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Editorial

Bangladesh Achieves Remarkable Milestone in Public Health: Visceral Leishmaniasis (Kala-azar) Eliminated

Mohammad Mazharul Islam

"I salute the great progress made by Bangladesh, in line with WHO guidance, in eliminating visceral leishmaniasis as a public health threat". -Dr Tedros Adhanom Ghebreyesus, WHO Director-General.

In a groundbreaking achievement for public health, Bangladesh has successfully eliminated visceral leishmaniasis, a potentially fatal parasitic disease transmitted by infected sandflies.[1] This milestone reflects the nation's unwavering commitment to healthcare, the concerted efforts of healthcare professionals, and collaborative initiatives with international organizations. Visceral leishmaniasis, commonly known as kala-azar, poses a severe threat to public health due to its debilitating symptoms and potentially fatal consequences. Bangladesh's success in eliminating this disease underscores the effectiveness of targeted public health strategies and the dedication of the country's healthcare system.[2]

Visceral leishmaniasis is a neglected tropical disease caused by the Leishmania parasite, transmitted through the bite of infected sandflies, primarily belonging to the genus Phlebotomus.[3] The disease affects vital organs such as the spleen, liver, and bone marrow, manifesting symptoms such as prolonged fever, weight loss, enlarged spleen and liver, and anemia. If left untreated, visceral leishmaniasis can lead to severe complications and, in some cases, prove fatal. Bangladesh faced the challenge of controlling and eliminating visceral leishmaniasis, prompting the implementation of a comprehensive strategy in collaboration with international partners such as the World Health Organization (WHO) and non-governmental organizations. Bangladesh prioritized early detection through active surveillance programs, ensuring prompt treatment with antimonial drugs.[4] This strategy aimed to interrupt the transmission cycle and prevent further spread of the disease.

Controlling the sand fly population is crucial in preventing the transmission of the Leishmania parasite. Bangladesh employed various vector control measures, including the use of insecticide-treated bed nets, indoor residual spraying, and environmental management to reduce sand fly breeding sites.[5]

Community involvement and awareness were pivotal in disease control. Bangladesh initiated educational programs to raise awareness about the disease, its symptoms, and preventive measures. This approach empowered individuals to actively participate in protecting themselves and their communities.[6]

Collaborative efforts with international organizations, notably the WHO, provided Bangladesh with technical expertise, financial support, and access to global resources. This collaboration facilitated the development and implementation of evidence-based strategies for disease control and elimination.[7]

Bangladesh's success in eliminating visceral leishmaniasis carries far-reaching implications, extending beyond immediate health benefits.The elimination of visceral leishmaniasis significantly improves the overall quality of life for affected individuals and their communities. Families are spared the physical, emotional, and financial burdens associated with the disease.[8]

The successful elimination of visceral leishmaniasis reflects the resilience and effectiveness of Bangladesh's healthcare system. It demonstrates the nation's capacity to implement effective public health measures and respond to emerging health challenges.[9]

Bangladesh's achievement contributes to global efforts to control and eliminate neglected tropical diseases. It serves as a model for other countries facing similar challenges, emphasizing the importance of collaborative approaches and sustained investment in public health.[2]

While celebrating the elimination of visceral leishmaniasis, continued vigilance is essential to prevent the reemergence of the disease. Sustained surveillance, a robust healthcare infrastructure, and ongoing community engagement are crucial components of post-elimination strategies. Bangladesh can leverage the lessons learned from this success to address other health challenges and further strengthen its healthcare system.[10]

Bangladesh's successful elimination of visceral leishmaniasis stands as a testament to the transformative power of determined public health efforts and collaborative initia-

tives.[1] This milestone not only improves the health and well-being of the nation's citizens but also contributes significantly to the global fight against neglected tropical diseases. As Bangladesh continues its journey toward sustainable health outcomes, the elimination of visceral leishmaniasis serves as a shining example of what can be accomplished through dedication, innovation, and international cooperation in the field of public health.

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Original article

Cervical IL-6 Level: A Potential Diagnostic Indicator for Cervical Cancer

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Background: Chronic infection by high-risk human papillomavirus (HR-HPV) causes more than 95% of cervical cancer and its precursor lesions. Along with infection, some immunomodulatory components like cytokine are also responsible for the progression of the disease. Interleukin 6 (IL-6) regulates other cytokine production and secretion and induces the proliferation of normal and neoplastic cervical epithelial cells. The levels of IL-6 were estimated in both HPV-infected cancer and healthy cervical fluids for the establishment of this cytokine as a diagnostic indicator for the detection of disease severity. ***Methodology:*** A cross-sectional study was conducted based on histopathology and HPV DNA status from the Gynaecological Oncology department of BSMMU, 30 cervical cancer patients and 20 HPV DNA-negative healthy individuals women were enrolled for this study and the levels of IL-6 were estimated in cervical smear by Flowcytometery. ***Result:*** The mean level of IL-6 was found to be considerably elevated in individuals diagnosed with cancer compared to healthy women ($p<0.05$). ***Conclusion:*** Early cervical cancer diagnosis by measuring cervical cytokine levels might halt the disease progression in HPV-infected women and allow them to use anti-inflammatory medications to prevent the disease progression.

