Associate Professor, Department of Otolaryngology, Monno Medical College and Hospital, Manikganj, Bangladesh; [2]Assistant Professor, Department of Otolaryngology, Tangail Medical College and Hospital, Tangail, Bangladesh; [3]Professor, Department of Orthopedics, Monno Medical College and Hospital, Manikganj, Bangladesh; [4]Associate Professor, Department of Dermatology and Venrelogy, Monno Medical College and Hospital, Manikganj, Bangladesh.

Abstract

Background: Occupational noise exposure remains a major public health concern, particularly in the textile industry, where workers are continuously exposed to high-intensity noise. Noise-induced hearing loss (NIHL) is one of the most prevalent occupational health hazards, often leading to irreversible hearing impairment and reduced quality of life. Objective: This study aims to determine the prevalence of NIHL among textile industry workers in Bangladesh and identify associated risk factors to inform workplace safety strategies. **Methodology:** This cross-sectional study was conducted in the Department of Otolaryngology, Monno Medical College and Hospital, Manikganj from March 2023 To February 2024 among 185 textile worker's exposed to occupational noise levels exceeding 85 dB. Participants underwent pure-tone audiometry to assess hearing impairment. Data were collected through structured questionnaires and analyzed using SPSS version 26. **Results:** NIHL prevalence was 58.38%, with a significant association with age ($p < 0.001$). Workers aged 38–47 years had the highest prevalence (80.95%). Bilateral hearing loss was most common (69.44%), with 4000 Hz being the most affected frequency. The highest risk was observed among loom shade workers (95–100 dB). **Conclusion:** The high prevalence of NIHL underscores the need for stricter noise control measures, mandatory hearing protection, and regular audiometric screening in the textile industry. Strengthening occupational health policies is crucial to safeguarding worker well-being.

Keywords: Noise-induced hearing loss; occupational noise; textile industry; audiometry; workplace safety

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Correspondence: Dr. Mohammad Sazzadul Haque, Associate Professor, Department of Otolaryngology, Monno Medical College and Hospital, Manikganj, Bangladesh
E-mail: sazzad.h.mitul@gmail.com, ORCID: https://orcid.org/0009-0000-9194-6376, Contact no-01916438183.

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Introduction

Noise is one of the most pervasive environmental pollutants in industrial workplaces, persisting as a significant concern since the advent of the industrial revolution. It remains the most ubiquitous pollutant in industrial settings, posing widespread risks to workers' health[1]. The industrial environment, particularly the textile sector, is among the highest-risk settings for noise-induced hearing loss (NIHL) due to continuous exposure to high-frequency noise from weaving machines [2]. Studies have reported noise levels in textile mills ranging from88 to 99 dBA, with weaving rooms often exceeding 95 dBA[3]. Repeated exposure to noise levels above 85 dB causes gradual hearing loss, with louder sounds accelerating this damage[4-5]. Globally, the prevalence of NIHL varies widely, with industrial populations exhibiting rates between 37% and 59.7% depending on the sector [6].

In developing countries like Bangladesh, industrial growth has led to increased noise exposure among workers, particularly in the textile industry, which employs a significant portion of the population[7]. A 2024 study in Bangladesh reported a 14.5% prevalence of NIHL among

textile workers, linked to prolonged working hours[8]. Among the adverse effects of occupational noise exposure, NIHL is particularly concerning. NIHL is a sensorineural hearing impairment caused by prolonged exposure to excessive sound levels. It typically begins with damage to higher-frequency hearing, ranging from 3,000 to 6,000 Hz, and gradually progresses over time[9]. This condition, though largely preventable, is the second most common type of sensorineural hearing loss after presbycusis, and its impact is especially pronounced in occupational settings[10]. Occupational hearing loss remains the leading cause of preventable sensorineural hearing impairment among adults, with noise being the most ubiquitous industrial pollutant[1]. Noise exposure in the workplace not only contributes to hearing loss but also significantly impacts non-auditory health, including sleep disturbances and psychological stress. Chronic noise exposure has been linked to poor sleep quality, hypertension, and reduced cognitive function, highlighting the need for preventive measures in industrial settings[11].

The consequences of occupational noise extend beyond auditory damage. Long-term exposure to high noise levels can increase stress, which negatively affects workplace productivity. Moreover, noise exposure is associated with various health problems, such as hypertension and cardiovascular diseases, underscoring the necessity of addressing these risks. Effectively managing noise in the workplace is essential for safeguarding both employees' physical health and overall work performance[12]. Occupational health and safety services in Bangladesh are still in the process of development. The occupational health and safety framework in Bangladesh, governed by the Factory Act of 1965 and the Factory Rule of 1979, lacks standard noise regulations, leaving workers vulnerable to excessive noise exposure[8]. Given the critical health implications of occupational noise exposure, particularly in the textile industry, this study aims to evaluate the prevalence of NIHL among textile workers in Bangladesh.

Methodology

Study Settings and Population: This cross-sectional study was conducted among workers employed in textile industries to assess the effects of occupational noise exposure. The research was carried out at department of Otolaryngology, Monno Medical College and Hospital, Manikganj Bangladesh. Data collection for the study was completed over one year period between March 2023 and February 2024. The target population included workers. The target population included workers exposed to noise levels exceeding 85 dB in their work environment. Total 185 participants who meet the inclusion and exclusion criteria were participated in this study. Participants were included if they were aged above 18 years, individuals with normal hearing status at the time of employment, workers who spent at least 16 hours in a noise-free environment before assessment and participants with at least five years of exposure to occupational noise. Participants with a history of middle ear diseases were not eligible. Participants with history of viral infections or drug therapies causing sensorineural hearing loss were not eligible.

Study Procedure: Data were collected through structured interviews conducted by trained data collectors. Each participant completed a questionnaire that addressed self-reported demographic information, such as age, gender, and monthly income, duration of service in the industry, department and sound history, number of patients with hearing loss, and characteristics of the hearing loss. To ensure clarity and reliability, the questionnaire was pretested prior to formal data collection. Audiometricians handled the audiometric hearing test section.

Measurement of Sound Levels: Environmental sound intensity levels were measured across various locations using a sound level meter. Participants underwent pure tone audiometric testing using a calibrated audiometer. The device was used to assess frequencies ranging from 125 Hz to 8000 Hz and sound intensity levels between -10 dB and 120 dB for each ear individually.

Statistical Analysis: All data were displayed in appropriate tables or graphs based on their relevance, with accompanying descriptions provided for clear interpretation. The data analysis was performed using SPSS version [26]. Descriptive statistics were reported as means and standard deviations for normally distributed data, and frequency with percentages for categorical variables. A p-value of less than 0.05 was considered statistically significant, indicating meaningful associations between variables.

Ethical Consideration: Participants were informed about the purpose and nature of the study and provided written consent before participation. They were allowed to withdraw from the study at any time without any consequences. Ethical approval for the study was obtained from a recognized review board.

Results

The study included textile industry workers with a mean age of 30.9 ± 6.1 years. The majority (54.59%) were aged 18-27 years, followed by 29.73% in the 28-37 age group. Males comprised 60.54% of the participants, while females accounted for 39.46%. Regarding monthly income, most workers (58.92%) earned between 4000-8999 Taka, while only 1.62% earned 24,000 Taka or more (Table 1).

Most of the participants had 5-10 years of experience (44.32%) and 19.46% had over 35 years. 80.54% worker worked in the loom shade department, where noise levels ranged from 95-100 dB. Other departments included drawing frame (8.65%, 77 dB), finishing (5.95%, 81-85 dB), preparation (3.78%, 85-87 dB), and sizing (1.08%, 85-87 dB) (Table 2).

About 58.38% participants were affected by hearing loss. The prevalence of hearing loss among textile workers was significantly associated with age ($p<0.001$). The highest prevalence was observed in the 38 to 47 age group (80.95%), followed by 28 to 37 years (74.55%). In contrast, only 46.53% of workers aged 18 to 27 had hearing loss. Gender-wise, hearing loss was more common among males (62.5%) than females (52.05%), though the association was not statistically significant ($p = 0.09$) (Table 3).

Among the 108 participants with hearing loss, the highest number of participants (69.44%) experienced bilateral hearing loss. A noise-induced notch was present in 58.33% of cases, with 44.44% having notches in both ears. The most affected frequency was 4000 Hz (47.22%), followed by 6000 Hz (42.59%) and 3000 Hz (10.19%) (Table 4).

Table 1: Demographic Characteristics of the Study Population (n=185)

Variable	Frequency (n)	Percentage
Age (years)		
18-27	101	54.59
28-37	55	29.73
38-47	21	11.35
>47	8	4.32
Mean±SD	30.9+6.1 years	
Gender		
Male	112	60.54
Female	73	39.46
Monthly income in Taka		
4000-8999	109	58.92
9000-13999	52	28.11
14000-18999	14	7.57
19000-23999	7	3.78
2400 and above	3	1.62

Table 2: Work-related information of the study population (n=185)

Variables	Frequency	Percent
Work Experiences		
5 to 10 Years	82	44.32
11 to 15 Years	6	3.24
16 to 20 Years	2	1.08
21 to 25 Years	25	13.51
26 to 30 Years	20	10.81
31 to 35 Years	14	7.57
More Than 35 Years	36	19.46
Department (sound intensity in dB)		
Drawing frame (77)	16	8.65
Finishing (81–85)	11	5.95
Loom shade (95–100)	149	80.54
Preparation (85–87)	7	3.78
Sizing (85–87)	2	1.08

Table 3: Prevalence of Hearing Loss among Textile I ndustry Workers by Age and Gender

Variable	Have hearing loss (n=108)		Normal hearing (n=77)		P value
Age Group (years)					
18-27 (n=101)	47	46.53	54	53.47	<0.001
28-37 (n=55)	41	74.55	14	25.45	
38-47 (n=21)	17	80.95	4	19.05	
>47 (n=8)	3	37.50	5	62.50	
Gender					
Male (n=112)	70	62.5	42	37.5	0.09
Female (n=73)	38	52.05	35	47.95	

Table 4: Hearing Loss Characteristics among Participants (n=108)

Variable	Frequency	Percent
Side of hearing loss		
Left	18	16.67
Right	15	13.89
Both	75	69.44
Presence of notch		
Yes	63	58.33
No	45	41.67
Side of notch		
Right	25	23.15
Left	35	32.41
Both	48	44.44
Frequency at which notch present		
3000Hz	11	10.19
4000Hz	51	47.22
6000Hz	46	42.59

Discussion

Occupational noise-induced hearing loss (ONIHL) is a major public health issue, especially in industries like textiles, where workers face prolonged noise exposure. Despite safety regulations, ONIHL remains prevalent, leading to permanent hearing impairment and reduced quality of life. Textile workers often experience noise levels exceeding safe limits, resulting in communication difficulties, cognitive issues, and economic consequences. This study explores ONIHL prevalence in textile workers to identify risk factors and support preventive strategies for better workplace safety and health. The demographic analysis revealed that the majority of participants were young adults (mean age: 30.9 ± 6.1 years), with a predominance of male workers (60.54%). This could be attributed to job distribution within industries, where men typically operate machines that generate higher noise levels, while women work in quieter areas. This may account for the higher prevalence of NIHL among males. This result aligns with the findings of a study That the high prevalence of workers in the lower-income category (4000–8999 Taka, 58.92%) reflects socio-economic challenges that may limit access to healthcare and protective measures[13]. The findings align with global trends, where textile workers often belong to economically vulnerable groups and are at heightened risk for occupational health hazards[14].

Work-related factors, particularly prolonged exposure to high-intensity noise, emerged as significant contributors to NIHL. The majority of workers (44.32%) had 5–10 years of work experience, with a considerable proportion (19.46%) exceeding 35 years of exposure. The loom shade department, characterized by the highest noise levels (95–100 dB), accounted for the largest proportion of workers (80.54%), reinforcing the link between chronic noise exposure and auditory damage. These findings support existing literature emphasizing the cumulative impact of noise exposure duration and intensity on hearing impairment[15]. Additionally, this pattern aligns with a study conducted in the United States, which reported NIHL prevalence rates of 75% among workers aged 20–29 years, 89% among those aged 30–39 years, and 100% among workers over 40 years in the construction industry 16. The prevalence of noise-induced hearing loss (NIHL) among textile industry workers was determined to be 58.38%. Globally, NIHL prevalence ranges between 37% and 59.7% [17]. The prevalence of hearing loss was markedly high (58.38%), with a significant age-related increase ($p<0.001$). Workers aged 38–47 years exhibited the highest prevalence 80.95%), suggesting a cumulative effect of noise exposure. Notably, the lower prevalence in workers over 47 years (37.50%) may indicate a "healthy worker effect," wherein individuals with severe hearing loss may have exited the workforce earlier. Gender differences were not statistically significant ($p=0.09$), implying that both male and female workers are equally susceptible to NIHL under similar exposure conditions. Our findings are comparable with the results of Abraham et al [15]. This study found that 69.44% of workers had bilateral NIHL, with a higher prevalence in the right ear. Similar findings have been reported in other studies [13]. The high rate of bilateral NIHL may be due to the uniform distribution of excessive noise in the workplace. Additionally, 58.33% of workers with NIHL exhibited a notch, with 44.44% affected in both ears and 47.22% at 4000 Hz, followed by 42.59% at 6000 Hz. These results differ from a study on musicians in the United States, where 45% had a notch, with 78% at 6000 Hz, 22% at 4000 Hz, and 2% at 3000 Hz [18]. The variation may be due to differences in noise exposure levels. Addressing this occupational health challenge through evidence-based strategies will enhance worker safety, productivity, and overall quality of life.

There are limitations of the study. Being conducted in a single region, it does not account for variations in noise exposure across different industrial settings. The cross-sectional design prevents establishing a causal relationship between noise exposure and hearing loss. Self-reported data on noise exposure and hearing protection use may be inaccurate due to recall bias.

Conclusion

This study highlights a significant prevalence of occupational noise-induced hearing loss (NIHL) among textile industry workers in Bangladesh, with higher risks associated with prolonged exposure and increased age. The findings emphasize the urgent need for workplace interventions, including the implementation of noise control measures, mandatory hearing protection programs, and regular audiometric screening to prevent further hearing damage. Additionally, occupational health policies in Bangladesh should be strengthened to enforce noise exposure regulations in industrial sectors. Addressing these concerns will improve worker safety, productivity, and overall quality of life.

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Original Article

Comparative Analysis of Hyaluronic Acid and Corticosteroid Injections in the Treatment of Temporomandibular Joint Osteoarthritis.

Mohammad Nasir Uddin[1], Mohammad Sazzadul Haque[2], Mohammad Ashrafuzzaman[3], Mohammad Nayeem Ullah[4]

[1]Professor, Department of Orthopedics, Monno Medical college and Hospital, Manikganj, Bangladesh. [2]Associate Professor (cc), Department of Otolaryngology, Monno Medical College and Hospital, Manikganj, Bangladesh; [3]Professor, Department of Surgery, Monno Medical College and Hospital, Manikganj, Bangladesh; [4]Associate Professor, Department of Radiology and Imaging, Monno Medical College Hospital, Manikganj, Bangladesh

Abstract

Background: Temporomandibular joint (TMJ) osteoarthritis is a common disorder that results in pain, dysfunction, and limited mouth opening. Intra-articular injections of hyaluronic acid (HA) and corticosteroids are frequently utilized in the management of TMJ osteoarthritis, yet their comparative efficacy remains unclear. **Objective:** The aims to compare the clinical outcomes of intra-articular hyaluronic acid versus corticosteroid injections in the treatment of temporomandibular joint osteoarthritis. **Methodology:** This randomized controlled trial was conducted in the Department of Department of Orthopedics, Monno Medical college and Hospital, Manikganj, Bangladesh and Department of Otolaryngology, Monno Medical College and Hospital, Manikganj, Bangladesh which was included 42 participants with TMJ osteoarthritis. Participants were randomly assigned to receive either intra-articular hyaluronic acid (n=21) or corticosteroid (n=21) injections. Clinical outcomes were assessed at baseline, and at 4-, 8-, and 12-weeks post-treatment. Primary outcomes included changes in visual analog scale (VAS) pain scores and mouth opening measurements. Secondary outcomes included adverse events. **Results:** Both treatment groups demonstrated significant improvements in VAS pain scores and mouth opening over the 12-week study period. However, at Week 12, the hyaluronic acid group showed a significantly greater reduction in pain (p=0.042) and improvement in mouth opening (p=0.022) compared to the corticosteroid group. No significant differences were observed between the groups regarding adverse events. **Conclusion:** Intra-articular hyaluronic acid injections provide superior long-term pain reduction and improvement in mouth opening compared to corticosteroid injections in the treatment of TMJ osteoarthritis. Both treatments were well-tolerated with minimal adverse effects.