Keywords: HR-HPV-High-risk Human Papillomavirus; IL-Interleukin; TNF-Tumor Necrosis Factor

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Introduction:

Cervicalcancer (Ca cervix)is mainly caused by *Human papillomavirus* (HPV), an oncogenic non-enveloped DNA virus and member of the papilomaviridae family. In 2021, about 82.8% of invasive cervical cancer were due to HPVs 16 or 18.[1] It is the most common sexually transmitted infection (STI), affecting approximately 12% ofthe world's population.[2] Approximately 200 varieties of HPVs are divided into high-risk, intermediate-risk and low-risk categories, around 40 infect the genital tract[3] primarily the cutaneous and muco-cutaneous epithelia of vertebrates and the high-risk varieties cause cervical cancer mainly.[4] About 10% of individuals with HPV infection of the female genital tract failed viral clearance and remain infected thoughit is believed that the host's immunological response to HPVinfectionis a critical role in the elimination of the virus.[5] Numerous genetic variants in immune-associated genes, such as interleukins(ILs) are a class of cytokines that have a role in immunological and inflammatory responses

as well as tumor growth and progression.[6] After infection, the tumor microenvironment secretes different polarizing cytokines such as IL-2, IL-4, IL-6, IL-8, IL-21, TNF-α and IL-10 which also progress the disease condition.[7]
Interleukin-6(IL-6) is one such inflammatory cutokine, involved in the proliferation and differentiation of malignant cells and has been detected in high concentrations in the serum and tumor tissues majority of malignancies. Tumor cells, as well as tumor-associated macrophages (TAMs) and CD4+T cells are the principal producers of IL-6 in the tumor microenvironment. In the tumor microenvironment, IL-6 promotes carcinogenesis by modulating the intrinsic and extrinsic activities of cancer cells and influencing stromal cells that indirectly promote tumorigenesis.[8] IL-6 has a major role in promoting the proliferation of cervical tumor cells. E6 oncoprotein with cancer-associated fibroblasts (CAF) releases more IL-6 and causing the aging of other normal fibroblasts (NF) and promoting cervical carcinoma.[9] This pleiotropic multifunctional interleukin might be the most therapeutically useful and investigated cytokine, with applications in chronic inflammation and cancers, including cervical cancer. The relationship between the levels of cytokine in the cervical fluid in HPV-induced cervical precancer and cancer is an important area of research. Moreover, the mechanisms of HR-HPV-induced chronic infection and progression of cervical cancer were analyzed to provide an immunity theory for preventing HR-HPV infection and disease progression. Thus this study is designed to determine and compare the level of IL-6 in cervical cancer patients and healthy women.

Methodology

In this cross-sectional study 30 patients who were diagnosed as cervical cancer by histopathology with HR-HPV infection by Hybrid Capture II (HC II) in the department of Gynecological Oncology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, with no history of pregnancy or postpartum in last 6 months, additional sexually transmitted diseases infection (STIs), hysterectomy or invasive cervical treatment, other any cancer chemotherapy or malignancies, chronic infection, or vaginal medication in the last 2 weeks were enrolled by purposive sampling. About 20 normal women who came for regular cervical screening also enrolled in this study with negative Hybrid Capture II reports. All patients and healthy participants were informed and gave their written consent and data on epidemiology were collected by survey. Cervical smear samples were collected by cytobrush of cervical sampler (Digene® HC-2 DNA Collection Device) and transferred into microcentrifuge tube and centrifuged (14,0000 RPM X 10 min) to separate the supernatant and cellular components. The level of IL-6 was measured by using cervical swab samples were estimated by cytometric bead array (CBA) method using the kit BD cytometric bead array (CBA) human soluble protein master buffer kit (Cat no: 558264) through BD Accuri™ C6 plus flowcytometer by IL-6 reagent (Cat no: 558276). The concentration was determined in pg/ml. Statistical analysis was done using SPSS/PC 25.0 software and Microsoft Excel. P-value of <0.05 was considered significant.

Result

Cytokine IL-6 levels were measured in samples collected from 30 cervical cancer women and 20 apparently healthy women. The mean age of the cervical cancer patients was 49.4±9.9 years (Mean ± SD), while the mean age of the HPV-DNA negative healthy participants was 37.5±7.2 years (Mean ± SD). The difference in mean age between the two groups was found to be statistically significant. In this study, there were about 40% healthy women in the age range of 31 to 40 years and 33.3% cancer women in the age group of 41 to 50 years (Figure 1). The study aimed to measure the levels of IL-6 in the collected samples. The results revealed that the mean level of IL-6 was significantly higher in cervical cancer patients compared to healthy individuals. In this study, IL 6 levels were significantly different among the age 31 to 60 years of cervical cancer and healthy women (Figure 2).

Table: Levels of IL-6 in pg/ml among cervical cancer and healthy women

Cytokine (Mean in pg/ml)	Cervical cancer (n=30)	Healthy participants (n=20)	p-value*
IL-6	2747.7	152.8	<0.05

*p-value was determined by independent T test.p< 0.05 considered as significant

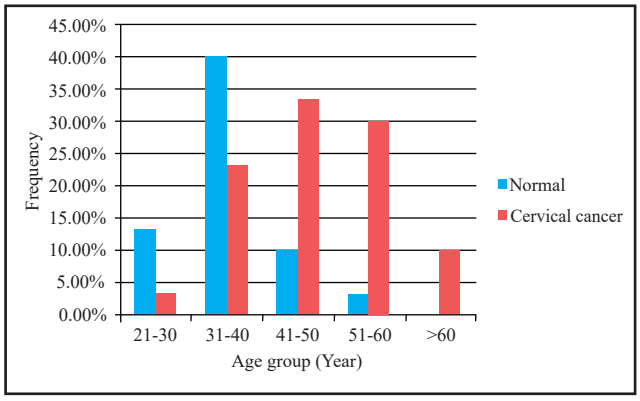
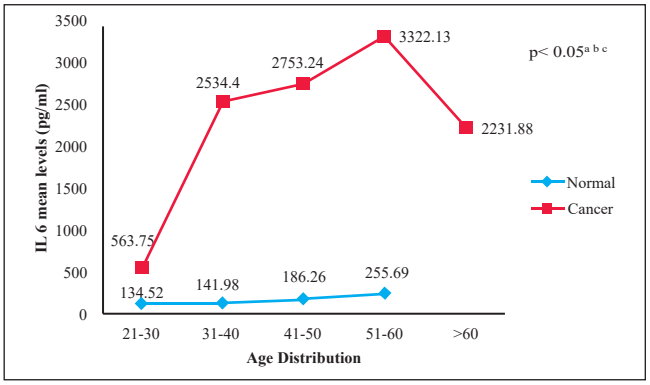