Keywords: Temporomandibular joint, osteoarthritis, hyaluronic acid, corticosteroids, intra-articular injections, pain management, mouth opening, clinical outcomes

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Correspondence:: Dr. Mohammad Nasir Uddin, Professor, Department of Orthopedics, Monno Medical college and Hospital, Manikganj, Bangladesh. E-mail ID-nasir.ms.ortho@gmail.com , ORCID: https://orcid.org/0009-0008-2613-3801; Contact no- 01747-230597

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Introduction

Temporomandibular joint (TMJ) disorders encompass a range of conditions affecting the joint and its surrounding tissues. According to the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD), there are twelve types of TMJ disorders, including myalgia, arthralgia, disc displacement, and degenerative joint diseases like osteoarthritis (OA)[1]. Among these, TMJ osteoarthritis (TMJ OA) is a prevalent condition, particularly involving degenerative changes in the articular surfaces of the condyle and fossa, often due to mechanical and biological factors such as bruxism, trauma, and genetic

predisposition[2]. TMJ OA, like other osteoarthritic conditions, leads to joint pain, dysfunction, and sometimes the degeneration of joint surfaces. The disease is typically characterized by symptoms such as pain, limited mouth opening, joint noise (crepitation), and radiographic evidence of surface wear and flattening of the condyle[3]. TMJ OA is a prevalent condition, with its occurrence reported to range from 8% to 60%[4]. In the context of treatment, the primary goals are to reduce pain, restore normal mandibular movements, and improve the patient's quality of life[5]. In the treatment of TMJ OA, non-invasive methods such as occlusal splints, physical therapy, and pharmacotherapy are commonly employed. However, when these conservative treatments fail, more invasive options such as intra-articular injections of steroids and hyaluronic acid (HA), are considered[6]. Corticosteroids are a type of steroid commonly used to reduce inflammation and pain in joint disorders, such as temporomandibular joint osteoarthritis, often administered through intra-articular injections for localized relief where hyaluronic acid, a type of glycosaminoglycan, is commonly chosen for intra-articular injections, are known for their role in joint lubrication and protection of cartilage, which is often compromised in osteoarthritic joints[7-9]. The concept of visco-supplementation, which involves restoring the concentration and molecular weight of HA in the joint, has shown therapeutic promise in various osteoarthritic conditions, including TMJ OA[10]. The efficacy of intra-articular HA injections in TMJ OA has been well-documented, with studies suggesting that these injections can improve symptoms by enhancing lubrication and promoting the repair of damaged cartilage[11].

However, steroid injections, while commonly used for their anti-inflammatory effects, may present potential side effects such as joint tissue damage, they remain a frequently used option due to their rapid pain relief and anti-inflammatory properties[12,13]. Studies indicate that both intra-articular injections of HA and steroids provide significant benefits for TMJ OA, with treatment choice influenced by disease severity and patient response[13]. The comparison between HA and steroids in TMJ OA is noteworthy, as the two treatments function differently. Steroids may have potential long-term effects on joint tissues, making HA a preferred option due to its safety profile and lower risk of side effects[12]. The therapeutic benefits of HA injections have been explored in other osteoarthritic joints, and preliminary findings suggest that similar benefits may be achieved in the TMJ[10]. The aim of the study was to compare the clinical outcomes of intra-articular hyaluronic acid versus corticosteroid injections in the treatment of temporomandibular joint osteoarthritis.

Methodology

Study Settings and Population: This prospective observational study was conducted in the Department of Orthopedics, Monno Medical college and Hospital, Manikganj, Bangladesh and Department of Otolaryngology, Monno Medical College and Hospital, Manikganj, Bangladesh from March 2022 to December, 2022. The research comprehensively examined the clinical outcomes associated with intra-articular injections of hyaluronic acid and corticosteroids for managing temporomandibular joint (TMJ) osteoarthritis. The study employed a purposive sampling method, enrolling all patients from the outpatient department to create a well-defined and representative study cohort. Participant selection was guided by stringent inclusion criteria to ensure the reliability and clinical significance of the findings. Patients were stratified into two groups based on the treatment protocol.

Inclusion Criteria: Patients aged 30 years or older, clinically diagnosed with TMJ osteoarthritis based on presenting symptoms such as pain, crepitus, and restricted mouth opening, corroborated by radiographic findings, experienced persistent symptoms for at least three months and had no prior history of TMJ injections or surgical interventions were included.

Exclusion criteria: Patients with systemic inflammatory conditions, such as rheumatoid arthritis, a history of TMJ trauma or fracture, known allergies to study medications, or those who were pregnant or lactating, were excluded from the study.

Allocation and Randomization: Injections were administered under strict aseptic conditions. The Hyaluronic Acid Group received 2 mL of high-molecular-weight hyaluronic acid, while the Steroid Group was treated with 1 mL of triamcinolone acetonide (40 mg/mL). The injections were delivered into the superior joint space of the TMJ using established anatomical landmarks to ensure precision.

Follow Up and Outcome Measures: Data were gathered using a structured and validated questionnaire. Baseline demographics and clinical parameters, including age, gender, body mass index (BMI), and duration of symptoms, were recorded. Pain levels were assessed with

the Visual Analog Scale (VAS),[14] and the range of mouth opening was measured using a digital caliper. Follow-up assessments occurred at 4, 8, and 12 weeks to evaluate pain reduction, improvement in mouth opening, and potential adverse events. Ethical approval for the study was obtained from the institutional ethics committee, and informed consent was secured from all participants.

Statistical Analysis: Demographic data and baseline characteristics were collected and compared between groups using independent t-tests and chi-square tests. Continuous variables were presented as mean ± standard deviation (SD). Repeated measures ANOVA was used to compare temporal changes in VAS scores and mouth opening within and between groups. A p-value of <0.05 was considered statistically significant. All analyses were performed using SPSS software (Version 26.0).

Ethical Clearance: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the Institutional research ethics. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and analyzed using the coding system.

Results

A total of 42 patients participated in this study, with an equal distribution between the Hyaluronic Acid group (n=21) and the Steroid group (n=21). The mean age was comparable, with 54.31±8.25 years in the Hyaluronic Acid group and 55.14±7.93 years in the Steroid group. Most patients were aged 46–55 years. Gender distribution was nearly equal, with no statistically significant difference (p=0.782). The mean BMI was slightly higher in the Steroid group (25.14±3.12 kg/m²) than in the Hyaluronic Acid group (24.76±2.87 kg/m²) (p=0.647). The mean duration of symptoms was 15.25±4.82 months in the Hyaluronic Acid group and 16.18±5. 29 months in the Steroid group (p=0.584). Baseline pain scores were 7.44±1.18 in the Hyaluronic Acid group and 7.64±1.32 in the Steroid group (p=0.683). Baseline mouth opening was 29.69±5.43 mm in the Hyaluronic Acid group and 30.26±4.91 mm in the Steroid group (p=0.746). No significant differences were observed between the groups in demographic characteristics (Table 1).

Table 1: Demographic Characteristics of Study Participants (n=42)

Variables	Hyaluronic Acid Group (n=21)	Steroid Group (n=21)	P value
Age Group			
• ≤45 Years	4(19.1%)	3(14.3%)	
• 46 to 55 Years	8(38.1%)	9(42.9%)	
• 56 to 65 Years	6(28.6%)	5(23.8%)	0.721**
• More Than 65 Years	3(14.3%)	4(19.1%)	
Mean±SD	54.3±8.25	55.1±7.93	
Gender			
• Male	10(47.6%)	9(42.9%)	0.782*
• Female	11(52.4%)	12(57.1%)	
BMI (kg/m²) (Mean±SD)	24.76±2.87	25.14±3.12	0.647*
Duration of symptoms (months)	15.25±4.82	16.18±5.29	0.584*
Baseline VAS pain score	7.44± 1.18	7.64±1.32	0.683*
Baseline mouth opening (mm)	29.69±5.43	30.26±4.91	0.746*

*Student t test was performed to see the label of significance; **Chi-square test was performed to see the label of significance

VAS pain scores were assessed at multiple time points. At baseline, the Hyaluronic Acid group had a score of 7.42±1.13 and the Steroid group 7.67±1.38 (p=0.682). At Week 4, pain scores decreased to 5.28±1.27 and 5.55±1.47, respectively (p=0.534). At Week 8, the Hyaluronic Acid group had a lower mean score (3.85±1.14) than the Steroid group (4.41±1.35) (p=0.115) (Table 2).

Table 2: Temporal changes in VAS pain score (Mean±SD)

Time Point	Hyaluronic Acid Group	Steroid Group	P value
Baseline	7.42±1.13	7.67±1.38	0.682
Week 4	5.28±1.27	5.55±1.47	0.534
Week 8	3.85±1.14	4.41±1.35	0.115
Week 12	3.16±0.93	3.83±1.26	0.042

By Week 12, the Hyaluronic Acid group showed a significantly lower score (3.16±0.93) than the Steroid group (3.83±1.26) (p=0.042). Mouth opening changes were also analyzed. At baseline, values were 29.62±5.43 mm in the Hyaluronic Acid group and 30.22±4.97 mm in the Steroid group (p=0.742). By Week 4, both groups showed improvement, reaching 32.14±4.88 mm and 31.74±5.15 mm, respectively (p=0.686). At Week 8, further increases were observed, with the Hyaluronic Acid group at 35.45±4.28 mm and the Steroid group at 33.62±4.75 mm (p=0.098). By Week 12, a significantly greater increase was seen in the Hyaluronic Acid group (37.16±3.97 mm) compared to the Steroid group (34.42±4.18 mm) (p=0.022) (Table 3).

Table 3: Temporal Changes in Mouth Opening (mm) (Mean±SD)

Time Point	Hyaluronic Acid Group	Steroid Group	P value
Baseline	29.62±5.43	30.22±4.97	0.742
Week 4	32.14±4.88	31.74±5.15	0.686
Week 8	35.45±4.28	33.62±4.75	0.098
Week 12	37.16±3.97	34.42±4.18	0.022

*Student t test was performed to see the label of significance

Pain reduction was greater in the Hyaluronic Acid group (4.28±1.24) than in the Steroid group (3.81±1.47) (p=0.194). Mouth opening improvement was also greater (7.47±2.18 mm vs. 4.16±1.82 mm) with a near-significant difference (p=0.071). Localized swelling was seen in 4.76% and 9.52% of patients, respectively (p=0.558). Pain at the injection site occurred in 9.52% and 14.29%, respectively (p=0.639). One infection was reported in the Steroid group (p=0.315) (Table 4).

Table 4: Comparison of Clinical Outcomes between Groups after 12 weeks (N=42)

Outcomes	Hyaluronic Acid Group	Steroid Group	P value
VAS pain score reduction(Mean±SD)	4.28±1.24	3.81±1.47	0.194*
Improvement in mouth opening (mm) (Mean±SD)	7.47±2.18	4.16±1.82	0.071*
Adverse events			
Localized swelling	1(4.8%)	2(9.5%)	0.558**
Pain at injection site	2(9.5%)	3(14.3%)	0.639**
Infection	0(0.0%)	1(4.7%)	0.315**

*Student t test was performed to see the label of significance;
**Chi-square test was performed to see the label of significance

Discussion

Temporomandibular joint osteoarthritis (TMJ OA) is a common condition that leads to pain, limited mouth opening, and functional impairment[15]. Intra-articular injections of hyaluronic acid (HA) and corticosteroids (CS) are frequently used treatments for symptom management[16]. HA is known to provide lubrication and reduce inflammation in the joint, while CS offers potent anti-inflammatory effects[16]. Both treatments have demonstrated efficacy in improving pain and function, yet their comparative long-term benefits remain unclear. In our study, the comparative analysis of hyaluronic acid (HA) and corticosteroid (CS) injections for the treatment of temporomandibular joint osteoarthritis (TMJ OA) demonstrated differential effects on pain reduction and functional improvement over a 12-week period. The findings align with prior research highlighting the efficacy of intra-articular injections in managing TMJ OA symptoms, though the magnitude of benefit varied between treatment modalities[15]. At baseline, both groups exhibited similar demographic characteristics, such as age, gender, BMI, and symptom duration.

These findings suggest that the two groups were comparable at the outset, reducing the potential for confounding variables in the subsequent analysis. With respect to pain relief, although both groups showed a progressive decline in visual analog scale (VAS) scores, the HA group exhibited a significantly lower pain score at 12 weeks compared to the CS group (3.16 ± 0.93 vs. 3.83 ± 1.26, p = 0.042). This finding aligns with previous studies indicating that HA has a prolonged analgesic effect by restoring joint lubrication and modulating inflammatory cytokines[17]. Conversely, corticosteroids provide rapid pain relief by suppressing inflammation but may have a shorter duration of efficacy due to their catabolic effects on cartilage[18]. The present study observed a steady increase in mouth opening in both treatment groups. At baseline, there was no significant difference in interincisal opening between groups. By Week 4, the improvements remained comparable. However, at Week 8, the HA group showed a greater improvement trend (HA: 35.45±4.28 mm, Steroid: 33.62±4.75 mm, p=0.098), which became statistically significant by Week 12 (HA: 37.16±3.97 mm, Steroid: 34.42±4.18 mm, p=0.022). This suggests that HA may provide better long-term functional recovery. This is consistent with the biomechanical role of HA in enhancing synovial fluid viscosity and promoting chondroprotection[19,20]. Notably, previous randomized

controlled trials have reported similar trends, suggesting that HA may facilitate long-term joint mobility improvements compared to steroids21. Although both treatments were well-tolerated, mild adverse events were observed. Pain at the injection site and localized swelling were slightly more common in the CS group, possibly due to its inflammatory response upon intra-articular administration. Additionally, one case of infection occurred in the CS group, which aligns with prior reports of an increased risk of local immunosuppression following steroid injections[22].

There are limitations of this present study. Every hospital-based study has some limitations and the present study undertaken is no exception to this fact. The observational design restricts causal conclusions, and a randomized controlled trial would provide stronger evidence. The 12-week follow-up period may not reflect long-term effects. Additionally, only mouth opening was assessed as a functional outcome, without considering other factors such as chewing ability or quality of life.

Conclusion

TMJ osteoarthritis is a debilitating condition that can significantly impact a patient's quality of life. The present study demonstrates that both hyaluronic acid (HA) and corticosteroid injections are effective in managing pain and improving mouth opening in patients with TMJ osteoarthritis. However, our results suggest that HA injections may offer superior outcomes, with a more significant reduction in pain and a greater improvement in mouth opening after 12 weeks. Both treatments were well-tolerated, with minimal adverse events, confirming their safety profiles. These findings provide valuable insights into the potential of HA as a treatment modality for TMJ osteoarthritis.

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Original Article

Serum Albumin Level in Third Trimester of Pregnancy and Non-Pregnant Women: A Comparative Study

Farzana Noor[1], Nuzat Tasnim[2], Farzana Yeasmin[3], Manira Khanam Nishi[4], Ridwana Rahman[5]

[1]Associate Professor (c.c), Department of Physiology, Ashiyan Medical College, Dhaka, Bangladesh; [2]Assistant Professor, Department of Physiology , Ashiyan Medical College, Dhaka, Bangladesh; [3]Curator, Department of Anatomy, Netrokona Medical College, Netrokona, Bangladesh; [4]Assistant Professor, Department of Pharmacology and Therapeutics, Shaheed Mansur Ali Medical College, Dhaka, Bangladesh; [5]Assistant Professor, Department of Physiology, Ad-Deen Women's Medical College, Dhaka, Bangladesh.

Abstract

Background: Serum albumin level is an predictor for preeclamplsia and eclampsia. **Objective:** The purpose of the present study was to compare the levels of serum albumin in third trimester of pregnancy with non-pregnant women of same age range. **Methodology:** This analytical type of cross-sectional study was carried out in the Department of Physiology, Mymensingh Medical College, Mymensingh, Bangladesh over one year. A total number of 140 subjects, age range between 20 to 35 years were included in this study. Among them, 70 healthy subjects were taken as control group (Group I) and 70 pregnant women of third trimester were taken as study group (Group II). The results were calculated and analyzed by using SPSS. Quantitative data were expressed as mean (±SE) and statistical significance of difference among the group was calculated by unpaired student's 't' test. **Results:** In this study we found that Serum albumin level of group I (Control group) was 4.03±0.36 gm/dl and group II (Study group) was 3.03±0.48 gm/dl. In group II Serum Albumin level was decreased in comparison to group I. The difference of mean Serum Albumin level between the groups was statistically highly significant ($p<0.001$). **Conclusion:** In conclusion, Serum Albumin significantly decreased in study group in comparison with control group.

Key Words: Third trimester of pregnancy; serum albumin; non-pregnant women
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Correspondence: Dr. Farzana Noor, Associate Professor (c.c), Department of Physiology, Ashiyan Medical College, Dhaka, Bangladesh; Email: noorfarzana045@gmail.com; ORCID: https://orchid.org/0009000585434565, Contact no: 01902-222345

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Introduction

Pregnancy is a biological stress associated with many complex and interrelated physiological, anatomical and biochemical alterations happening in the body[1]. Serum albumin is produced by liver and comprises about half of the total protein in blood[2]. The physiological changes during pregnancy predispose women to increased blood volume, hormonal fluctuations, and changes in vascular permeability. Serum albumin, a critical plasma protein responsible for maintaining oncotic pressure and regulating fluid balance, plays a key role in these processes[3]. They sum-up to about 55.0% to 60.0% of the protein in human serum and thus ensure normal water balance between blood and tissues via osmotic mechanisms. They also function as blood transport protein for less soluble substances that can bind to them[4]. More so, in the blood, albumin aids to prevent the escape of fluid into the body tissues. In addition, albumin in urine could be an indication of kidney disease[5]. More often, some people experience high levels of protein in their urine during pregnancy. When it's accompanied by high blood pressure, it's likely a sign of preeclampsia[6]. Albumin is decreased under many other

circumstances, such as presence of stress or disease, malnutrition or Kwashiorkor[7]. Hypoalbuminaemia occurs before the early stage of toxemia of pregnancy[8]. WHO estimates incidence of preeclampsia to be seven times higher in developing countries than in developed countries[9]. A study by Chien et al[10] evaluated that in severe hypoalbuminemia during pregnancy hypertension, ascites and abruption occurred more frequently. In another study by Taimoor et al[6] serum albumin level was significantly reduced in 3rd trimester preeclamptics and there was also a significant difference in albumin levels in both cases and controls in 2nd and 3rd trimesters[6]. This is in accordance with another study which determined decrease in albumin levels in preeclamptics in 3rd trimester compared to controls[11].

Albumin possesses a long half-life and a large pool in the circulation, and the serum concentration has been widely tested in clinical sites for the evaluation of long-term variations in protein nutritional status[12]. Low birth weight (LBW) acts as a critical risk factor for infant morbidity and mortality[13]. Maternal low serum albumin level in the third trimester was found to be associated with low birth weight in a study done by Tabata et al[14]. In the findings of a study by Akter et al[15] suggest that pregnant women with low serum albumin level carry higher risk of giving LBW newborns then women with normal serum albumin level[15]. Birth weight of babies correlated between half-siblings of the same mother but not of the same father because of the possible contribution of maternal albumin[16]. It has been argued that the likely effects of maternal albumin deficiency on the birth weight of babies depend on the stage of gestation. Xiong et al[17] concluded in his study that a higher maternal serum albumin in the late trimester is associated with a lower risk of infant birth weight. The data suggests that maternal serum albumin in the late trimester may serve as a simple and effective tool for the assessment of the low-birth-weight risk in clinical practice[17].

Thus, the purpose of the present study was to find and compare the difference between serum Albumin level in Pregnant women of third trimester and non-pregnant women of same age range for further better management in clinical aspect for a pregnant women and her baby's valuable life.

Methodology

Study Settings and Population: The study was a cross-sectional analytical study. It was conducted in the Department of physiology, Mymensingh Medical College, Mymensingh from January 2019 to December 2019 for a period of one year. The subjects were obtained from the Department of Gynecology and Obstetrics Mymensingh Medical College & Hospital, Mymensingh, Model Family Planning clinic, Mymensingh and locality of Mymensingh.

Study Procedure: The subjects were selected by convenient sampling. After proper counseling, written informed consent was taken. Ethical permission was taken from the Institutional Review Committee of Mymensingh Medical College. A total number of 140 subjects, age range between 20-35 years were included in this study. Among them, 70 apparently healthy women were taken as control group (Group II) and 70 pregnant women of third trimester were taken as study group (Group I). Those who were diagnosed case of pregnancy with gestational diabetes mellitus, hypertension, and women with other chronic diseases, renal or cardiovascular disease, drug induced abnormal liver function test and women over age 35 and less than 20 years were excluded from the study. Patients who disagreed to donate blood samples were also excluded. Laboratory analysis of Serum Albumin was done by Bromocresol Green Method. Data were expressed as mean (±SD) and statistical significance of difference among the group was calculated by unpaired students' test.

Statistical Analysis: Statistical analysis was done by using Statistical package of social service (SPSS) for windows version 21.00. P value <0.05 was considered as significant.

Ethical Clearance: The work approval was taken from the Ethical Review committee of Mymensingh Medical College, Bangladesh. Written informed consent was obtained from the participants to ensure their voluntary participation before preceding the questionnaire and specimen collection.

Results

Those women (in both Control and study group) who were diagnosed case of diabetes mellitus, gestational diabetes mellitus, hypertension, and women with other chronic diseases, renal or cardiovascular disease, drug induced abnormal liver function test and women over age 35 and less than 20 yrs age were excluded from the study. Subjects' ages were ranged from 20 to 35 years and the mean age of Group I was 26.01±3.54 years and Group II was 25.06±3.67 years (Table 1).

Table 1: Distribution of Age of Both Control and Study Groups

Group	Mean ± SD
Group I	25.06 ± 3.67
Group II	26.01 ± 3.54

The mean (±SD) of Serum Albumin of group I and group II were 3.03±0.48 gm/dL and 4.03±0.36 gm/dL respectively. In group II Serum Albumin level was decreased in comparison to group I. The difference of mean Serum Albumin between the groups was statistically highly significant (p<0.001) (Table 2).

Table 2: Statistical Analysis of Serum Albumin Level between Control Group (Group I) and Study Group (Group II) of two groups (n=140)

Groups	n	Mean±SD	Mean difference	P value
Group I	70	3.03±0.48	-0.994	<0.001
Group II	70	4.03±0.36		

Group I = Control group (non-pregnant women aged 20-35 yrs); Group II =Study group (Pregnant women of third trimester aged 20-35 yrs); n= Total numbers of subjects in each group

Discussion

This study was carried out to compare the levels of serum Albumin between third trimester of pregnancy age ranged 20 to 35 years and non-pregnant women of same age range. In this study decrease in level of serum albumin in pregnant women was significantly proved by comparing the level of serum albumin with non-pregnant control group. This association of decrease in level of albumin in pregnant women was analyzed by applying T- test for comparing case and control groups. In this study, decrease in serum albumin is very much significant in third trimester of pregnancy (p<0.001).

The liver produces serum albumins which are dissolved in blood plasma, containing about 55.0%, and the most abundant blood protein. Albumin serve as transport of lipids, hormones, vitamins and minerals, and also assist immune systems with maintaining 80.0% of colloidal osmotic pressure[18]. In the current study, there were significant heterogeneity of level of serum albumin between third trimesters and the non-pregnant women. The current work corroborates with the views of Abbasi et al[18], Shakhmatova et al[19], Cassaza and Yazdi[20] respectively. Similar findings also reported from a study of Agbeca et al[11] where he found serum albumin level was significantly lower in pregnant group compared to the controls and also revealed a significant lower serum albumin in third trimester group than in second trimester and controls. Ogbodo et al[21] found in his study that significant decrease in serum albumin occurred in third trimester than non- pregnant control group. The postulated mechanism of decreased serum albumin may be due to increased albuminuria during pregnancy[22]. The sum effect of volume expansion, increases in cardiac output and pulse rate, reduced systemic vascular resistance, renal vasodilation and increased renal plasma flow. This is considered a major contributor to the hyperfiltration and increase in glomerular filtration rate observed during pregnancy[23]. The hyperfiltration combined with the reduction of tubular reabsorption is thought to increase urine albumin excretion[24] Another cause of hypoalbuminemia during pregnancy may be the mild systemic inflammation that occurs in normal pregnancy due to multitude secretion of inflammatory factors as cytokines and growth factors from the placenta into the maternal circulation[25].

During pregnancy, the protein synthesis and protein breakdown in the body increase of pregnant woman, whereas they generally possess a positive nitrogen balance[26-27]. Under the effect of blood dilution during pregnancy, plasma protein begins to be reduced from early pregnancy, mainly manifested as a decrease in albumin[28]. The half-life of serum Albumin in women during pregnancy is 21 days. Moreover, its concentration acts as a typical marker of (protein energy) malnutrition, which is likely to mirror the quality of diet[5]. The above-mentioned observations were consistent with our finding that no pronounced difference existed in serum Albumin concentration in third trimester.

Albumin can maintain the constant osmotic pressure of plasma colloid and ensure sufficient blood supply of uterus and placenta[29]. Albumin, a non-specific transport protein, is capable of transporting different nutrients from the mother to the fetus and promote its growth and development[20]. Yemane et al[30] in his investigation about level of hemocystine during normal pregnancy found that Homocysteine levels were directly correlated with albumin levels, which decreased during pregnancy.

The reduction of albumin concentration is easy to shorten gestational age, such that there might be a close correlation of birth weight and albumin level in these studies[31]. Changes in level of serum albumin during pregnancy have been well documented in number of studies[32]. These changes in level of serum albumin have been attributed to many factors. In a study decrease in level of serum albumin during pregnancy has found to be caused by harmonic changes which occurs during pregnanacy[33]. Estrogen and Progesterone increase progressively during whole period of gestation. These hormones reach maximum at third trimester. Most of the metabolic and biochemical changes

are attributed to these harmonic changes[34]. Liver function is also affected which itself bring about changes which are manifested in number of biochemical, physiological and metabolic changes.

Conclusion

In conclusion, pregnancy has an appreciable effect on serum albumin level compared to non- pregnant. Therefore, it is important to consider undergoing screening of serum albumin for prevention of complication related to low serum albumin level for prevention of complication related to pregnancy for wellbeing of both mother and foetus. Adequate and proper dietary habit may also prevent complication related to low serum Albumin.

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Original Article

Changing Trends in Antibiotic Sensitivity of Urinary Tract Infections by *Escherichia coli* at a Tertiary Care Hospital

Ferdows Ara Mollika[1], Tarana Jahan[2], Tashmin Afroz Binte Islam[3], Farjana Majid[4], Premananda Das[5], Nahla Islam Neeva[6]

[1]Assistant Professor, Department of Microbiology, Tairunnessa Memorial Medical College & Hospital, Gazipur, Bangladesh; [2]Associate Professor, Department of Microbiology, Monno Medical College, Manikgonj, Bangladesh; [3]Associate Professor, Department of Microbiology, Tairunnessa Memorial Medical College & Hospital, Gazipur, Bangladesh; [4]Professor & Head, Department of Microbiology, Tairunnessa Memorial Medical College & Hospital, Gazipur, Bangladesh; [5]Professor & Head, Department of Microbiology , Sylhet MAG Osmani Medical College, Sylhet, Bangladesh; [6]Associate Professor(cc), Department of Microbiology, Monowara Sikder Medical College, Shariatpur, Bangladesh.

Abstract

Background: Urinary tract infection (UTI) is one of the most common causes of bacterial infections worldwide. Rising antibiotic resistance among urinary pathogens to commonly prescribed antibiotics has become a significant therapeutic challenge. **Objective:** Our study aims to investigate the patterns of antibacterial susceptibility in E. coli among patients residing in the densely populated industrial area of Gazipur, Bangladesh. Methodology: This cross-sectional study was conducted in the Department of Microbiology at Tairunnessa Memorial Medical College, Gazipur, Bangladesh from July 2023 to January 2024. The study included 100 adult patients admitted to the Medicine Indoor Department with confirmed UTI, based on clinical symptoms, signs, supportive investigations, and urine culture results (positive or negative). **Results:** Urine samples were taken several times from the 100 patients. Among the 1000 urine samples analyzed, 256 tested positive for pathogenic organisms. Escherichia coli was isolated in 128(50.0%) of the positive samples, followed by Klebsiella species (28.0%,), Pseudomonas species (13.7%), Enterococcus species (5.5%) and Proteus species (2.8%). Escherichia coli exhibited the highest sensitivity to nitrofurantoin (92.5%), meropenem (92.5%), amikacin (84.6%), and gentamicin (71.8%). However, it showed resistance to commonly used antibiotics such as cefixime (78%), cefuroxime (77.5%), ciprofloxacin (62.5%) and ceftriaxone (62.5%). **Conclusions:** Gram-negative bacilli were identified as the primary causative agents of UTI, with Escherichia coli being the most prevalent pathogen. The most effective antibiotics were nitrofurantoin, meropenem, amikacin, and gentamicin. In contrast, frequently prescribed antibiotics like cefixime, cefuroxime, cotrimoxazole, ciprofloxacin, and ceftriaxone demonstrated high resistance rates against E. coli.

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Correspondence: : Dr. Ferdows Ara Mollika, Assistant Professor, Department of Microbiology, Tairunnessa Memorial Medical College & Hospital, Kunia, Board Bazar, Gazipur, Bangladesh. Email: ferdowsmollika@gmail.com, **ORCID**: https://orcid.org/0009-0002-5960-2551, Contact no: 01877657848

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Introduction

Urinary tract infections (UTIs) are common conditions primarily caused by the ascent of normal intestinal bacteria through the urethra into the bladder. UTIs can affect any part of the urinary system, including the urethra, ureters, bladder, and kidneys, triggering an inflammatory response in the urothelium due to invading pathogens[1]. Women face a high lifetime risk of developing cystitis, estimated at 60.0%, whereas men have a lower risk of only 13% cases[2]. Approximately 3.0% of girls and 1.0% of boys

experience at least one UTI episode before the age of 11 years[3]. The main bacterial culprits behind UTIs include Escherichia coli, responsible for 80.0% of cases, along with Klebsiella pneumoniae, Citrobacter species, Enterobacter species, Pseudomonas aeruginosa and various Staphylococcus species[4-5].

While antibiotics play a crucial role in treating UTIs, their overuse and misuse contribute significantly to the development of resistance among uropathogenic bacteria[6-7]. In hospitals, a substantial percentage, ranging from 20.0% to 50.0%, of prescribed antibiotics are unnecessary or inappropriate[8]. Globally, there is a troubling rise in UTIs caused by multidrug-resistant uropathogens, posing severe health risks, particularly in developing nations[9-10]. Recent studies have highlighted that over 75.0% of Escherichia coli strains causing UTIs are resistant to third-generation cephalosporins[11]. It is imperative to closely monitor infection etiology and antibiotic resistance patterns to ensure appropriate antibiotic selection for empirical therapy and to combat the overuse and misuse of antibiotics.

Methodology

Study Settings and Population: This cross-sectional study was conducted at Tairunnessa Memorial Medical College, Gazipur, Bangladesh from July 2023 to January 2024. Data were collected from 100 patients aged 18 to 75 years through purposive sampling technique and samples were taken several times from each patient. A total of 1000 clean catch-midstream urine samples were collected from inpatients suspected of having UTI. Samples were subjected to Gram staining, microscopic identification, colony morphology identification, and biochemical tests following the Clinical Laboratory Standard Institute (CLSI) procedures[12].

Selection Criteria: Patients diagnosed clinically with UTI based on symptoms such as fever, dysuria, and increased frequency of urination were included in the study. Patients receiving antibiotic therapy within one week prior to sample collection were excluded.

Sample Collection Procedure: Urine samples were collected in sterile containers and transported to the laboratory within thirty minutes. Upon arrival, samples were stored at 4°C until further processing and analysis.

Culture Specimen: All specimens were cultured on HiCrome UTI Agar media (HiMedia Laboratory Pvt Ltd, India), blood agar, and MacConkey's agar media. A wire loop with 0.001 mL of urine was used for inoculation, followed by aerobic incubation at 37°C for 24 hours. Urine cultures with colony counts ≥ 10^5 CFU/ml were considered as positive for significant growth. Gram staining was performed on significant single colonies, and further identification was carried out using biochemical tests such as Indole, Citrate Utilization, and Triple Sugar Iron (TSI).

Antimicrobial Sensitivity Testing: Antimicrobial sensitivity testing was conducted using the Kirby-Bauer disc diffusion method. Interpretation of sensitivity (Sensitive or Resistant) was based on the diameters of zones of inhibition of bacterial growth according to disc manufacturer recommendations. The antibiotics tested for sensitivity included Ceftriaxone, Ciprofloxacin, Cefixime, Cefuroxime, Amikacin, Imipenem, Gentamicin, Nitrofurantoin, and Amoxicillin. Demographic data and antibiotic sensitivity profiles were recorded and analyzed for the six-month study period.

Statistical Analysis: Findings were recorded and analysed. Collected data were checked and edited first and processed with the help of the software Statistical Package for Social Sciences (SPSS) version 21 and analysed. Statistical analyses were done by using appropriate statistical tools. Qualitative data were expressed as frequency and percent.

Ethical Clearance: Ethical clearance was given from Head of the department of Microbiology of Tairunnessa Memorial Medical College, Gazipur, Bangladesh.

Results

A total of 1000 urine samples were cultured, 256 samples showed significant growth, whereas majority of the samples showed no growth. This study determines the antibiotic susceptibility of 128 isolates of E. coli from 256 positive urine culture and their sensitivity pattern pertaining to a period of 06 months (July 2023 to January 2024) were analyzed. We noted that UTI was more common in females 65.6% than males 34.4% cases (Table 1).

Table 1: Distribution of Study Patients by Gender

Gender	Frequency	Percent
Male	88	34.4
Female	168	65.6
Total	256	100.0

Among the study population mean age was 33 years (Table 2).

Table 2: Distribution of Study Patients by Age Group

Age	Values
Mean	33.039
Std. Deviation	16.5206
Minimum	0.9
Maximum	85.0

Among the study population the most frequent causative organisms isolated were Escherichia coli 50% followed by, Klebsiella 28% Pseudomonas 13.7%, Enterococcus species 5.5% and Proteus 2.8% (Table 3).

Table 3: Microbiological Pattern of UTI Patients

Traits	Frequency	Percent
Escherichia coli	128	50
Klebsiella	72	28
Pseudomonas	35	13.7
Enterococcus	14	5.5
Proteus	7	2.8

Escherichia coli was found to be most sensitive to nitrofurantoin (92.5%), meropenem (92.5%), amikacin (84.6%) and gentamycin (71.8%) and resistant to most commonly used drugs like cefixime (78.0%), cefuroxime (77.5%), ciprofloxacin (62.5%), ceftriaxone (62.5%). All organisms are mostly sensitive to nitrofurantoin (86.2%), meropenem (93.1%), amikacin (77.2%), and gentamycin (64.9%) and mostly resistant to cefixime (83.3%), cefuroxime (81.4%), and ceftriaxone (66.9%) (Table 4).