Figure 1: Cervical cancer and healthy women among different age groups



*p-value was determined by independent T test.p< 0.05 considered as significant
a= p value for 31-40 years age group
b= p value for 41-50 years age group
c= p value for 51-60 years age group

Figure 2: Line chart of the mean IL-6 levels in different age groups

Discussion

HPV causes cervical cancer which is the second most common cancer in Bangladeshi 15–44 years women.[10] Globally, there were an estimated 6, 04,000 new cases and 3, 42,000 deaths from cervical cancer in 2020. More than 95% of cervical cancer is due to persistent high-risk HPV infection.[11] During HPV infection, the local immune response plays a significant role and releases different cytokines. Cytokines were considered to be immune system messengers that guided leucocytes to the areas of inflammation.[12] However, it has been established that dysregulation of cytokine is associated with most neoplastic tissues and may play a role in malignant transformation, proliferation, survival, angiogenesis, invasion and metastasis.[13] The pro-inflammatory cytokine IL-6 was tested for its ability to regulate epithelial cervical cell cytokine production and secretion and to induce the proliferation of normal and neoplastic epithelial cervical cells.[14] The incidence of cervical cancer steadily increased with age, about 33.3% in 41 to 50 years (Figure 1). Nessa et al.[15] demonstrated that the conversion of high-grade lesions to cancer occurred usually in the elderly population. There is an inverse relationship between immune function and the development of several types of cancer; as immune function declines with age, the incidence of cancer rises.[16] The result of this study showed that the level of the pro-inflammatory cytokine, IL-6 in cervical cancer patients increased significantly compared to the HPV DNA-negative healthy participants ($p < 0.05$). Some authors also found the levels of IL-6 were significantly raised in HPV DNA-positive patients as well as cervical cancer and precancer patients.[5,17] During the development and progression of HPV infection, IL-6 levels rise dramatically without any auto-inhibition. Therefore, infected cells continue to produce huge amounts of IL-6 and prevent tumor cell death as well as cause uncontrolled atypical cell proliferation.[18] However, according to Mhatre et al.[19] the levels of IL-6 were not significantly elevated in HR-HPV-induced different stages of cervical neoplasia. A persistent inflammatory condition, precancerous lesions and cervical cancer stimulate the immune system and release several cytokines. As such the current study observed that the mean level of IL-6 was increased significantly in cervical cancer patients than in healthy individuals with increasing age (Figure 2).

Limitation

Despite the significance of our findings regarding the elevated IL-6 levels in cervical secretions of cervical cancer patients, it is essential to acknowledge certain limitations inherent in our study. A more comprehensive understanding could have been achieved if all patients were of the same age or if we could observe the same cytokine levels over a specified period. Unfortunately, due to constraints in time and resources, we were unable to design the study in such a controlled manner. Prospective studies using a more uniform sample and a longitudinal methodology might provide more understanding the correlation between IL-6 levels and the progression of cervical cancer.

Conclusion

The mean level of cervical IL-6 is more in cervical cancer patients than in healthy individuals. Detection of IL-6 early in cervical cancer by measuring the levels of cervical cytokine may pave the way for the use of anti-inflammatory medications such as steroids or monoclonal antibodies against the raised cytokine as a treatment protocol and halt the disease progression.

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Conflict of interest

The authors thereby declare no conflict of interest exists.

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Original article

Exploring the link between Sexual Behavior and Depression among Male Methamphetamine Users Engaging in Injection of Other Drugs in a Chosen District of Bangladesh

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Background: Drug abuse is a growing challenge in Bangladesh, with most of the drug abusers are young and middle age, between 8 and 50 years. Methamphetamine (Yaba) abusers are vulnerable for their risky sexual behavior and unstable mental health. There are lack of information about sexual behavior and mental health of Yaba abusers. We need research to assess current situation among young people of Bangladesh. To fill up the gap of knowledge a study was conducted with the objective of assess the current status of sexual behavior and depression among Yaba abuser in selected drop in center (DIC) of Save the Children in Cumilla district. ***Methodology:*** The study was cross-sectional and done from January to December 2018. Adult male aged between 18-69 years who were enrolled in 'Harm Reduction Services' and were diagnosed as methamphetamine drug user by verbal consent. The modified semi structured CDC questionnaire data was collected from 263 young study respondents in two DICs by face-to-face interview. Before collection both written and verbal consents was obtained from respondents. 'Depression' was accessed by Montgomery-Asberg depression rating scale and graded score range from 0 to 60. Data was analyzed by SPSS software version 2.1 where we observed descriptive and bivariate analysis with frequency, percentage and cross tabulation. ***Results:*** The result found that more than half 151(57.4%) of respondents were 26-35 years of age, 253(96.2%) as Muslim. 252(95.8%) was married, 183(69.6%) had business as occupation, 240(91.3%) had with family, 201(76.4%) have 5 or more siblings. Half of the respondents' Yaba consumption was >24 months, 140(53.2%) consumed Yaba with 1-4 friends. The prevalence of any kind of sexual contact except wife (Extramarital affair) was 47(17.9%) and prevalence of any kind of sexual contact without condom in last 12 months except wife was 32(12.2%). Majority respondents reported they always use condom when have sexual contact with sex workers. More than one third 101(38.4%) respondents bought condoms from pharmacy and 105(39.9%) population bought condom from HIV program. Majority 258(98.1%) respondent said they experienced decrease satisfaction on condom use. Around 53(20.2%) population had discontinued condom use. Majority respondents 252(95.8%) perception was Yaba affects discontinuation of condom use and 37(14.2%) respondents said they were affected by STDs. The prevalence of depression among Yaba abuser was 107(41.0%) and among them 62(58.2%) asymptomatic, 34(31.9%) had mild depression, 7(6.8%) had moderate depression and 3(3.1%) had severe depression. Conclusion: Methamphetamine abuse is a rising problem for young people. They have risky sexual and mental health problem.

Keywords: Sexual Behavior and depression, male Methamphetamine users

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Introduction

Drug addiction is a substantial concern for Bangladesh and the global community. Over the past two decades, Bangladesh has witnessed a prolonged history of drug usage, primarily involving substances such as opium and cannabis. The issue of drug use first manifested in Bangladesh during the 1980s. Notable categories of available drugs in Bangladesh include: A. Opium (including Heroin, Pethedine, and Cocaine) B. Cannabis (comprising Ganja, Chorosh, Bhang, and Hashish) C. Stimulants (such as Methamphetamine, Ecstasy, and Viagra) D. Sleeping pills (including Tranquilizers and Diazepam) E. Cough Syrup (marketed under the trade name Phensidyl, containing codeine, pseudoephedrine, and chlorpheniramine).Moreover, there exists a significant association between the use of methamphetamine and risky sexual behavior, as indicated by numerous research articles spanning various countries. This correlation underscores the need for comprehensive interventions and preventive measures to address the multifaceted challenges posed by drug addiction in Bangladesh and beyond.[1]

In recent years, numerous studies have indicated a noteworthy escalation in the prevalence of methamphetamine usage. Amphetamines, ranking as the second most widely utilized class of substances globally, follow closely behind cannabis. Over the past two decades, a considerable surge in the accessibility of amphetamine-type stimulants (ATS) has been observed on a global scale. In the United States, adolescents constitute 20% of all admissions, and this percentage continues to exhibit an upward trajectory with each passing day.[2] The geographical areas exhibiting the highest prevalence of amphetamine utilization encompass North America, Europe, Southeast Asia, and Australia. Within the global population of 200 million individuals who engaged in drug consumption during the years 2009 and 2010, approximately 35 million individuals were identified as users of amphetamine-like stimulants.[3] Owing to its accessibility in transportation and substantial demand among student populations, it occupies a prominent position within the drug trafficking industry. The global prevalence of risky sexual behaviors among individuals grappling with substance addiction has been substantiated by numerous studies. The term "risky sex" encapsulates a spectrum of behaviors that place an individual at heightened vulnerability for unintended pregnancy, sexually transmitted infections (STIs), sexual violence, or other adverse outcomes.[4] Methamphetamine exhibits greater potency than amphetamine due to its inherent lipophilic properties, facilitating enhanced penetration of the central nervous system.[5] Upon the prompt ingestion of Methamphetamine, individuals undergo a series of effects characterized by notably pleasurable sensations. These sensations encompass euphoria, instigated by elevated dopamine levels. Additionally, Methamphetamine usage is linked to heightened productivity, increased attentiveness and curiosity, hyper sexuality, diminished anxiety, and augmented energy levels.[6] Hence, the prevalence of precarious sexual conduct may be elevated as a consequence of the impact of methamphetamine on hyper sexuality. The administration of an excessive dose of methamphetamine has been associated with severe health complications, including cerebrovascular hemorrhage, acute cardiac failure, and hyperthermia.[7] No formal studies have been conducted concerning the correlation between risky sexual behavior and the utilization of methamphetamine in Bangladesh. It is noteworthy that methamphetamine has been observed to augment sexual desire and arousal in both men and women. However, protracted usage has been linked to sexual dysfunction, with certain reports indicating instances of erectile and orgasmic dysfunction.[8] One international study finding which include intravenous drug use and risky sexual behavior stated that, Twenty one percent of the total participants reported condom use during the last sexual intercourse. Another study stated that, Condom use during the last sexual intercourse was highest in Nepal (37%) and lowest in Bangladesh (10%) among south East Asian countries.[9]

In an additional investigation, a noteworthy univariate association was identified between individuals engaging in unprotected anal intercourse and the abuse of amphetamines within the gay population. Furthermore, a study conducted in Colombia revealed that adolescents who disclosed elevated levels of drug consumption concurrently exhibited increased numbers of sexual partners and higher frequencies of engaging in unprotected sexual activities. It is pertinent to note that substantial data lacunae persist in

certain countries, notably India and China. Additionally, in some countries within the region, reported data on recreational drug use relies on expert estimates rather than formally collected data.[10] Now a day's depression also a great issue which also endanger public health. Drug addiction due to depression is a well-known trend. Depression is highly co morbid with substance abuse. There is little knowledge about clinical course and outcome of methamphetamine.[11] Depression often occurs among person who uses methamphetamine.[12] In Thailand, association between methamphetamine and high depression level is shown in a study.[13] In that study they find approximately 12% of individual who reported ever using methamphetamine had high levels of depressive symptoms. One limitation of that study is that the study was based on adolescent, parents did not give consent and many difficultly arise regarding giving consent of adolescent due to Thai stigmatization. Another limitation of that study was, no study was done in rural region of Thailand where there was epicenter of an existing methamphetamine epidemic in Thai rural areas. My study population was adult (18-69), so no parental consent needed. I shall go to Cumilla to collected sample which represent rural area of Bangladesh to encounter previous study limitations.[14] In country like Bangladesh depressive patient is increasing day by day. A study in Bangladesh showed that 2.88% of outdoor patient were suffering from substance use disorder. From another study from National Institute of Mental Health 7.66% of their outdoor patient was suffering from substance related disorder. Another study conducted in a private psychiatric clinic in Dhaka showed 29.6% of admitted patient were also suffering from substance related disorder.[15] In relation to individuals experiencing homelessness, it has been observed that 69% of males engaged in the utilization of various forms of substances.[16] However, there exists a notable dearth of comprehensive investigations aimed at elucidating the simultaneous occurrence of depression and methamphetamine use.[17] Despite the recognized association between methamphetamine and depression, the precise prevalence of depressive conditions among methamphetamine users remains indeterminate. It is imperative to undertake additional research endeavors to ascertain the contemporary prevalence of coexisting depression and methamphetamine use within the Bangladeshi population.[18]

Materials and methods:

The Study was cross-sectional study. Study site was Harm reduction program was started from 2008 under the supervision of Save the Children. There are 21 DIC (drop in center) in 7 districts under Save the Children supervision. My study areas were 2 DIC (drop in center) of save the children in Cumilla, Bangladesh. Total People who inject drugs (PWID) is about 815 which is enlisted in Save the Children DIC (drop in center), Cumilla. 2 DIC (drop in center) is located in Chalkbazar and Race course in which 410 methamphetamine addict enlisted out of 520 in race course and 292 methamphetamine addict enlisted out of 295 in Chalkbazar.