Table 4: Antibiotic Sensitivity Pattern of Escherichia coli

Antibiotics	Sensitive	Resistant
Amoxicillin	58.6%	42.4%
Levofloxacin	47.2%	52.8%
Cefixime	22%	78.0%
Cotrimoxazole	40.5%	59.5%
Cefuroxime	22.5%	77.5%
Nitrofurantoin	92.5%	7.5%
Ciprofloxacin	37.5%	62.5%
Ceftriaxone	37.5%	62.5%
Meropenem	92.5%	7.5%
Amikacin	84.6%	15.4%
Gentamycin	71.8%	28.2%

Discussion

Urinary tract infections (UTIs) represent a major clinical challenge in both community and healthcare settings. Epidemiological data indicate that UTIs contribute to approximately seven million outpatient visits and one million emergency department admissions annually in the United States, with 100,000 cases requiring hospitalization, positioning them among the leading causes of bacterial infections in ambulatory care[13]. The economic burden is equally substantial, with yearly costs estimated at $1.6 billion[13].

The study revealed that females (65.6%) were more susceptible to UTI than males (34.4%), which is also like other studies[14,15]. The increased incidence of the urinary tract infection in women is conditioned by favoring anatomic factors, by hormonal changes and by the urodynamic disturbance occurring with age[16].

The predominant number of pathogens isolated in our study were Gram negative bacilli rather than Gram positive pathogens. Bacteriological studies usually reveal the involvement of Gram-negative enteric organisms that commonly cause UTI, such as *E. coli, Klebsiella* species and *Proteus* species[17]. Similarly, in another study, the most predominant pathogens isolated from UTI were Gram negative bacilli[18]. The higher prevalence of Gram-negative enteric organisms in UTI cases may be due to the better chances of these organisms getting access to urinary tract from the intestine where they inhabit as normal flora. In our study, majority of isolated bacteria were also the Gram-negative *Escherichia coli* 50.0%, and Klebsiella 28.0% and *Pseudomonas* 13.7% and Gram-positive *Enterococcus* species 5.5% isolates. Our study, along with previous studies, shows that *E. coli* is the predominant etiology of UTI[19,20,21].

Antimicrobial resistance patterns demonstrate significant geographical and temporal variability[22]. In this study, *Escherichia coli* isolates showed high sensitivity to nitrofurantoin (92.5%), meropenem (92.5%), and amikacin (84.6%), but substantial resistance to first-line agents like cefixime (78.0%) and ciprofloxacin (62.5%). These findings mirror a Bangladesh-based study reporting efficacy of imipenem and amikacin against uropathogens as well as Philippine data highlighting amikacin sensitivity[23-25]. Notably, resistance patterns in high-income countries with stricter antimicrobial stewardship differ, with stable susceptibility profiles despite rising *Escherichia coli* and ESBL prevalence[24].

Aggregate antimicrobial susceptibility analysis revealed high resistance to amoxicillin (52.0%), cotrimoxazole (66.9%), and third-generation cephalosporins (68.9 to 83.3%), consistent with prior Bangladeshi research[25]. Conversely, meropenem (93.1%), nitrofurantoin (86.2%), and amikacin (77.2%) demonstrated robust efficacy, aligning with observations from a Saudi Arabian tertiary care center[26]. These findings underscore the need for region-specific antibiotic stewardship to optimize empirical therapy.

Conclusion

UTI among female is more prevalent and the most predominant pathogen was E-coli. Most effective antimicrobial agents for *E coli* are nitrofurantoin,

meropenem, amikacin and gentamycin. It is resistant to most commonly used drugs like cefixime, cefuroxime, ciprofloxacin, levofloxacin and ceftriaxone. Therefore, the choice of antibiotic therapy should integrate the local sensitivity pattern of the infecting organisms. Periodic evaluations of predominant organisms and their antibiotic susceptibility pattern are essential as it is changing over.

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Original Article

Detection of Extended Spectrum Beta-lactamase (ESBL) Producing Gram Negative Bacteria from Different Clinical Specimens of Largest Teaching Hospital in Dhaka City of Bangladesh.

Bhuiyan Mohammad Mahtab Uddin[1], Mosfika Mahjabin[2], Md. Anisur Rahman[3], Zubair Ahmed Ratan[4], Mohammad Nahid Salman[5], Maruf Ibne Monowar[6], Md. Faizur Rahman[7]

[1]Associate Professor, Department of Microbiology, Enam Medical College, Dhaka, Bangladesh; [2]Assistant Professor, Department of Pharmacology, Enam Medical College, Dhaka, Bangladesh; [3]Assistant Professor, Department of Microbiology, Noakhali Medical College, Noakhali, Bangladesh; [4]Lecturer and Project Coordinator, School of Health and Society University of Wollongong Wollongong, Australia; [5]Associate Professor, Department of Physiology, Ad-din Akij Medical College, Khulna, Bangladesh; [6]Associate Professor, Department of Anatomy, Enam Medical College, Dhaka, Bangladesh; [7]Lecturer, Department of Microbiology, Dhaka Medical College, Dhaka, Bangladesh.

Abstract

Background: Detection of Extended spectrum beta lactamase (ESBL) enzyme producing bacteria in hospital settings is very important as ESBL genes are transmissible. **Objective:** This study was carried out to determine the distribution of ESBL producing gram negative isolates at a tertiary care hospital in Dhaka city which deals with different types of patients. **Methodology:** Clinical specimens were collected from the patients attending the microbiology laboratory of Dhaka Medical College from outpatient and inpatient department, Dhaka, during the period of July 2014 to June 2015. **Results:** Out of 191-gram negative bacteria 73 (38.22%) were positive for ESBL production by DDS test. Those out of 73 ESBL producers 50 (68.49%) were positive for ESBL encoding gene *bla*CTX-M-15 and 36 (49.32%) for *bla*OXA-1 by PCR. By DDS test, Among the ESBL producers, *Escherichia coli* was the highest (43.84%%) which was followed by *Pseudomonas* species (15.07%), *Klebsiella species* (15.07%), *Citrobacter spp*. (8.22%) and *Proteus species* (6.85%). Out of 24 *Esch. coli* isolated from outpatient department, 7(29.2%) were positive for ESBL. On the other hand, out of 30 Esch coli isolated from inpatient department, 25 (83.33%) were positive for ESBL. The difference was statistically significant ($p<0.001$). **Conclusion:** In conclusion, the distribution of ESBL producers is more among the hospitalized patients than the patients of the community.

Keywords: Bangladesh, Dhaka Medical College Hospital, ESBL, Gram negative bacteria
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Correspondence: Dr. Bhuiyan Mohammad Mahtab Uddin, Associate Professor, Department of Microbiology, Enam Medical College, Dhaka, Bangladesh; email: mahtab.sbmc@gmail.com, **Orchid ID:** 0000-0002-5109-9851.Contact number- 01682780492.

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Introduction

Gram negative bacteria intrinsically can produce both chromosomal and plasmid mediated beta lactamases enzymes due to selective pressure created by beta lactam substances produced by soil organisms. TEM-1 was the 1st plasmid mediated beta lactamase enzyme described in early 1960. Subsequently extended spectrum beta lactamases (ESBLs) are identified[1]. ESBLs are 2be group enzymes of Bush-Jacoby-Medeiros classification and some of group 2d enzymes which has similar functional properties like group 2be enzymes[2]. These enzymes are produced by members of Enterobacteriaceae such as *Esch. coli*, *K. pneumoniae, Citrobacter* spp, *Proteus* spp, *Enterobacter spp, Morganella morganii, Serratia marsescens* and other gram-negative bacteria such as *Pseudomonas aeruginosa* and A*cinetobacter baumannii1*.

ESBLs are efficiently capable of hydrolyzing penicillins, early cephalosporins such as cephaloridine and cephalothin except cephamycins, the oxyimino group containing cephalosporins like cefotaxime, ceftazidime, and monobactam and are usually inhibited by beta lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam[3,5].

In addition, ESBLs genes are frequently intermingled with other antibiotic resistance genes such as tetracycline, aminoglycosides, trimethoprim, sulphonamide, chloramphenicol and quinolones making them multidrug resistance[4]. High prevalence of ESBL producers are documented from all over the country. Prevalence of ESBLs differs significantly geographically and depends on various factors6.Enterobacteriaceae are the most common group of gram-negative rods isolated in clinical laboratories[7]. Hence Detection of ESBL production by Enterobacteriaceae and other gram-negative bacteria have paramount importance to ensure appropriate antibiotic treatment. With this view this study was designed to find out the distribution of ESBL producing bacteria isolated from different clinical specimens of Dhaka Medical College and Hospital in Dhaka city of Bangladesh.

Methodology

Study Settings and Population: This cross-sectional study was conducted in the Departmetn of Microbiology at Dhaka Medical College, Dhaka, Bangladesh from July 2014 June 2015 for a period of one year. The clinical specimens were collected from the patients attending the microbiology laboratory of Dhaka Medical College, Dhaka, Bangladesh from outpatient and inpatient department. In total 191-gram negative bacteria were isolated and identified from different types of samples such as urine, wound swab, endotracheal aspirate and blood. Samples were collected by aseptic standard procedures.

Detection of Bacterial Species: Blood agar and MacConkeys's agar media were used for the primary isolation of the bacteria. Identification of every particular gram-negative bacterium was done by Gram staining, observing colony morphology, oxidase test, inoculation into Triple sugar iron (TSI), Motility indole urea (MIU) and Simmons citrate agar media. ESBL producing bacteria was detected by screening test that is Double disc synergy test (DDST). Then detection of ESBL encoding genes such as *bla*CTX-M-15 and blaOXA-1 were detected by PCR. PCR was taken as gold standard. *Klebsiella pneumoniae* ATCC 700603 was used as reference strain for ESBL positive . control. The strain of *Esch. Coli*, which was sensitive to ceftazidime, ceftriaxone, cefotaxime and aztreonam was used as negative control.

Screening Test: Standard inoculum of bacterial suspension matched to 0.5 McFarland was made and Mueller Hinton Agar (MHA) plate was inoculated properly with bacterial suspension. Ceftazidime (30 g), Ceftriaxone (30µg), Cefotaxime (30µg) and Aztreonam (30µg) discs (Oxoid, England) were placed onto MHA plate and incubated overnight at 37ºC. When inhibition zone of any isolate to Ceftazidime ≤22mm or Aztreonam ≤27mm, or Cefotaxime ≤27mm or Ceftriaxone ≤25mm alone or in combination was found then the isolate was taken as screening test positive.

Double Disc Synergy Test (DDST) test[8-9]: The MHA plate was inoculated with bacterial suspension matched to 0.5 McFarland. Ceftazidime (30µg), Ceftriaxone (30µg), Cefotaxime (30µg) and Aztreonam (30µg) discs were placed 15 mm distance centre to centre from amoxiclav disc (20mg amoxicillin and 10mg of clavulanic acid) which was placed at middle. Any extension of inhibition zone of antimicrobial discs (one or more) towards amoxiclav disc confirmed the presence of ESBL.

Molecular Characterization ESBL Producers by PCR[10] test: The presence of ESBL genes such as *bla*CTX-M-15 and *bla*OXA-1 genes among the ESBL producers were detected by polymerase chain reaction (PCR). To prepare bacterial pellets, a loop full of bacterial colonies was inoculated into a Falcon tube containing trypticase soy broth. After incubation overnight at 37ºC, the Falcon tubes were centrifuged at 4000 ×g for 10 minutes, after which the supernatant was discarded. A small amount of sterile trypticase soy broth was added into the Falcon tubes with pellets and mixed evenly. Then an equal amount of bacterial suspension was placed into 2 to 3 to microcentrifuge tubes. The microcentrifuge tubes were then centrifuged at 4000×g for 10 minutes and the supernatant was discarded. The microcentrifuge tubes containing bacterial pellets were kept at -20ºC until DNA extraction. Bacterial DNA was extracted by the boiling method[7]. The following pairs of previously used primers were used to yield PCR products: for b1aCTX-M-15-CACACGTGGAATTTAGGGACT (forward), GCCGTCTAAGGCGATAAACA (reverse) and for blaOXA-1- ACCAGATTCCAACTTTCAA (forward), TCTTGGCTTTTATGCTTG (reverse)[8]. The following cycling parameters were used: initial denaturation at 95ºC for 10 minutes, then 30 cycles of denaturation at 95º C for

one minute, annealing at 63ºC (for blaNDM-1), 52º C (for *bla*IMP), 52º C (for blaVIM) for 45 seconds, extension at 72ºC for one minute and 30 seconds, and a final extension at 72º C for 10 minutes. The amplified DNA were loaded into a 2% agarose gel, electrophoresed at 100 volts for 30 minutes, stained with 1% ethidium bromide, and visualized under UV light.

Statistical Analysis: Statistical analysis was performed by Windows based software named as Statistical Package for Social Science (SPSS), versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data were expressed as mean, standard deviation, minimum and maximum. Categorical data were summarized in terms of frequency counts and percentages. Every efforts were made to obtain missing data.

Ethical Consideration: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration 2013) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local ethics committee. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and were analyzed using the coding system.

Results

Total isolated gram-negative bacteria were 191 and the most frequently isolated bacteria was Escherichia coli. Out of 54 Escherichia coli, 32 (4.24%) was confirmed as ESBL producers by PCR test (Figure I).

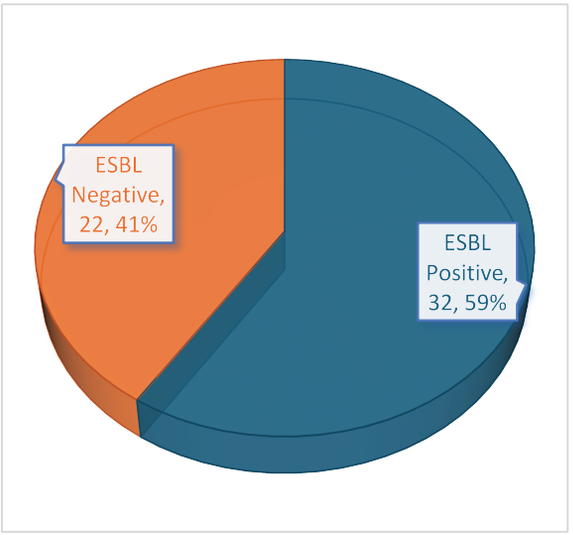

Figure I: Showing the ESBL Producing Escherichia coli (n=54)

Significantly highest (83.33%) percentage of ESBL producing *Escherichia coli* had been identified from inpatient department (IPD) and a much less amount of 29.2% isolates. ESBL producing *Escherichia coli* was detected from outpatient department (OPD). Urine was the most common sample from which *Escherichia coli* was isolated. Sixty six percent of total *Escherichia coli* was isolated from urine samples. Out of 24 *Escherichia coli* isolated from urine samples of inpatient department, 21(87.5%) were ESBL producers and 5(41.7%) were ESBL producers among 12 Escherichia coli isolated from urine of outpatient department. Difference of ESBL production by *Escherichia coli* between inpatient and outpatient department was statistically significant (Table 1).

Table 1: ESBL producing *Escherichia coli* detected by Different Methods

Test	Inpatient Department			Outpatient Department		
	ESBL		Total	ESBL		Total
	Positive	Negative		Positive	Negative	
Screening test	32	04	36	07	11	18
DDST	20	16	03	03	15	18
PCR	27 ESBL (27.0%)	09	05	05 ESBL (27.0%)	13	18

About 32 (59.26%) ESBL producing *Escherichia coli* out of total 54 *Escherichia coli*, and isolated 25.0% of *Pseudomonas* species, 14.29% of *Acinetobacter baumannii* and 42.31% of *Klebsiella* species were ESBL producers. Next to *Escherichia coli*, *Pseudomonas* species numbered second, *Acinetobacter baumannii* numbered third and *Klebsiella* species numbered fourth position among 191 bacterial isolations. ESBL producing *Pseudomonas* species were 11(25.0%) out of 44, ESBL producing *Acinetobacter baumannii* were 4(14.3%) among 28, ESBL producing *Klebsiella* species were 11(42.31%) out of 26, ESBL producing *Citrobacter* species were 6 (40.0%) among 15 and ESBL producing Proteus species were 5(38.46%) out of 13. In all strains number of inpatient ESBL producing isolates were higher than outpatient department (Table 2).