Study populationwasadult population, male aged between 18-69 years who were enrolled in "Harm reduction services" conducted by Save the Children in Cumilla. Sampling technique was purposive sampling. By using sample size for cross sectional study, $N=Z^2 *P(1-P)/d2$ our sample size for this study is 263

A pretested semi-structured modified questionnaire derived from CDC Atlanta and from other literature review for sexual behavior status and Depression is rated by Montgomery-Asberg Depression Rating scale (MADRS). Regarding modified CDC questionnaire and other from other literature review, we did not find any languages barrier which is not understandable to Bangladeshi people. After doing pretesting from 10 participants, I took expert opinion from my supervisor if any change is needed. Section A was containing different socio demographic information such as age, marital status, educational status etc. Section B was included question about sexual behavior like number of multiple sexual partners, sexual contact without condom except wife etc. Section C was included according to Montgomery – Asberg Depression rating Scale which already have validate questionnaire including Bangali Language 20scoring range from 0 to 60 where 0 to 6 is asymptomatic, 7 to 19 is mild depression, 20 to 34 moderate depression and more than 34 represent severe depression.

Face to face interviews- pretesting were conducted in another DIC center in Dhaka, where 10 methamphetamine drug users were included. The questionnaire had containing sections which include -The socio-demographic characteristics, sexual behavior and depression status among methamphetamine drug user who also inject other drugs.

Data management procedure was after completion of data collection all questionnaires was checked and edited by researcher. A data base in SPSS (v-21) was developed according to questionnaire. Data regarding all questions were entered in the developed data base. Outliers and missing values were checked and corrected. Data was cleaned, entered and analyzed by Statistical Package for the Social Sciences (SPSS) software version [21]. All the question data was entry in the developed data based by using questionnaire. Outliers and missing values were checked and corrected. Data exploration was done to visualize and general feature of data. After exploration percentage was used to assess to level of socio demographic status. Data was presented by using tables and pie chart only. Depres-

sion was accessed by Montgomery-Asberg depression rating scale and graded as an overall score (range 0 to 60). Cut-off points include: 0 to 6 – symptoms absent, 7 to 19 – mild depressions, 20 to 34 – moderate, more than 34 – severe depressions. It was presented by various graphs and table.[22]

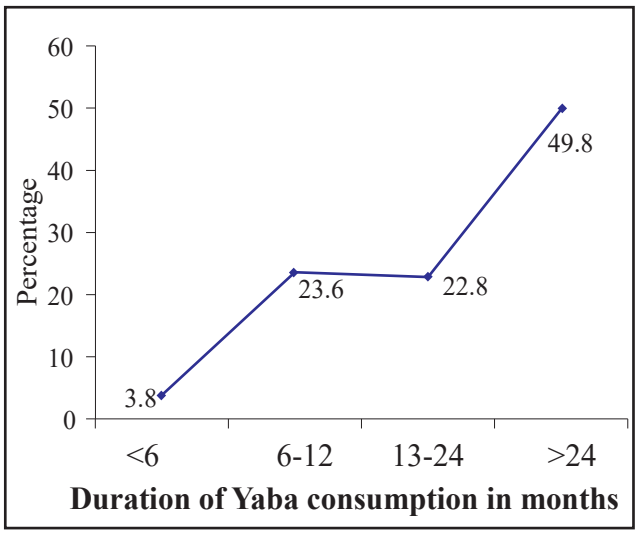
Result:

Table 1: Distribution of the study populations by socio demographic characteristics (N=263)

Socio demographic variable	Number (n)	Percentage (%)	Mean±SD
Age (in years)			33.21±7.65
18 - 25	33	12.5	
26 - 35	151	57.4	
36 - 45	56	21.3	
46 - 55	21	8.0	
>55	2	0.8	
Education			
No institutional education	55	20.9	
Do not complete primary education	79	30.0	
Complete primary education	104	39.5	
Complete secondary education	17	6.5	
Complete higher secondary education	5	1.9	
Complete graduation	3	1.1	
Religion			
Islam	253	96.2	
Hindu	10	3.8	
Marital status			
Unmarried	14	5.3	
Married	252	95.8	
Occupation in last 12 months			
Business small	237	90.1	
Day labourer	26	9.9	
Present occupation			
Business	183	69.6	
Service	54	20.5	
Unemployeed	26	9.9	
Stay with family			
Yes	240	91.3	
No	23	8.7	
If no where you stay			
House	254	96.6	
Home and road	9	3.4	
Siblings			
≤ 5	201	76.4	4.54±2.14
>5	62	23.6	

Table 1 shows the distribution of the study populations by socio demographic characteristics. It was observed that more than half 57.4% population belonged to age 26-35 years. The mean age was found 33.21±7.65 years with ranged from 18 to 57 years. More than one third 39.5% population were completed primary education than 30.0% in did not complete primary education, 20.9% had no institutional education, 6.5% had complete secondary education, 1.9% had complete higher secondary education and 1.1% had complete graduation. Majority 96.2% population was Muslim and 3.8% Hindu. Majority 95.8% population was married and 5.3% unmarried. Majority 90.1% were business small and 9.9% in day laborer. More than two third 69.9% were business than 20.5% in serves and 9.9% in unemployed. Majority 91.3% population had stay with family. Three fourth 76.4% population belonged to 5 siblings. The mean number of sibling was 4.54±2.14 with ranged from 0 to 14.

Figure 1: Distribution of the study population by duration of Yaba consumption in months (N=263)



Line chart shows the distribution of the study population by duration of Yaba consumption in months. It was observed that almost half 49.8% study respondents belonged to duration of Yaba consumption >24 month. The mean duration of Yaba consumption was 42.61±31.84 months with ranged from 1 to 120 months.

Table 2: Distribution of the study respondent by Yaba consumption and condom use (N=263)

Yaba consumption with friends	**Number (n)**	**Percentage (%)**
0	110	41.8
1-4	140	53.2
>4	13	4.9
Always use condom when have intercourse with female sex worker		
Yes	245	93.2
No	18	6.8
Most condom bought in last 1 month		
Pharmacy	101	38.4
DICCenter	91	34.6
Did not brought condoms in last 1 month	71	27.0
Price of condom when use last time		
0	105	39.9
1-15	97	36.9
16-25	48	18.3
>25	13	4.9

Table 2 shows the distribution of the study population by Yaba consumption with friends. It was observed that more than half 53.2% study respondents belonged to 1-4 Yaba consumption with friends, 41.8% in 0 and 4.9% in >4 Yaba consumption with friends. The distribution of the study population by always use condom when have intercourse with female sex worker. It was observed that majority 93.2% study respondents had always use condom when have intercourse with female sex worker. Source of condom buying in last 1 month of the study population. It was observed that more than one third 38.4% study respondents brought condoms from pharmacy, 34.6% from DIC center and 27.0% did not brought condoms in last 1 month than. The distribution of the study population price of condom when use last time. It was observed that 39.9% population had 0 price of condom when use last time than 36.9% in 1-15 price of condom when use last time, 18.3% in 16-25 price of condom when use last time and 4.9% in >25 price of condom when use last time.

Table 3: Distribution of the study respondent by HIV history (N=263)

Brought condom ever from HIV program	**Number (n)**	**Percentage (%)**
Yes	105	39.9
No	158	60.1
Ever test HIV		
Yes	224	85.2
No	39	14.8

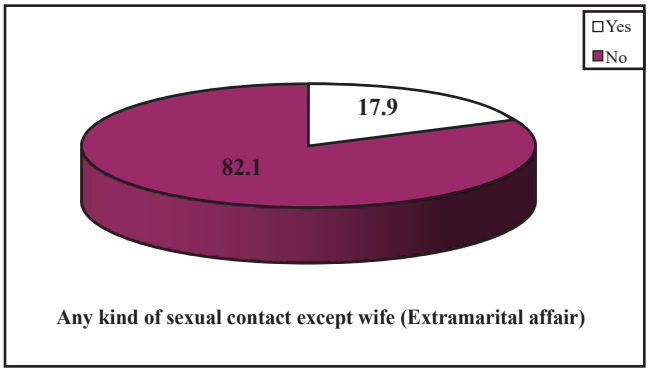
Table 3 shows the distribution of the study population brought condom ever from HIV program. It was observed that more than one third 39.9% population had brought condom ever from HIV program. The distribution of the study population ever tests HIV. It was observed that majority 85.0% population had ever test HIV. The distribution of the study population knows HIV test result. It was observed that all 100.0% population had known HIV test result.

Table 4 shows the decrease Satisfaction on condom use according to Yaba abuser. It was observed that majority 98.1% population had decrease satisfaction on condom use according to Yaba abuser. It was observed that 20.2% population had discontinued condom use during sexual intercourse. Majority respondent (95.8%) perception was that, Yaba had affect on discontinuation of condom use. It was observed that 14.2% population had ever effected by sexual transmitted diseases.

Table 4: Percentage distribution of Yaba affect on condom user (N=263)

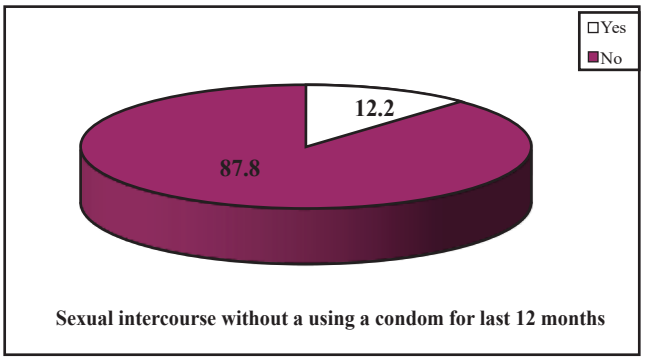
Dissatisfaction on condom use	**Number (n)**	**Percentage (%)**
Yes	258	98.1
No	5	1.9
Discontinue condom use during sexual intercourse		
Yes	53	20.2
No	210	79.8
Perception on discontinuing using condom due to Yaba		
Yes	252	95.8
No	11	4.2
Ever effected by sexual transmitted diseases		
Yes	37	14.2
No	226	85.8

Figure 2: Distribution of the study population by sexual contact except wife (N=263)



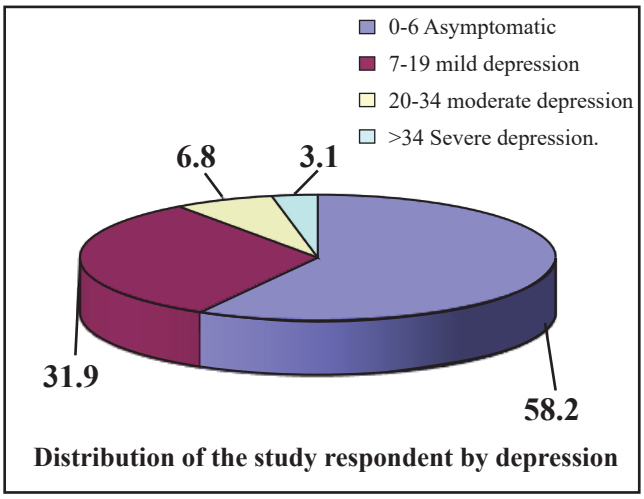
Pie chat shows the distribution of the study population by percentage of sexual contact except wife. It was observed that the prevalence of any kind of sexual intercourse except wife (Extramarital affair) was 17.9%.