Bacteria Name	Inpatient Department			Outpatient Department			ESBL producers out of total respective organisms
	ESBL		Total	ESBL		Total	
	Positive	Negative		Positive	Negative		
Esch. Coli	27	09	36	5	13	18	32 (59.25%)
Pseudomonas spp.	9	21	30	2	12	14	11 (25%)
Klebsiella spp.	8	10	18	3	5	8	11 (42.31%)
Citrobacter spp.	5	7	12	1	2	3	6 (40%)
Proteus spp.	4	6	10	1	2	3	5 (38.46%)
Acinetobacter spp.	4	22	26	0	2	2	4 (14.28%)

Discussion

Over the years beta lactamase antibiotics are prescribed for both hospital acquired and community acquired infections. The continued use of these antibiotics produces selective pressure for pathogenic and commensal bacteria to produce and maintain beta lactam antibiotic destroying mechanisms. Discovery of different types of beta lactamase enzymes are the best example of this long-continued pressure. Now a days multiple broad-spectrum beta lactamases produced by multi drug-resistant *Klebsiella pneumoniae*, *Acinetobacter* species, *Pseudomonas* aeruginosa and *Enterobacter* species have disseminated through gram-negative pathogens[11].

In this research work ESBL producing isolates were identified from clinical specimens of outpatient (OPD) and inpatient department (IPD). ESBL strains obtained from outpatient department figured out community involvement. In contrast ESBL infections of inpatient department denoted nosocomial participation. This study reported 32(54.2%) ESBL *Escherichia coli* out of total 59 *Escherichia coli*, in which 81.5% was from inpatient department and 31.25% from the outpatient department. Significant presence of higher percentage of ESBL producing *Escherichia coli* in inpatient department in present study indicates certain degree of nosocomial spread of infections. Significantly higher number of ESBL producing Escherichia coli was detected from urine of inpatient department which is consistent with findings of several studies[6,12,13]. Isolated 43.75% of *Enterobacter* species, 54.55% of *Proteus* species and 57.1% of *Klebsiella* species were ESBL producers. Vinodini et al., found low percentage of ESBL Enterobacter species and *Proteus* species and Rao et al., showed higher percentage of ESBL *Proteus* species[14,15].

ESBL *Enterobacter* species, ESBL Proteus species and ESBL *Klebsiella* species isolation numbers were high in IPD than OPD. Identification of ESBL *Acinetobacter* species was very important because this is one of the multidrug resistant pathogens[11] and now a day it is being isolated from various biological specimens. This study also documented ESBL *Pseudomonas* species from IPD samples as reported by other study[15]. Isolations of ESBL *Acinetobacter* (7.24%) and ESBL *Pseudomonas* strains (1.45%) were alarming because they are environmental bacteria, difficult to control[9]. All isolated *Serratia* species were identified from IPD blood samples sent for blood culture and all of them were ESBL producers. *Serratia* infections are clearly related to hospitalization[9]. Comparable findings wer e documented by other studies[16-21].

Conclusion

The present study reveals significant number of ESBL producing gram negative bacteria which demands routine practice of ESBL testing in microbiology laboratory of Dhaka Medical College hospital for reporting.

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Original Article

Efficacy and Safety of Adapalene Gel and Benzoyl Peroxide Gel in the Treatment of Mild to Moderate Acne Vulgaris: A Single Centre, Double Blind, Parallel Arm Randomized Controlled Trial Hospital in Dhaka City of Bangladesh.

Md. Abul Kalam[1], Kamrul Islam[2], Tarana Jahan[3], Md. Jahangir Hossain[4], Sazzadul Haque[5], Md. Abdullah Yusuf[6]

[1]Associate Professor, Department of Dermatology & Venereology, Tairunnesa Memorial Medical College, Tongi, Gazipur. Bangladesh; [2]Associate Professor, Department of Dermatology &Venereology, Monno Medical College, Manikganj, Bangladesh; [3]Associate Professor, Department of Microbiology, Monno Medical College, Manikganj, Bangladesh; [4]Professor and head, Department of Psychiatry, Monno Medical College, Manikganj, Bangladesh; [5]Assistant professor, Department of ENT & Head-Neck Surgery, Monno Medical College, Manikganj, Bangladesh; [6]Associate Professor, Department of Microbiology, National Institute of Neurosciences & Hospital, Dhaka, Bangladesh.

Abstract

Background*:* Acne is a common dermatological disorder of teen age group. There are different modalities of topical and systemic treatments are available to manage this disease. **Objective:** The purpose of the present study was to evaluate the efficacy and safety of Adapalene 0.1% gel in comparison to Benzoyl Peroxide (BPO) 2.5% gel for the treatment of mild to moderate acne vulgaris. **Methodology:** This single centered randomized controlled trial was conducted in the Department of dermatology and venereology Tairunnesa memorial medical college Hospital, Tongi, Gazipur, Bangladesh from October 2023 to April 2024 for a period of six month. A total number of sixty patients of clinically diagnosed acne vulgaris were primarily selected and randomly divided into two equal group (group-A and group-B). Group A was given adapalene 0.1% gel for 12 weeks & Group B was given BPO 2.5% once daily in the evening for same duration. Patients were clinically assessed at baseline and at week 4, 8 and 12. At each visit, the investigator rated, scaling, erythema, dryness, burning, pruritus on a scale ranging from 0 (none) to 3 (severe). Adverse events were evaluated at each visit. **Results:** A total number of 60 patients were recruited after fulfilling the inclusion and exclusion criteria. Among them predominant age group was 20 to 24 years which were 27 (45.0%). Male: female ratio was 1:2.52. 51(85%) patients had oily skin type and 9(15%) had dry skin type. Adapalene 0.1% gel was significantly more effective than BPO 2.5% gel, with significant differences in total lesion counts observed as early as 4 weeks of treatment. Adverse event frequency and cutaneous tolerability profile was significantly favorable for adapalene gel in the treatment of acne vulgaris. **Conclusions:** Once daily Adapalene gel provides significantly greater efficacy and safety for the treatment of mild to moderate acne vulgaris compare to Benzoyl Peroxide gel.

Keywords: Efficacy and Safety; Adapalene Gel; Benzoyl Peroxide Gel; Mild to Moderate Acne Vulgaris
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Correspondence: Dr. Md. Abul Kalam, Associate Professor, Department of Dermatology & Venereology, Tairunnesa Memorial Medical College, Tongi, Gazipur. Bangladesh; Email: drmakalam@yahoo.com, **ORCID:** https://orcid.org/0009-0009-0033-870X , Contact no: 01915526201.

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Introduction

Acne vulgaris is a chronic disease of the pilosebaceous unit. Usually occur at puberty but can also be seen in adult age. A multifactoral pathophysiology including sebaceous gland hyperplasia with seborrhea, altered follicular growth and differentiation, Propionibacterium acnes proliferation, and inflammation[1]. Most cases of acne consist of comedones, papules, pustules, and nodules. Although the course of acne may be self-limiting, but sometime pitted or hypertrophic scar may persist for lifelong[2]. Lesions of acne vulgaris can be divided into four grades – 1, 2, 3, and 4. Grade 1 consists of comedones and occasional papules. Grade 2 consists of papules, comedones and few pustules. Grade 3 consists of predominant pustules, nodules, and abscesses. Grade 4 consists of mainly cysts, abscesses, and widespread scarring[3].

Drugs used for Grades 1 and 2 (mild-to-moderate case) of acne vulgaris are topical comedolytics, antibacterials, and retinoids as monotherapy or combination therapy. Grades 3 and 4 (severe cases) of acne vulgaris require systemic antibacterials along with topical agents[4]. Topical treatments such as adapalene and benzoyl peroxide are popular in mild to moderate acne vulgaris[5]. Among topical treatments, adapalene and benzoyl peroxide (BPO) are widely used for mild to moderate acne. Benzoyl peroxide, a potent topical antibacterial agent, exerts its effects by oxidation and free radical generation, leading to the reduction of C. acnes. In addition to its antibacterial properties, it has comedolytic and mild anti-inflammatory actions. BPO is effective as monotherapy or in combination with other topical agents and is associated with minimal resistance[6]. Adapalene, a third-generation topical retinoid, is a naphthoic acid derivative that selectively targets retinoic acid receptors. It exhibits anti-inflammatory, comedolytic, and anti-comedogenic properties, making it a rational choice for acne treatment.

Adapalene demonstrates favorable tolerability and effectiveness, both as a standalone treatment and in combination with antimicrobial agents such as BPO[6,7]. Benzoyl peroxide is a potent topical antibacterial agent, acts through oxidation and formation of free radicals causing a reduction of P. acnes. It also has comedolytic property and mild anti-inflammatory actions. It is usually used alone or in combination with other topical anti-acne medications[3]. Adapalene is a topical retinoid. It is a receptor-selective naphthoic acid derivative with anti-inflammatory, comedolytic, and anti-comedogenic properties[7]. It is recognized as an effective topical retinoid with a favorable tolerability profile and is therefore a rational selection for acne treatment in alone or combination with an antimicrobial agent[8]. Clinical studies comparing adapalene and BPO have focused on their efficacy and tolerability in mild to moderate acne vulgaris. Both agents effectively reduce inflammatory and non-inflammatory lesions; however, adapalene's anti-inflammatory properties offer a potential advantage in minimizing erythema and irritation[9]. BPO, while effective in reducing bacterial proliferation, may cause dryness or peeling, particularly at higher concentrations. Combining the two agents has shown superior efficacy compared to monotherapy, enhancing lesion reduction while mitigating the risk of bacterial resistance[10].

Acne vulgaris treatment requires a tailored approach based on the severity of the condition[8]. For mild to moderate acne, topical therapies such as adapalene and BPO remain cornerstone treatments. Both agents exhibit unique mechanisms of action and are effective individually or in combination[11]. Further studies are warranted to optimize their use, considering efficacy, safety, and patient tolerability. By understanding the comparative benefits of these treatments, clinicians can make informed decisions to improve outcomes for patients with acne vulgaris. This study was planned to compare the efficacy and side effects of topical application of Adapalene alone and BPO alone in the treatment of mild to moderate acne vulgaris.

Methodology

Study Settings & Population: This single centered randomized controlled trial was conducted in the Department of dermatology and venereology Tairunnesa memorial medical college Hospital, Tongi, Gazipur, Bangladesh from October 2023 to April 2024 for a period of six month. Patients between 15 and 30 years of age with mild to moderate facial acne vulgaris, assessed using the Investigator Global Assessment Scale with a minimum of 10 inflammatory lesions, 10 to 100 non-inflammatory lesions, and no more than one nodule or cyst on the face, were included in this study. Patients suffering from nodulo-cystic acne, pregnant women and lactating mother, patient taking any medication for acne, persons having hypersensitivity to adapalene and benzoyl peroxide and patients with other dermatologic conditions interfering with the treatment of acne vulgaris were excluded in this study.

Allocation and Blinding: A total number of sixty patients

of clinically diagnosed acne vulgaris were primarily selected and randomly divided into two equal group (group-A and group-B). Group A was given adapalene 0.1% gel for 12 weeks & Group B was given BPO 2.5% once daily in the evening for same duration.

Follow up and Outcome Measures: Patients were clinically assessed at baseline and at week 4, 8 and 12. The primary efficacy variables were success rate (the percentage of subjects rated "clear" or "almost clear" on the investigator's global assessment scale [IGA] of acne severity) and percentage of lesion reduction from baseline (total, inflammatory, and non-inflammatory). Safety and tolerability were assessed through evaluations of local facial tolerability and adverse events. At each visit, the investigator rated, scaling, erythema, dryness, burning, pruritus on a scale ranging from 0 (none) to 3 (severe). Adverse events were evaluated at each visit.

Statistical Analysis: Computer based statistical analysis were carried out with appropriate techniques and systems. All data were recorded systematically in preformed data collection form (questionnaire) and quantitative data were expressed as mean and standard deviation and qualitative data were expressed as frequency distribution and percentage. Statistical analysis was performed by using window-based computer software devised with Statistical Packages for Social Sciences (SPSS-25) (SPSS Inc, Chicago, IL, USA). A 95% confidence limit was taken. Probability value <0.05 was considered as the level of significance.

Ethical Consideration: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the Institutional research ethics. 3.Formal ethics approval was granted by the IRB of Monno Medical College. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and analysed using the coding system.

Results

A total number of 60 patients were recruited after fulfilling the inclusion and exclusion criteria. Among them predominant age group was 20 to 24 years which were 27 (45.0%) followed by 15 to 19 years which were 21(35.0 %) and 25 to 29 years which were 12(20.0%) (Table 1).

Table: 1. Distribution of Patients According to Age Group

Age Group	Frequency	Percent
15 to 19 Years	21	35.0
20 to 24 Years	27	45.0
25 to 29 Years	12	20.0
Total	60	100.0

Female predominance was observed 43 (71.7%) in comparison to male 17(28.3%). Male: female ratio was 1:2.52 (Table 2).

Table:2. Distribution of Patients According to Gender

Gender	Frequency	Percent
Male	17	28.3
Female	43	71.7
Total	60	100.0%
Ratio	1: 2.5	

Among 60 patients, 41 (69.3%) were unmarried and 19 (31.7%) were married (Figure I).

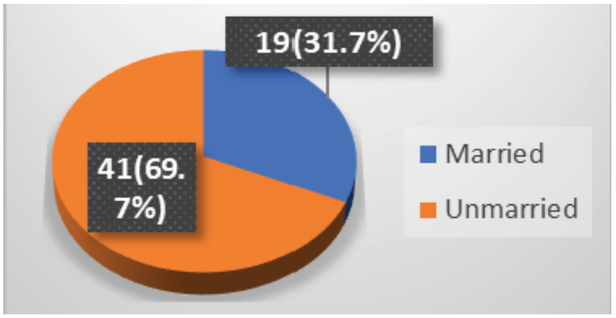

Figure I: Distribution of patients according to marital status

Among 60 patients, 51(85%) patients had oily skin type and 9 (15%) had dry skin type (Figure II).

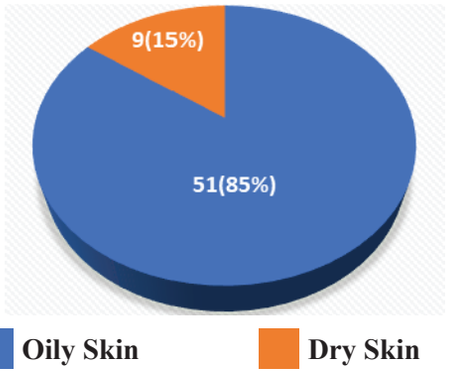

Figure II: Distribution of patients according to skin type

Among 60 patients, face was the common site 56(93.33%) in all the patients, followed by back 34(56.7%) and chest 8(13.33%) (Figure III).

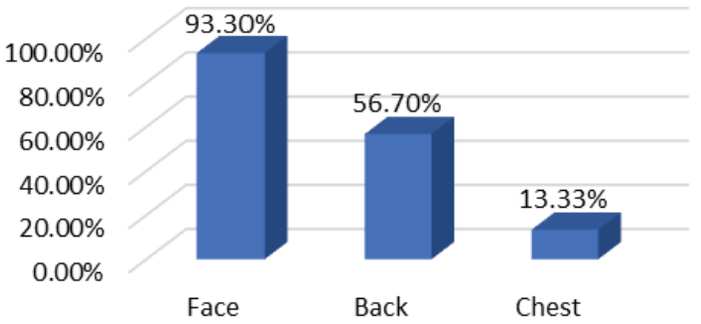

Figure III: Distribution of patients according to site

Mean score for open comedone, closed comedone, papule and pustule was identical between two groups at base line (p>0.05). Significantly better reduction of acne score for open comedone, closed comedone, papule, pustule and total acne score was noticed at 2nd and 3rd follow up (p<0.005) in the group A than the group B. Percent reduction of acne severity from base line to final follow up was 94.76% in group A and 83.42% in group B and it was statistically significant between two group (p=0.001) (table 3).

Table: 3. Efficacy of treatment between two groups

Follow up	**Group A (Adapalen)** (mean lesions count)	**Group B (BPO)** (mean lesions count)	**p-value**
Baseline (0 week)	34.66±6.40	33.66±4.96	0.45
4th week	31.56±3.86	24.0±3.27	0.188
8th week	11.10±2.22	15.26±3.04	<0.001
12th week	2.66±1.17	6.30±1.58	<0.001
Reduction from baseline To 3rd follow up	93.66%	82.32%	<0.001

After 12 week of treatment, in Group-A 6.6% patient had dryness of skin and 3.3% had erythema, whereas in Group -B, who had applied benzoyl peroxide, among them 10% suffered from dryness, 6.6% erythema and 3.3% burning .There was a significant difference of adverse effects between two groups(p>0.05) with favorable to Adapalene (Figure IV).

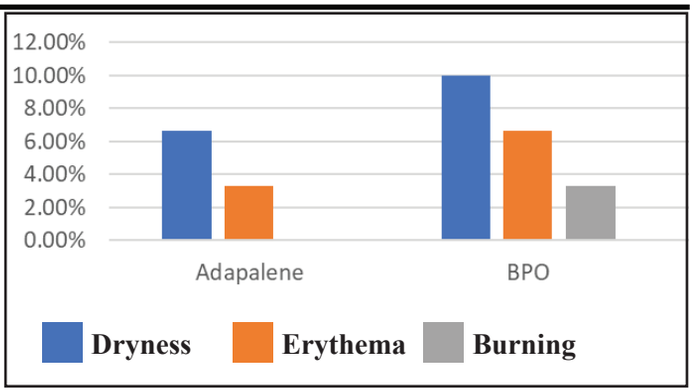

Figure IV: Show Adverse effects at 3rd follow up.

Discussion

To evaluate the on efficacy and safety of Adapalene gel and Benzoyl peroxide gel in the treatment of mild to moderate Acne Vulgaris 60 patient has been selected according to inclusion and exclusion criteria. Among them predominant age group was 20 to 24 years. Despite its spontaneous regression in most patients, acne persists in 10.% of those patients over the age of 25 years[11]. Female predominance was observed. Male: female ratio was 1:2.52. Among 60 patients, 51(85%) patients had oily skin type and face was the common site 56(93.33%) in all the patients, followed by back 34(56.7%) and chest 8(13.33%). Additionally, a significant number of patients reported similar findings which is consistent to other study.

For mild to moderate acne vulgaris, topical therapy is the standard treatment[12]. The retinoids and benzoyl peroxide are frequently used drugs for the topical treatment. Benzoyl peroxide is a bactericidal agent that has moderate comedolytic and anti-inflammatory action[9]. Topical retinoids reduce the abnormal growth and development of keratinocytes within pilosebaceous duct[13]. Adapalene, which is a topical retinoid, binds to specific retinoic acid nuclear receptors, and modulates the cellular differentiation, keratinization and inflammatory processes[14]. Despite the fact that there are a lot of studies with benzoyl peroxide, or adapalene alone, there are only a few studies which compare these two drugs[7]. In our study, reduction of non-inflammatory lesion, inflammatory lesion and total lesion counts from baseline values were highly significant in both the groups (p<0.001) and between the groups also, there was a statistically significant difference present in different visits (p<0.05).

These findings conclude the better efficacy of adapalene as compared to benzoyl peroxide for the treatment of mild to moderate acne vulgaris. This data correlates with the study conducted by another study, they found adapalene to be

significantly more superior to benzoyl peroxide in reducing the lesions of mild acne vulgaris[15]. But they found a faster onset of action of benzoyl peroxide (at month 1) against inflammatory lesions, which they concluded to be due to its more rapid and superficial antibacterial and anti-inflammatory functions. Like our study, this study was also a single centre, randomized study comparing efficacy and safety of adapalene 0.1% gel and benzoyl peroxide 2.5% gel for a study period of 3 mont16. A similar comparative clinical study of efficacy and safety of adapalene 0.1% gel versus benzoyl peroxide 2.5% gel for the treatment of acne vulgaris. They found that a better efficacy and safety of adapalene than benzoyl peroxide in the treatment of mild to moderate acne vulgaris[17].

In a similar study, it has also compared the efficacy and safety of adapalene 0.1% gel and benzoyl peroxide, but the concentration of benzoyl peroxide was different, they used 4% benzoyl peroxide, and unlike our study, they found benzoyl peroxide to be more efficacious than adapalene on non-inflammatory and inflammatory lesions at 2 weeks and 5 weeks18. Compared these two drug monotherapies with their combination, and concluded that the combination therapy has no superior efficacy over adapalene or benzoyl peroxide monotherapy. This was also an open-label, prospective study but unlike our study, they used 5% benzoyl peroxide lotion[19]. The adverse events are also major determinants in selecting a topical medication for acne patients. In our study, we found lesser side effects with adapalene group than those with benzoyl peroxide. The patients treated with adapalene suffered from 6.6% dryness, 3.3% erythema whereas those treated with benzoyl peroxide had 10% dryness, 6.6% erythema and 3.3% burning. The adverse events did not interfere with the completion of the treatment. Similarly, a study conducted by Babaeinejad and Fouladi20, mild and transient side effects were found in both adapalene and benzoyl peroxide groups. It has been justified that benzoyl peroxide 2.5% is as effective as higher concentrations (5% and 10%) in treating acne vulgaris while causing fewer side effects[21-23].

In the study conducted both adapalene and benzoyl peroxide were found to be safe drugs although they used 4% benzoyl peroxide. In another study concluded that there were no significant differences between the 3 groups (adapalene, benzoyl peroxide and adapalene-benzoyl peroxide combination) with regard to erythema, dryness or burning. All the side effects diminished with the continuation of the treatment[22].

Conclusion

Adapalene has better efficacy and safety than benzoyl peroxide, thus adapalene monotherapy can be used for the treatment of mild to moderate acne vulgaris, whereas severe inflammatory lesions of acne should be treated with combining adapalene with other drugs, along with the systemic therapy as suggested by previous studies.

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Original Article

Seroprevalence of Rubella Virus among Pregnant Women in Relation with Sociodemographic Change in Sylhet District of Bangladesh

Nasreen Ahmed[1], Md. Moynul Haque[2], Syed Anwarul Hoque[3], Ishrat Jahan Karim[4], Mohammad Thoufiqul Islam[5], Tarana Jahan[6]

[1]Assistant Professor, Department of Microbiology, Sylhet Central Dental College, Sylhet, Bangladesh; [2]Professor and Former Head, Department of Microbiology, Sylhet MAG Osmani Medical College, Sylhet, Bangladesh; [3]Professor and Head, Department of Microbiology, Park View Medical College, Sylhet, Bangladesh; [4]Associate Professor, Department of Obstetrics & Gynaecology, Sylhet MAG Osmani Medical College, Sylhet, Bangladesh, [5]Assistant Professor, Department of Cardiology, Sunamganj Medical College, Sunamganj, Bangladesh; [6]Associate Professor, Department of Microbiology, Monno Medical College, Manikganj, Bangladesh

Abstract

Background: Clinical or subclinical infection of rubella in pregnant women can give rise to congenital rubella infection of her baby. **Objectives:** The purpose of the study was to see the prevalence of rubella virus specific IgG antibody among pregnant women. **Methodology:** This cross-sectional study was conducted in the Department of Microbiology in collaboration with the Department of Obstetrics & Gynaecology at Sylhet MAG Osmani Medical College, Sylhet, Bangladesh during the period from January to December 2019 for a duration of one year. By simple random sampling pregnant women with the age between 16 to 38 years attending antenatal clinic were selected according to inclusion and exclusion criteria. Anti-rubella antibody (IgG) titre was measured by Enzyme-Linked Immunosorbent Assay (ELISA) method. **Results:** A total number of 207 individuals were included in this study. Among them 186(89.9%) participants had protective immunity and 21(10.1%) participants had no protective immunity against rubella. Protective immunity was higher in age group 26-30 years and then it was declined as age increases (p=0.042). Protective immunity was found in 61 (82.43%) participants of 1st trimester, 64 (94.12%) participants of 2nd trimester and 61(93.85%) participants of 3rd trimester(p=0.031). Protective immunity was in 103 (90.35%) urban participants and 83 (89.25%) in rural participants (p=0.794). Protective immunity was found in 70 (93.33%) participants of lower class, 97 (90.65%) participants of middle class, 19 (76%) participants of higher class (p=0.042). **Conclusion:** In conclusion, the substantial percentage of pregnant women are susceptible for rubella infection.

Keywords: protective immunity; antibody; rubella infection; vaccination
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Correspondence: Dr. Nasreen Ahmed, Assistant Professor, Department of Microbiology, Sylhet Central Dental College, Sylhet, Bangladesh; Email: nasreen4nbmc41@gmail.com; Cell no: +8801724575487; ORCID: https://orcid.org/0009-0006-4962-3905

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Introduction

Rubella is an acute febrile illness characterized by a rash and lymphadenopathy and caused by rubella virus[1]. Rubella is considered one of the most teratogenic viruses during pregnancy. Congenital infection depends on the time of exposure to the virus[2]. In the first 10 weeks of gestation congenital rubella syndrome occurs in about 90.0%, at 11 to 12 weeks about 50.0% and at 13 to 16 weeks about 33.0%. After 17 to 18 weeks defects due to rubella are rare. The risk of congenital malformation again rises to 60.0% at 31 to 36 weeks[3].

The worldwide pandemic of rubella in 1962 to 1965

highlighted its importance. The last major rubella epidemic in the United States occurred from 1964 to 1965. An estimated 12.5 million people got rubella, 11,000 pregnant women lost their babies, 2,100 newborns died, and 20,000 babies were born with congenital rubella syndrome[1]. This pandemic stimulated the development of a safe and effective rubella vaccine. World Health Organization established goal to eliminate rubella and CRS by 2020. CRS rates are highest in the African and South-East Asian regions where vaccine coverage is lowest[4]. Worldwide over 100,000 babies are born with CRS every year[5]. In Bangladesh, a study was carried out in 2003 to 2004 on 134 pregnant women. The overall prevalence of seropositivity for IgG was 84.3% and 15.7% were seronegative[6]. A study conducted in Ethiopia showed that seropositivity was higher in urban area than in rural area[7].

Rubella vaccine is available as combined measles, mumps and rubella (MMR) vaccine, or measles and rubella (MR) vaccine[5]. In October 2012, Bangladesh replaced a single antigen measles vaccine with a measles-rubella (MR) vaccine in the routine immunization schedule at 9 and 15 months of age. Unfortunately, adolescent and adult girls of child bearing age, who are presently at risk are not included in this programme[8]. It is advisable that all women of child bearing age are screened for rubella antibody and susceptible women should be included in the routine vaccination programme. The vaccine is live and therefore contraindicated in pregnancy and pregnancy should be avoided in the month after vaccination[9]. Hence, active surveillance is required to determine the seropositivity of rubella in pregnant women and to identify those who are at risk of giving birth to congenitally malformed babies. Therefore, the present study is carried out to detect the seropositivity of IgG for rubella in pregnant women to see their immune status and vulnerability to rubella infection.

Methodology

Study Settings and Population: This cross-sectional observational study was conducted in the Department of Microbiology in collaboration with the Department of Obstetrics & Gynaecology, Sylhet MAG Osmani Medical College, Sylhet, Bangladesh. The study was carried out during the period from 1st January 2019 to 31st December 2019 for a duration of one year. All pregnant women with the age between 16 to 38 years attending antenatal clinic in the Department of Obstetrics & Gynaecology.

Study Procedure: Study population was selected on the basis of some enrollment criteria. Inclusion criteria were pregnant women in the age group of 16 to 38 years and persons excluded who were diagnosed case of diabetes mellitus, AIDS, malignancy and taking immunosuppressant drugs or steroid therapy. After selection of study population who were mostly available, easily accessible and convenient to include were identified against a serial number. All the participants were thoroughly informed about their roles and the procedure of this research work. Data were collected by predesigned data collection sheet. Informed written consents were obtained from all the subjects. All information was kept confidential with due respect to the participants wish and without any force or pressure. After taking all aseptic precautions 3 ml of venous blood was drawn by sterile disposable 5 cc syringe into a vacutainer tube and was allowed to clot at room temperature for about 30 minutes. Serum was separated by centrifugation at 3000 rpm for 10 minutes and then 100 µL of serum was transferred carefully into Eppendorf tube, properly capped, labelled and stored at -20^{oC} until further analysis. Serum IgG antibody against rubella virus was measured by Enzyme-Linked Immunosorbent Assay (ELISA) method following the instructions provided by manufacturers package insert. Manufacturer of the reagent: Rubella IgG ELISA EIA-1800, DRG International, Inc., Lot no RN-59479 and 60091, USA. All reagents were kept in proper temperature before use. All steps of procedure were completed without interruption.

Statistical Analysis: All data were processed and analyzed with the help of SPSS (Statistical Package for Social Sciences version 21). Quantitative data were expressed as mean and standard deviation and qualitative data as frequency and percentage. Association was analyzed by Pearson's Chi square (X^2) test. A probability (P) value of <0.05 was considered statistically significant.

Ethical Clearance: Approval of the research protocol and ethical permission were obtained from the Ethical Review Committee of Sylhet MAG Osmani Medical College, Sylhet. All the ethical committee guidelines were followed during the study period.

Results

A total number of two hundred seven pregnant women were recruited after fulfilling the selection criteria. The mean ± SD of serum IgG level was 47.61 ± 30.84. In this study protective immunity was determined as serum IgG level of 15 IU/ml. Out of 207 participants 186(89.9%) cases had protective immunity against rubella and

21(10.1%) cases had no protective immunity against rubella (Figure I).

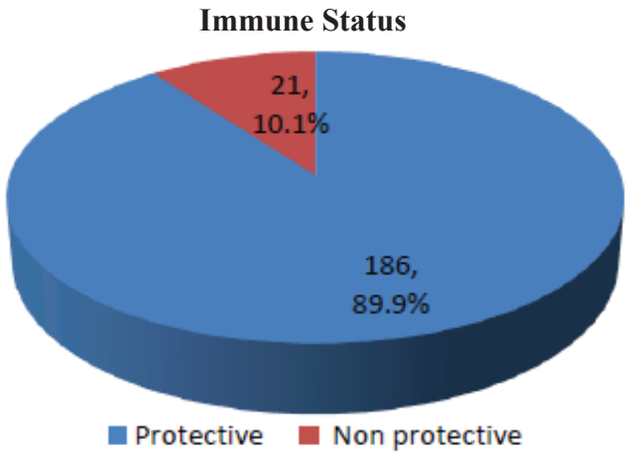

Figure I: Pie chart showing Distribution of the Participants according to Protective immunity against Rubella

Protective immunity against rubella was found in 39 (90.69%) participants of aged between 16-20 years, in 63 (92.64%) participants of aged between 21-25 years, in 58 (93.55%) participants of aged between 26-30 years and in 26 (76.47%) participants of aged between 31-38 years. Protective immunity differed significantly in different age group (p=0.042) (Table 1).

Table 1: Protective Immunity against Rubella in Different Age Group

Age Group Years	Protective Immunity	Non-Protective Immunity	Total	P value
16 to 20	39 (90.69%)	4 (9.31%)	43(100.0%)	0.042
21 to 25	63 (92.64%)	5 (7.36%)	68(100.0%)	
26 to 30	58 (93.55%)	4 (6.45%)	62(100.0%)	
31 to 38	26 (76.47%)	8 (23.53%)	34(100.0%)	
Total	**186**	**21**	**207**	

Protective immunity against rubella was found in 103 (90.35%) urban participants and 83 (89.25%) rural participants. Protective immunity did not differ significantly among different residential area (p=0.794) (Table 2).

Table 2: Protective Immunity against Rubella in Different Residence

Residence	Protective immunity	Non protective immunity	Total	P value
Urban	103(90.35%)	11(9.65%)	114(100.0%)	0.794
Rural	83(89.25%)	10(10.75%)	93(100.0%)	
Total	**186**	**21**	**207**	

Protective immunity against rubella was found in 70 (93.33%) participants of lower class, in 97 (90.65%) participants of middle class, in 19 (76%) participants of higher class. Protective immunity differed significantly among different socioeconomic status (p= 0.042) (Table 3).

Table 3: Protective Immunity against Rubella in Different Socioeconomic Status

Socioeconomic status	Protective immunity	Non protective immunity	Total	P-value
Lower Class	70(93.3%)	5 (6.67%)	75(100.0%)	0.042
Middle Class	97(90.6%)	10(9.4%)	107(100.0%)	
Higher Class	19(76.0%)	6(24.0%)	25 (100.0%)	
Total	**186**	**21**	**207**	

Protective immunity against rubella was found in 61 (82.43%) participants of 1st trimester, in 64 (94.12%) participants of 2nd trimester, in 61 (93.85%) participants of 3rd trimester. Protective immunity differed significantly among different trimester (p=0.031) (Table 4).

Table 4: Protective Immunity against Rubella in Different Trimester

Trimester (Weeks)	Protective immunity	Non protective immunity	Total	P-value
Up to 12	61(82.43%)	13(17.57%)	74(100.0%)	0.031
13 to 24	64(94.12%)	4(5.88%)	68(100.0%)	
25 to 38	61(93.85%)	4(6.15%)	65(100.0%)	
Total	**186**	**21**	**207**	

Discussion

To see the immune status of Rubella among pregnant women 207 participants were selected. All pregnant women attending antenatal clinic in the Sylhet MAG Osmani Medical College Hospital were the target population. By simple random sampling those who fulfilled the selection criteria were enrolled as the study

population. Among 207 participants, 186 (89.9%) had protective immunity and 21 (10.1%) had no protective immunity against rubella infection. A study conducted by Wondimeneh et al in Ethiopia showed that the overall protective immunity was 89% and 11% of the pregnant women were non protective which is almost similar to this study[10]. Another study conducted in Iran in 2013, where 88.9% had protective immunity and 10.4% had no protective immunity against rubella[11].

This study showed that protective immunity against rubella was found in 39 (90.69%) participants of aged between 16 to 20 years; 63 (92.64%) participants of aged between 21 to 25 years; 58 (93.55%) participants of aged between 26 to 30 years and in 26(76.47%) participants of aged between 31-38 years. Protective immunity differed significantly in different age group (p=0.042). It was also supported by other studies which showed that protective immunity was higher in 20 to 30 years age and decreased after 30 years[6,12]. Protective immunity against rubella was found in 61 (82.43%) participants of 1st trimester, 64 (94.12%) participants of 2nd trimester, 61(93.85%) participants of 3rd trimester. Protective immunity differed significantly among different trimester (p=0.031). It was also supported by other studies which showed that seropositivity was lower in 1st trimester and higher in 2nd and 3rd trimester[6,7,13,14].

Protective immunity against rubella was higher in urban area (90.35%) than rural area (89.25%) but this did not differ significantly between different residence (p=0.794). It was also supported by other studies which showed that seropositivity rate was higher in urban area than in rural area[7,15].