Figure 3: Distribution of the study population by sexual intercourse without using a condom for last 12 months (N=263) except wife



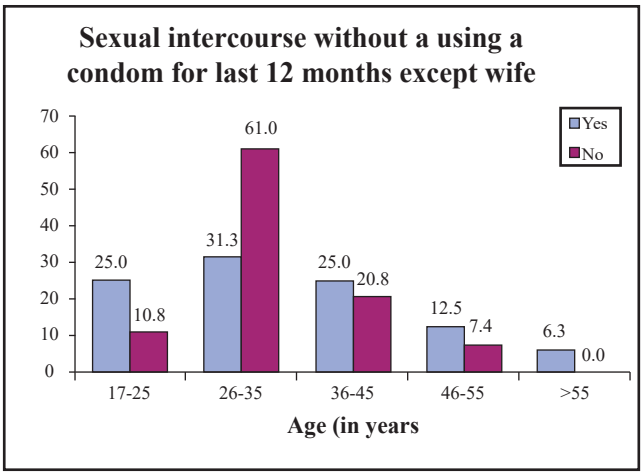
Pie chart shows the distribution of the study population by percentage of sexual contact except wife. It was observed that the prevalence of any kind of sexual intercourse without using a condom for last 12 months except wife was 12.2%.

Figure 4: Distribution of the study population by depression (N=263)



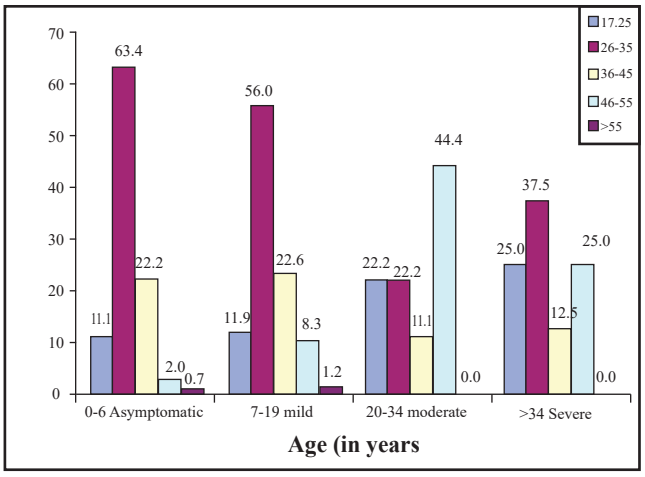
Pie chart shows depressionof the study population. It was observed that more than half 58.2% study respondents had 0-6 Asymptomatic, 31.9% 7-19 mild depression, 6.8% 20-34 moderate depression and 3.1% >34 Severe depression.The prevalence of depression among methamphetamine user was 41%

Figure 5: Comparison between age with sexual intercourse without a using a condom for last 12 months (N=263) except wife



Bar diagram shows comparison between age with sexual intercourse without a using a condom for last 12 months. It was observed that among 32 study respondents almost one third 31.3% belonged to age 26-35 years

Figure 6: Comparison between age with depression (n=263)



Bar diagram shows comparison between age with depression. It was observed that Asymptomatic and mild depression was higher in 26-35 years of age (Asymptomatic 63.4% and mild 56.0%) where total asymptomatic population were 153 and total mildly depressed population were 84. Whereas moderate and severe depression was higher in 46-55 years of age (moderate 44.0% and severe 25.0%) where total moderately depressed population were 18 and severely depressed population were 8.

Discussion:

In the present study, an examination of the educational background of the population revealed that 39.5% had completed primary education, while 30.0% did not complete primary education, and 20.9% had no institutional education. Moreover, 6.5% completed secondary education, 1.9% completed higher secondary education, and 1.1% had completed graduation. These findings are consistent with prior research by Islam and Hossain (2017),[13] who reported that 46.0% of drug users were undergraduates, 30.0% were graduates, and 24.0% were master's or postgraduate degree holders. Comparable observations regarding education status were also noted in studies conducted by Zhang et al. (2015), DiMiceli et al. (2016),[7] and Borders et al. (2013).[1] However, Maruf et al. (2016)[23] reported differing results, with 38.1% having graduated, 28.6% holding higher secondary qualifications, 17.1% having secondary education, 9.5% being postgraduates, and only 6.7% having completed primary education.

In terms of religious affiliation, the current study observed that 96.2% of the population identified as Muslim, while 3.8% identified as Hindu. This aligns with findings from Maruf et al. (2016),[23] where 98.1% identified as Muslim, 1.0% as Hindu, and 1.0% as Christian. Additionally, DiMiceli et al. (2016)[7] reported 100% Thai ethnicity and 99% Buddhist affiliation in their study.

Marital status in the present study indicated that 95.8% of the population was married, while 5.3% were unmarried. This contrasts with Maruf et al. (2016),[23] where 57.1% were single, 34.3% were married, 1.0% were widowed, 1.0% were separated, and 6.7% were divorced. Similarly, DiMiceli et al. (2016)[7] found that 79.0% were single, separated, widowed, or divorced.

Occupationally, the majority of participants in the current study (90.1%) were engaged in small businesses, with 9.9% working as day laborers. In contrast, Maruf et al. (2016)[23] reported unemployment at 31.4%, business involvement at 31.4%, students at 20.0%, service-holders at 7.6%, housewives at 3.8%, and retirees. Another study by Glasner-Edwards et al. (2009)[21] found 60.0% of their subjects were employed.

Family composition in the present study revealed that 91.3% of the population resided with their families, consistent with findings by Islam and Hossain (2017),[13] where 90.0% of recent drug users lived in nuclear families. Housing arrangements indicated that 96.6% lived in houses, with 76.4% belonging to families with five or fewer siblings.

Regarding Yaba consumption duration, almost half (49.8%) of the respondents in the current study had been consuming Yaba for over 24 months. This contrasts with findings by DiMiceli et al. (2016),[7] where 19.0% reported ever consuming methamphetamine, and 31.0% of lifetime users reported recent methamphetamine use within the past 3 months. The current study also highlighted that 53.2% of respondents engaged in Yaba consumption with friends, possibly for financial benefits and sexual pleasure.[7]

Sexual behavior outcomes in the current study indicated that 17.9% engaged in extramarital affairs, though potential bias due to respondents concealing their sexual history was acknowledged. Borders et al. (2013)[1] reported similar findings in rural stimulant users, emphasizing a significant reduction in the odds of multiple sexual partners over time. Additionally, the study noted that crack cocaine, non-prescribed pharmaceutical opioids, and alcohol to intoxication were associated with greater odds of multiple sexual partners.