In this study protective immunity against rubella was found in 70 (93.33%) participants of lower class, 97 (90.65%) of middle class, 19 (76%) participants of higher class. Protective immunity differed significantly among different socioeconomic status (p= 0.042). It was also supported by other studies which showed that seropositivity rate was much higher in lower socio-economic status group, and lower in upper socioeconomic status group[6,16,17].

Conclusion

This study showed that protective immunity against rubella declined as age increases, it was significantly higher in lower class but no significant difference between rural and urban area. Protective immunity was significantly lower in 1st trimester and the substantial percentage of pregnant women were susceptible for rubella infection. Therefore, rubella vaccination programme especially for female of reproductive age will reduce prevalence of rubella infection and rubella related complications.

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Conflict of Interest: We do not have any conflict of interest (financial or others).

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Review Article

Epidemiology, Molecular Characteristics and Genotypic Resistant Profiles of *Acinetobacter baumannii*: A Narrative Review

Sharmeen Sultana[1], Md. Abdullah Yusuf[2], Narjis Maliha Kawsar[3], Tarana Jahan[4], Md. Asifudduza[5]

[11]Associate Professor, Department of Microbiology, Monno Medical College, Manikganj, Bangladesh; [2]Associate Professor, Department of Microbiology, National Institute of Neurosciences and Hospital, Dhaka, Bangladesh; [3]Professor and Head, Department of Microbiology, Monno Medical College, Manikganj, Bangladesh; [4]Associaste Professor, Department of Microbiology, Monno Medical College, Manikganj, Bangladesh; [5]Consultant, Labaid Cardiac Hospital, Dhanmondi, Dhaka, Bangladesh

Abstract

Acinetobacter baumannii is an important opportunistic pathogen and is often involved in various nosocomial infections, such as bacteremia, urinary tract infection, surgical site infection, and nosocomial and ventilator associated pneumonia, especially in patients admitted to ICU. Acinetobacter baumannii is notable for its remarkable innate and acquired resistance to multiple antimicrobial classes, including extended-spectrum cephalosporins and carbapenems. Resistance to carbapenems is the most concerning, as carbapenems have a potent activity against Acinetobacter spp and are often used as a last resort for the treatment of infections due to multidrug resistant Acinetobacter baumannii isolates. In developing countries also, the misuse and underuse of antimicrobials due to lack of awareness of patients, medical workers and financial problems emerged the antimicrobial resistant strains. Due to rapid globalization of human population by travel and other factor these resistant strains spread easily between developed and developing countries making it a global problem.

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Correspondence: Dr. Sharmeen Sultana, Associate Professor, Department of Microbiology, Monno Medical College, Monno City, Gilondo, Manikganj, Bangladesh; Email: nila21786@gmail.com; Cell no.: +8801724819455; ORCID ID: https://orcid.org/0000-0002-0503-0693

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Introduction

Multidrug resistant Acinetobacter baumannii is a rapidly emerging opportunistic pathogen associated with a variety of nosocomial infection, including ventilator-associated pneumonia, bacteremia, surgical site infections, secondary meningitis and urinary tract infections[1]. Artificial ventilation and other invasive procedures, exposure to antibiotics, colonization pressure, environmental contamination in ICU and underlying illness facilitate the spread of these multidrug-resistant species in ICU[2].

Formerly, *Acinetobacter* species were susceptible to beta-lactam antibiotics, mainly ceftazidime and carbapenems. In recent times, the clinical isolates demonstrating resistance to cephalosporins and carbapenems are very high[3]. High level of resistance was recorded for ampicillin (86.3%), cefazolin (93.2%), gentamicin (61.5%), cefotaxime (65.8%), ceftriaxone (61.5%) and ciprofloxacin (69.2%)[4]. However, several studies have suggested that tigecycline and colistin may be effective in infections caused by carbapenem resistant strains of *Acinetobacter baumannii*[5-6]. Reduced Acinetobacter baumannii susceptibility to these drugs has recently been reported from several countries across the world[7-8]. The production of beta-lactamases, changes in permeability, increase in the efflux pump and modification of penicillin binding proteins (PBPs) have been described

regarding resistance to beta-lactam antibiotics[9]. Several mechanisms for acquiring colistin resistance have been described in *Acinetobacter baumannii*. Mutations in the genes encoding the two-component signaling protein pmrA and PmrB and mutation in lipopolysaccharide biosynthesis genes lpxA, lpxC and lpxD mediate colistin resistance[10-11]. Tigecycline non susceptibility in *Acinetobacter bauamnnii* isolates has been associated with over expression of a variety of efflux pumps such as AdeABC, AdeIJK, AdeFGH, AbeM and AdeDE[12].

The genus Acinetobacter

Acinetobacter was first described in 1911 by a Dutch microbiologist by the name of Martinus Willem Beigerinck as *Micrococcus calco-aceticus*. Since then, it has had several names, becoming known *Acinetobacter* in the 1950s[13]. The genus *Acinetobacter* can presently be defined as gram-negative, strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive and oxidase-negative coccobacillary bacteria with a DNA G and C content of 39% to 47%[1]. Nonetheless, gram-staining of *Acinetobacter* can be variable and the morphologic characteristics may change depending on the growth phase[14].

Acinetobacter species

At least 33 species within the Acinetobacter genus have so far been identified, including 24 named species and 9 currently described as genomic species (gen.sp.) given that no phenotypic properties have been found to differentiate them from other species[15]. In 1986, twelve *Acinetobacter* genomic species within the *Acinetobacter* genus were identified by DNA-DNA hybridization[16]. Six of these DNA groups could be differentiated by phenotypic properties and were given the following formal species names: *Acinetobacter calcoaceticus* (*Acinetobacter* gen. sp. 1), *Acinetobacter baumannii* (*Acinetobacter* gen. sp. 2), *Acinetobacter haemolyticus* (*Acinetobacter* gen. sp. 4), *Acinetobacter* junii (*Acinetobacter* gen. sp. 5), *Acinetobacter* johnsonii (*Acinetobacter* gen. sp. 7) and *Acinetobacter lwoffii* (*Acinetobacter* gen. sp. 8). The study reported an uncertain genotypic and phenotypic differentiation of *Acinetobacter* gen. sp. 9 from *Acinetobacter lwoffii*. In 2001 and 2003, Nemec identified three novel species names as *Acinetobacter schindleri*, *Acinetobacter* ursingii and *Acinetobacter* parvus[15]. Concurrently seven novel species were identified (*Acinetobacter baylyi*, *Acinetobacter bouvetii, Acinetobacter towneri*, *Acinetobacter tandoii*, *Acinetobacter grimontii*, *Acinetobacter tjernbergiae* and *Acinetobacter gerneri*)[17]. However, *Acinetobacter grimontii* was later re-classified within the *Acinetobacter* junii species. One novel species was identified and named as *Acinetobacter* septicus in 2008 although it was soon after re-classified within the *Acinetobacter ursingii* species[18]. Three novel species (*Acinetobacter soli*, *Acinetobacter beijerinckii* and *Acinetobacter gyllenbergii)* were also identified in 2008 and 2009 by two different research groups[19]. Furthermore, *Acinetobacter* gen. sp. 10, *Acinetobacter* gen. sp. 11, *Acinetobacter* gen. sp. 3 and *Acinetobacter* gen. sp. 13TU have recently been named *Acinetobacter berezinae*, *Acinetobacter guillouiae, Acinetobacter pittii* and *Acinetobacter nosocomialis*, respectively, given that they can phenotypically be differentiated from other species within the genus *Acinetobacter*[20].

Natural Habitat

Members of the genus *Acinatobacter* are considered ubiquitous organism. This holds true for the genus *Acinetobacter*, since *Acinetobacters* can be recovered after enrichment culture from virtually all samples obtained from soil or surface water[21]. The organism does not always act as an infecting pathogen, as it is widely distributed in nature and has tremendous colonizing potential[14].

The organism prefers moist environment, therefore, its colonization among damaged tissues is common[22]. *Acinetobacter* species are apparently the only group of gram-negative bacteria that may be natural residents of human skin[23]. A study from Germany reported high carriage rates of *Acinetobacter* spp. on human skin and mucus membrane among in patients (75%) and control non-hospitalized persons (43.0%). The most frequently isolated species in that study were *Acinetobacter lwoffii* (47.0%) and *Acinetobacter johnsonii* (21.0%). Unpredictably, the clinically important *Acinetobacter baumannii* and *Acinetobacter nosocomialis* species (0.5% and 1.0%, respectively) were not found to be common human skin colonizers[23].

In patients hospitalized on a regular ward, the carriage rate of *Acinetobacter* species was even higher, at 75.0%[23]. *Acinetobacter* species are fecal carriage and carrier rate is 25.0% among healthy individuals, with *Acinetobacter johnsonii* and *Acinetobacter genomic* species 11 predominating[24]. In contrast, *Acinetobacter baumannii*, the most important nosocomial *Acinetobacter* species, was

found only rarely on human skin, 0.5% and 3.0% respectively[25].

Epidemiology

The ecology of bacteria belonging to the genus *Acinetobacter* is diverse. These organisms have been recovered from soil, surface water, vegetables, animals, human body lice and humans[21]. Bacteria of this species have been isolated mainly from hospitalized patients, but also from hospital environment[26]. There are indications that skin and mucous membrane colonized by clinically relevant species is an important source of infections in hospitalized patients, thereby contributing to the development and persistence of outbreaks[14]. *Acinetobacter baumannii* has the capacity to survive on inanimate surfaces, such as ventilation equipment and bedding materials for upto five months[27].

The incidence of *Acinetobacter baumannii* infections varies widely: from less than 1% in different European hospitals to 32.0% among ventilated patients in a Taiwanese hospital[28]. *Acinetobacter baumannii*, *Acinetobacter pittii* and *Acinetobacter nosocomialis* are the species most frequently involved in these infections[29]. *Acinetobacter baumannii* strains have become endemic in multiple centres and outbreaks have been observed worldwide[1].

A systematic review of published nosocomial outbreaks in the intensive care units (ICU) setting from 2005 to 2010 has revealed that *Acinetobacter baumannii* was responsible for almost 25% of ICU infection outbreaks[30]. Three major lineages of genetically highly related *Acinetobacter baumannii* strains, designated European (EU) clone I, II and III are frequently implicated in outbreaks[31]. A recent striking manifestation is the occurrence of *Acinetobacter baumannii* infections in soldiers severely injured during the conflicts in Iraq and Afghanistan[32]. Although *Acinetobacter* is mainly associated with nosocomial infection, several cases of community-acquired pneumonia, mostly associated with underlying diseases, have been reported[33].

Morphology and Identification

Typical Organism: Members of the genus *Acinetobacter* are gram-negative *coccobacilli*, during periods of rapid growth (exponential phase), the organism typically appear bacillary or *coccobacillary* 1-1.5 by 1.5-2.5 microns in size. Notably they become more coccoid or diplococcal as the culture ages (stationary phase). The Acinetobacter are non-motile but occasionally an odd twitching motility can be demonstrated[34].

Culture Characteristics: *Acinetobacter* is easily isolated in standard cultures but is relatively nonreactive in many biochemical tests commonly used to differentiate among gram-negative bacilli. *Acinetobacter* are non-lactose fermenters but may produce a slight pinkish hue that could be mistaken for lactose fermentation. The Older cultures frequently capsulated, occasionally causing problems with destaining the crystal violet. *Acinetobacter* are strictly aerobic and are capable of growing at a wide range of temperatures. For the most part, the species are not fastidious and capably grow on the standard nutritional medium used in the laboratory. Occasionally, strains may be encountered that are fastidious, failing to grow in nutrient broth and forming smaller colonies in Blood agar. For clinical isolates, growth on MacConkey agar is variable and presenting as either colorless or light pink colonies. A. c*alcoaceticus-A. baumannii* complex colonies resembles those of *Enterobacteriaceae,* with a diameter of 1.5 to 3 mm after overnight culture, whereas most of the other *Acinetobacter* species produce smaller and more translucent colonies. Unlike the Enterobacteriaceae, some *Acinetobacter* species outside the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex may not grow on MacConkey agar[1]. The colonies are 1-2 mm in diameter (smaller than typical Enterobacteriaceae) and are typically doomed, smooth to slightly mucoid and opaque. Pigmentation is usually grayish-white, although some strain may appear pale yellow. Hemolytic activity on Blood agar is variable, although a diffusible brown pigment has been observed when glucose has been added to the medium[35].

Growth characteristics: Most clinical strains of Acinetobacter grow optimally at 37°C, while the environmental isolates prefer low temperatures. Utilization of carbohydrates varies considerably among the species. Nitrates are not reduced to nitrites. The ability to hydrolyze gelatin and urease is variable. Members of the genus Acinetobacter are gram negative, catalase positive and oxidase negative[1].

Virulence Factors

Despite the increasing clinical importance of Acinetobacter baumannii infections, relatively little is known about the factors that contribute to its pathogenesis. Of the studies addressing Acinetobacter baumannii that have been carried out over the preceding decades, the majority either describe the epidemiology, risk factors, and outcomes of infections caused by these bacteria or aimed to optimize antibiotic regimens for the treatment of

infections produced by MDR strains. While these studies provide important information regarding the epidemiology and clinical management of *Acinetobacter baumannii* infections, they do not address the underlying biological basis for the increasing success of this organism as a human pathogen[36]. Prevalence of Virulence Factors (VF) contributed to pathogenesis in bacteria[37]. Virulence factors help bacteria to colonize on the epithelium, evade and inhibit the host's immune response through biofilm formation and obtain nutrition from the host[38-39]. During the past decades, new virulence factors have been described in *Escherichia coli*. Pathogenicity associated islands (PAI) are blocks of virulence factor genes that provide a mechanism to coordinate horizontal transfer of virulence factor genes between lineages, and even between species, and have emerged as characteristic of diverse pathogenic bacteria, including *uropathogenic* E. coli strains[40]. Recognized or determine virulence factor in *uropathogenic* E. coli include diverse adhesins, as P fimbriae (pap genes), S and F1C fimbriae (sfa), Drantigen family (afa/dra), type 1 fimbriae (fimH) and curli fibers (csg), fibronectin receptor (fbn), toxins, as cytotoxic necrotizing factor (cnf), siderophores, as yersiniabactin (fyuA) and aerobactin (iutA); invasins as IbeA; polysaccharide coatings as group II and III capsules (kpsMT); serum resistance (traT) and colicin V production (cvaC)[40-45]. Identification of virulence factors in *Acinetobacter baumannii* is a kee to fighting this pathogen. Genes coding for some recognized virulence factors identified in uropathgenic E. coli strains were detected in *Acinetobacter baumannii*[46-49].

Antibiotic Resistance Profiles

The wide array of antimicrobial resistance mechanisms for *Acinetobacter baumannii* is impressive and rivals those of other non-fermentative gram-negative pathogens[50]. Definitions of multidrug-resistant *Acinetobacter* species vary, referring to a wide array of genotype and phenotypes. Two of the most common definitions of multidrug resistance are carbapenem resistance or resistance to 3 classes of antimicrobials[51]. *Acinetobacter baumannii* is considered the paradigm of multidrug-resistant bacteria as the organism has an ever-increasing list of resistance determinants that can rapidly nullify most of the therapeutic armamentarium. Both acquired and intrinsic resistance mechanisms can contribute this multi-resistance. The ability to acquire such resistance for multiple drugs may be due to either the acquisition of genetic elements carrying multiple resistant determinants or mutations affecting the expression of porins and/or efflux pumps, which can minimize the activity of unrelated antimicrobial agents[52]. The genetic surroundings of these resistance determinants provided more evidence for genetic promiscuity, with an array of broad-host-range mobile genetic elements identified, including three class I integrons, transposons and insertion sequence (IS) elements[2].

Resistance to β-lactams: The main resistance mechanisms to multiple antibiotics in Acinetobacter spp. can be summarily outlined as follows (i) production of hydrolysing enzymes for e. g, β-lactam hydrolysis by different kinds of β-lactamases (Class A to D β-lactamases), (ii) changes in penicillin-binding proteins (PBPs) that prevent the action of β-lactams, (iii) alterations in the structure and number of porin proteins that result in decreased permeability to antibiotics through the outer membrane of the bacterial cell and (iv) the activity of efflux pumps that further decrease the concentration of antibiotics within the bacterial cell[53]. Attempts have been done to sort β-lactamases since 1968. The classifications are derived from two major approaches; the first one is rooted on functional criteria and second one is based on molecular structure (amino acid sequence) of the enzyme. Some researcher cited the latest functional classification of β-lactamases founded on enzyme inhibition profile and antimicrobial substrate profile[54]. According to their classification scheme, β-lactamases are segregated into groups (1-4) and subgroups (a-f). Group 1 are cephalosporinases, not well inhibited by clavulanic acid, group 2 β-lactamases are penicillinase or both penicillinase and cephalosporinases, generally inhibited by β-lactamase inhibitors and group 3 are penicillinase, cephalosporinases and carbapenemase (metallo-β-lactamases), poorly inhibited by all classical β-lactamase inhibitors except EDTA and p-chloromercuribenzoate (pCMB) and group 4 are penicillinases, not inhibited by clavulanic acid. Depending on nucleotide and amino acid sequence of the enzymes, Ambler first recommended the Structural classification of β-lactamases. As only four amino acid sequences were known during that time, the author distinguished serine-based class A penicillinase from class B MBL[55]. Afterward a new class of serine based β-lactamases designated as class C that has only a few sequences homology to class A enzymes, was detected[56]. Subsequently the class D enzyme was described; the OXA β-lactamases bear little resemblance to either class A or class C[57]. The classification schemes of β-lactamases and its correlation with moleculer structure are shown in table 2.2.

Table 1: Classification schemes for β-lactamases and itscorrelation with molecular structure54

Bush-Jacoby-Medeitors group	Bush group	Richmond-Skyes class	Mitsuhashi-Inouetype	Molecular class	Preferred substrate	Inhibited by: CA	EDT	Representative enzyme
1	1	Ia, Ib, Id	CSase	C	Ceph	-	-	Ampc of gram (-ve) bacteria; MIR-1
2a	2a	NI	Pcase V	A	Pen	+	-	Pcase from gram positive bacteria
2b	2b	III	Pcase I	A	Pen, ceph	+	-	TEM-1, TEM-2, SHV-1
2be	2b	NI except KI	CXase	A	Pen,narrow-spectrum and extended spectrum ceph.monobac	+	-	TEM-3toTEM-26,SHV-2 toSHV-6,*K.Oxytoca* KI
2br		NI	NI	A	Pen	+/-		TEM-30 toTEM-36, TRC-1
2c	2c	II, V	Pcase IV	A	Pen, carben	+	-	PSE-1, PSE-3, PSE-4
2d	2d	V	PcaseII, PCaseIII	D	Pen, cloxa	+/-		OXA-1 to OXA-11, PSE-2 (OXA-10)
2e	2e	1e	CXase	A	Ceph	+	-	Inducible Csase from *p.vulgaris*
2f		NI	NI	A	Pen, ceph, carbapenem	+	-	NMC-A from *Entrobactercloacae,* Sme-1 from *Serratia marcescens*
3	3	NI	NI	B	Most β-lactams, includingcarbap	-	+	LI from *Xanthomonas maltophilia,* CerA from *Bacteroids fragilis*
4	4	NI	NI	ND	Pen	-	?	Penicillinase from *P.cepacia*

Note: CA: clavulanic acid, Carbap: carbapenem, Carben: carbenicillin, Ceph: cephalosporin, CSase: cephalosporinase, CXase: cefuroxime-hydrolyzing ß-lactamase, Monobac: monobactam, ND: not determined, NI: not included, PCase: penicillinase, pen: penicillin.

The over-expression of intrinsic and/or the horizontal obtaining of acquired β-lactamase genes encoding enzymes from the four different moleculer classes A to D is the main mechanism of Acinetobacter resistance to β-lactams[58-59].

A-class: A wide range of class A β-lactamases including the narrow- spectrum (TEM-1, TEM-2, CARB-5 and SCO-1), extended-spectrum (TEM-92, TEM-16, SHV-2, SHV-5, SHV-12, CTX-M-2, CTX-M-3, CTX-M-43, PER-1, PER-2, PER-6, VEB-1, VEB-1a, VEB-3, GES-11 and GES-12) and carbapenem- hydrolyzing (GES-14, KPC-2, KPC-3, KPC-4 and KPC-10) variants have been identified mainly in *Acinetobacter baumannii* but also among *Acinetobacter* isolates from other species[60]. The molecular class A beta-lactamases of the KPC family are a group of potent carbapenemases identified initially in a *Klebsiella pneumoniae* isolate from the United States and later in other members of the *Enterobacteriaceae* family and in other geographical regions world wide[61]. *Pseudomonas aeruginosa* positive for the *bla*KPC gene has been recently identified in Colombia, Puerto Rico, and Trinidad and Tobago[62]. Up to date, eight different KPC variants (KPC-2 to -9) have being identified differing by 1 or 2 two amino acid substitutions. KPC-2 and -3 are the most common variants identified in *Enterobacteriaceae* and P. aeruginosa. KPC-6, -7, and -8 have been identified only in *Klebsiella pneumoniae*, while KPC-9 was detected in *Escherichia coli* and KPC-5 in P. aeruginosa. All the KPC variants except for KPC-7 and -9 have been detected in Puerto Rico[63]. The presence of the KPC gene in clinical isolates of *Acinetobacter* species in Puerto Rico is identified as a novel KPC variant, KPC-1064. The presence of this gene suggests the possibility of horizontal transmission, as

this carbapenemase has been associated with mobile genetic elements (transposons) which can be transferred from one bacterium to another[65]. Class A β-lactamase genes are generally considered to be less widespread among *Acinetobacter* than *Enterobacteriaceae* species[66]. However, assessment of the true prevalence of extended –spectrum class A β-lactamase in *Acinetobacter* might be underestimated since it has been hindered by difficulties with laboratory detection, especially in the presence of intrinsic AmpC enzyme[67].

B-class: Class B β-lactamases (metallo-β-lactamases, MBLs) confer high levels of carbapenem resistance as well as resistance to all other β-lactams except for aztreonam. MBLs are characterized from other classes of β-lactamases by being susceptible to EDTA inhibition due to the requirement of zinc ions (Zn+2) in the active site[68]. Several IMP (IMP-1, IMP-2, IMP-4, IMP-5, IMP-6, IMP-8, IMP-11) and VIM (VIM-1, VIM-2, VIM-4, VIM-11) variants have been detected among isolates from the *Acinetobacter baumannii-Acinetobacter calcoaceticus* complex[69]. SIM-1 was first described in *Acinetobacter baumannii* isolates from Korea[70]. The study reported a lower level of carbapenem resistance conferred by SIM-1 compared with that coferred by IMP and VIM variants[70]. NDM-1, the new Delhi metallo-β-lactamase, is a novel metallo-β-lactamse (MBL) conferring resistance to almost all β-lactam antibiotics, including carbapenems, recently identified in *Klebsiella pneumoniae* and *Escherichia coli* isolates from a Swedish patient who travelled to New Delhi, India[71]. Following the first description, sporadic cases of infected patients have been reported in India, the UK and the USA72-73. Kumarasamy et al[73] have recently reported the emergence and spread of 180 cases of patients infected with bacteria carrying the NDM-1 encoding gene from Pakistan, India and the UK. Interestingly, most patients from the UK had travelled to India or Pakistan within 1 year and had been hospitalized in these countries, suggesting that these organisms were acquired from a local source in Asia. Since August 2010, other cases have been reported worldwide, including in the USA, Canada, Europe, Japan, Africa, Oman and Australia[74].

The rapid spread and dissemination of these multidrug-resistant bacteria worldwide represents a major public health problem, thus the US Centers for Disease Control and Prevention (CDC) has recently planned to add NDM-1 producing MDR bacteria as agents of communicable diseases and hospitals must immediately report any suspect cases, particularly those for which the patient received medical treatment in India or Pakistan. The aim of this work was to develop a rapid real-time polymerase chain reaction (PCR) assay to detect the NDM-1 encoding gene in bacteria. Interestingly, resistance to carbapenems mediated by the coexistence of *bla*NDM-1, *bla*OXA-23 and *bla*IMP has been detected in pan-drug resistant *Acinetobacter baumannii* isolates from China. The *bla*NDM-1-positive strain was more resistant to antibiotics than the strains that were harbouring both OXA-23 and IMP. Fortunately, it was found that this *bla*NDM-1-positive *Acinetobacter baumannii* strain was susceptible to several fluoroquinolone antibiotics and to polymyxin *B75*.

NDM-2, a variant of NDM-1 with only one amino acid substitution, has recently been described in an *Acinetobacter baumannii* isolate recovered from a patient transferred to Germany from Egypt[76]. In *Acinetobacter baumannii*, six IMP variants belonging to three different phylogroups have been identified and reported namely IMP-1 in Italy, Japan and South Korea; IMP-2 in Italy and Japan; IMP-4 in Hongkong; IMP-5 in Portugal; IMP-6 in Brazil and IMP-11 in Japan[68]. In addition, IMP-4 has been identified in clinical isolates of *Acinetobacter* junii in Australia[77].

On the contrary, there are only few studies that have documented VIM type of MBL in *Acinetobacter*. Surprisingly, P. aeruginosa isolates collected from the same hospital showed the presence of VIM type of MBL and there was no cross transmission observed. VIM-2 producing *Acinetobacter* spp. have been isolated in the Far East and in Germany, while the VIM-1 determinant has been reported only in Greece[78-80]. VIM-4 is nothing but a point mutant of VIM-1 and that has been previously identified only in *Enterobacteriaceae* and *Pseudomonas* spp.[81].

C-class: Class C β-lactamases (AmpC cephalosporinases) are enzymes able, when over-expressed, to hydrolyze most penicillins, cephalothin, cefazolin, cefoxitin, ceftazidime and β- lactamase inhibitor/β-lactam combination, but generally not cefepime or carbapenem. So far, the chromosomal-encoded AmpC cephalosporinase genes have only been identified in a few *Acinetobacter* species (A.*baumannii*, A pittii and A. baylyi)[82].

Constitutive over-expression of AmpC β-lactamases in gram-negative organisms occurs either by deregulation of the AmpC chromosomal gene or by acquisition of a transferable AmpC gene on a plasmid or other transferable element. The transferable AmpC gene products are commonly called plasmid-mediated AmpC β-lactamases[83]. Several plasmid encoded AmpC beta-lactamases (MIR-1, CMY-1 to CMY-11. BIL-1, FOX-1 to FOX-5, LAT-1 to

LAT-4, ACT-1, MOX-1, MOX-2, ACC, DHA-1 and DHA-2) have been isolated from *Klebsiella pneumoniae*, K. oxytoca, Salmonella spp., Proteus mirabilis, *Escherichia coli*, Citrobacter freundi and Enterobacter aerogens. Plasmid mediated enzymes are grouped into six families based on similarities with enzymes of chromosomal origins[84]. Plasmid mediated genes that encode extended-spectrum β-lactamases (ESBLs) or AmpC enzyme, can spread to other organisms within hospital setting[85]. Multiple β-lactamases within one organism (e.g., multiple ESBLs or ESBL-AmpC combinations) can make phenotypic identification of the β-lactamases difficulty. Unfortunately, for these reasons, plasmid-mediated AmpC β-lactamase resistance goes undetected in most clinical laboratories[86].

D-class: The most common carbapenemases detected in *Acinetobacter* are CHDLs (carbapenem hydrolyzing oxacillinases) that are also referred as Classs -D oxacillinases. Among the nine clusters of carbapenem hydrolysing oxacillinases, four have been identified in *Acinetobacter baumannii*. These included members of OXA-23, -24, -51 and -58 families. In addition, recently a novel class D enzyme named OXA-143 has been reported from germany, OXA-58 oxacillinase was the first enzyme to be identified in an *Acinetobacter baumannii* isolate in France and subsequently this has been reported among *Acinetobacter baumannii* isolates in several countries[87].

Recently, a new OXA class D β-lactamase Oxa-97 has been reported in Tunisia which belongs to Oxa 58-like (subgroup) in Africa[88]. In one instance, a novel Oxa-143-CHDL in *Acinetobacter baumannii* which is not associated with insertion sequence (IS) elements[89]. Oxa-143 is a class D carbapenemase is similar to OXA-66/OXA-51-like enzyme that contributes to imipenem resistance, which was first reported from Taiwan. Off late, OXA-72 oxacillinase has been also reported in several carbapenem resistant *Acinetobacter baumannii* in Taiwan[90].

Resistance to colistin: The rapid development of carbapenem-resistant MDR *Acinetobacter baumannii* has led to the use of polymyxins (in particular polymyxin B and colistin or polymyxin E) as the drug of "last resort"[91]. Polymyxins are cyclic, positively charged peptide antibiotics capable of posing antimicrobial activities to a broad variety of gram-negative pathogens, including *Acinetobacter baumannii*, due to their interaction with the lipid A moiety of lipopolysaccharide (LPS). This leads to the disorganization and disruption of the outer membrane integrity, causing cytoplasmic leakage[92]. Unfortunately, the intensive use of the polymyxins in recent years has led to the emergence of polymyxin heteroresistant and resistant *Acinetobacter baumannii*, as high as 40.7% reported in Spain and 30.6% in Korea[93-94]. The basis of polymyxin resistance in *Acinetobacter baumannii* has only recently been investigated and several mechanisms have been proposed. Several genetic loci have been implicated in the resistance towards polymyxins in *Acinetobacter*, namely, the pmrCAB operon and the lpxA, lpxC, lpxD, and lpxB genes, that are involved in LPS biosynthesis[11,95-96]. Resistance can arise through mutations in the two component system PmrAB, in which the downstream target PmrC catalyzes the addition of phosphoethanolamine to the lipid A component of LPS[95]. This modification reduces the net negative charge of the outer membrane thus reducing the affinity of polymyxins for the target. Mutations or insertions in the genes encoding the lipid A biosynthesis machinery, namely, the lpxA, lpxC, or lpxD genes, also mediate polymyxin resistance by abolishing the production of LPS, thereby eliminating the target of polymyxins[11].

Resistance to Tigecycline: Tigecycline, a new class of glycylcyclines, is modified by addition of a 9-t-butyl-glycylamido side chain to minocycline[97]. The drug binds to bacterial ribosomes with high affinity and therefore evades the major resistance mechanisms of tetracycline, retaining activity against a broad range of both gram-positive and gram-negative bacteria, including multidrug-resistant *Acinetobacter baumannii*[98]. However, tigecycline resistance has emerged recently and been detected during treatment with this agent[99]. Tigecycline nonsusceptibility in *Acinetobacter baumannii* isolates has been associated with overexpression of a variety of efflux pumps. The major clinically relevant efflux pumps, such as AdeABC, AdeIJK, AdeFGH, AbeM, and AdeDE, have all been identified in *Acinetobacter baumannii*. These efflux pumps display broad substrate specificity, and tigecycline is one such substrate[100].

Clinical Symptoms

The most frequent clinical manifestations of Acinetobacter infection are ventilator-associated pneumonia and bloodstream infections[101]. Vascular catheters and the respiratory tract have been the most frequent sources of *Acinetobacter bacteremias* for which crude mortality rates parallel those attributed to other gram-negative bacilli (28 to 32%)102-103. *Acinetobacter pneumonia* occurs predominantly in ICU patients who require mechanical

ventilation and tends to be characterized by a late onset. Affected patients spend more days in the ICU and on a ventilator before having positive cultures than do patients with pneumonias caused by other gram-negative bacilli or uninfected patients[14]. The clinical effect of ventilator-associated pneumonias has been variable. A recent study showed higher mortality among patients with multidrug-resistant *Acinetobacter* infections than among patients infected with susceptible Acinetobacter strains or uninfected patients. The severity of illness is more in multidrug-resistant *Acinetobacter* infections in patients who had hospitalized in ICU[104]. In other studies, mortality among patients with pneumonia due to multidrug-resistant *Acinetobacter* was similar to that among patients with infection caused by other pathogens[105].

Prevention and Control

Antimicrobial resistant bacteria are the emerging current threats. The followings are some control and preventive measures should be taken to minimize their developments, spread and to promote development of new therapeutics. Most of the infections spread and occur from the contact of infected persons and lack of hygienic practices. Proper sanitation and hygiene maintenance in food and other things can reduce the spread of superbugs. Inappropriate use of antibiotics occurs due to unnecessary length of treatment, wrong prescription and its use without infections[106]. Both physicians and people education about it can check the development of resistant strains. Some policies and regulations should be practiced in both developing and developed countries to check the unnecessary drug promotions[107]. Antibiotics are used vividly in food animals like chicken, cattle, pigs, agricultural fields and fish farming methods. These uses establish a direct link for the appearance of resistance in humans[108]. Attempts should be taken to check the spread of antimicrobial resistances by restricting human to human transmission of resistant strains, decreasing the use of broad spectrum antimicrobial and developing new and novel antimicrobials[109]. Steps should be taken to prevent infections by inhibiting key gene products involved in the infection process[110].

Conclusion

Acinetobacter baumannii is one of the main pathogens of nosocomial infection and clinical opportunity infection, and it is also the most important strain causing outbreak of *Acinetobacter* in hospital environment. With the extensive application of broad-spectrum antibacterial drugs and the popularization of interventional procedures, *Acinetobacter baumannii* is resistant to a variety of antibiotics, and gradually develops into multi-drug resistance and even total drug resistance. Vivid research and application of Nanotechnology for identification of resistant bacteria and therapy for combating superbugs should be practiced.

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Authors must submit electronic version in MS Word and two hard copies of the manuscript with a 'Cover letter' with sequences and contributions as well as signatures of all authors (a sample is given below) to the Editor-in-Chief via e-mail (jmomc2015@gmail.com, jmomc@monnomch.edu.bd) or surface mail or by hand to the address on right:

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C.2.1. Stage 1: Editorial Scanning (usually completed in 1st month of submission)

a. Received papers are entered into receive register giving an ID and acknowledged;
b. Editorial scanning- checked for appropriateness, integrity and plagiarism;
c. Primary author response- sent to corresponding author for primary response.

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a. Processed for Peer reviews (select Peer(s), sent to reviewers with timeline);
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a. Information of 'Acceptance'/ 'Rejection' communicated with the corresponding author;
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