In terms of condom use, 12.2% reported engaging in sexual intercourse without using a condom for the last 12 months, and 20.2% discontinued condom use during sexual intercourse. Borders et al. (2013)[1] found inconsistent condom use among rural stimulant users and noted associations with substance use, emphasizing the need for expanded sexual risk prevention programs.

Concerning HIV testing, the present study indicated that 85.0% of the population had undergone HIV testing, possibly influenced by community outreach efforts. In contrast, Kumar et al. (2008)[24] reported that 25.0% of participants in five countries had not heard of HIV/AIDS, with awareness varying across regions.

Depression prevalence in the current study showed that 58.2% had asymptomatic to mild depression, 6.8% had moderate depression, and 3.1% had severe depression. These findings align with prior research linking methamphetamine use to depression. Additionally, the study highlighted potential contributing factors such as social and political unrest, job dissatisfaction, low job security, financial issues, familial problems, and future uncertainty.[10]

In summary, this discussion has provided a detailed overview of various demographic and behavioral aspects observed in the current study, comparing and contrasting findings with relevant literature. The results underscore the multifaceted nature of substance use, emphasizing the importance of tailored interventions and comprehensive understanding in addressing associated issues.

Conclusion:

Drug abuse is a multidimensional problem, and it should be viewed from multidimensional perspective and be addressed accordingly. This study was undertaken to explore the sexual behavior and depression status among male inhaled methamphetamine user who also inject other drugs in selected district of Bangladesh. Most of the population age belonged to age 26-35 years, poor education level, came from Muslim family, married, involve with business, Stay with family and sibling belonged to <5. Most of the study respondents belonged to duration of Yaba consump-

tion >12 months, consumption with friends. The prevalence of any kind of sexual intercourse except wife (Extramarital affair) was 17.9% and that the prevalence of any kind of sexual intercourse without using a condom for last 12 months except wife was 12.2%. Majority of study respondents always used condom when have intercourse with female sex worker. More than one third population had brought condom from HIV program and most population had decrease satisfaction according to Yaba abuser. Nearly one fourth population had stop using condom during sexual intercourse, most of the respondent perception had Yaba effect on condom nonuse according to Yaba user. Ever effected by sexual transmitted diseases found 14.2% of the population. Majority (85.0%) population had ever test HIV and all had know HIV test result. More than half of the study respondents had 0-6 Asymptomatic. Almost one third belonged to age 26-35 years had sexual intercourse without a using a condom for last 12 months except wife. Most of the mild depression observed in age belonged to 26- 35 years. These findings highlight the need for management and prevention strategies for substance use in Bangladesh.

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Original article

Management of open fracture shaft of tibia with Ilizarov external fixator

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Abstract

Background: *The aim of this study was to evaluate the outcome of application of Ilizarov external fixator (IRF) in the management of open fracture shaft of tibia.* ***Methodology:****It was a prospective multicenter observational study which was carried out between June 2018 and December 2022. 36 patients with open fracture shaft of tibia with and without infections reported in outpatient department were included in the study. Both radiological and clinical evaluation was done. Skeletal, and functional results were obtained by using Association for the Study and Application of Methods of Ilizarov (ASAMI) scoring system.* ***Results:****Thirty-six patients with open fracture shaft of tibia were managed by IRF. Out of them, 21(58.3%) were male and 15(41.6%) were female.26(72.2%) patients had age below 40 years, 8(22.2%) were between 40 to 60 years and 2(5.5%) were above 60 years of age. 4(11.1%) of them had Gustilo II, 19(52.7%) had Gustilo IIIA, 11(30.5%) had Gustilo IIIB and 2(5.5%) had Gustilo IIIC fractures, respectively. 17(47.2%) patients needed soft tissue coverage, among them split thickness skin grafting was done in 5(13.8%) cases and flap coverage was needed for 12(33.3%)patients. Fracture was successfully united in every patient. The average time for successful union was 6.3 months (4-9 months). The average duration for frame removal was at 6 months (5-10 months). Results were made by the ASAMI scoring system and in our study bone results were excellent in 29, good in 5, fair in 2 and no poor. Functional results were excellent in 30, good in 5, fair in 1 and no poor. Most frequently occurring complication was pin track infection. Other complications were loosening of the frame, breakage of Ilizarov wires, poor quality of bone regenerate. In our study, no major complication occurred.* ***Conclusion:****Ilizarov ring fixator is an excellent choice of treatment for open fracture of tibia regarding the management of bone loss, eradication of infection, limb-length equalization and limb function.*

Keywords: Ilizarov;Open fracture;Tibia;ASAMI, IRF

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Introduction:

Tibial fractures are the most common long bone fractures, with around 25% being open fractures. The majority of open comminuted diaphysealtibial fractures result from high velocity trauma such as road traffic accidents and falls from height. Management of open diaphysealtibial shaft fractures can be comminuted due to lack of soft tissue coverage and blood supply of the tibialshaft. Prognosis depends on displacement, comminution, and soft tissue injury. Advanced bone reconstruction and soft tissue coverage is required to poses bone and soft tissue healing. Fracture shaft of tibia occurs very frequently and open fracture occurs most commonly in tibia. For fracture of tibia high energy trauma is required like road accident or fall from height etc.[1-4] Open fracture shaft of tibia frequently associated with many complications, like malunion,non-union, infection, soft tissue injury etc.[5-9] Infected open fracture is very difficult and challenging problem to treat. The incidence of nonunion in closed tibial fractures is 2.5%, and it increases many times for open fractures with contaminated wound and severe soft-tissue lossand infection. Due to various local problems, such as bone deformity, bone loss, and prior implant failure treatment of open fracture of tibia become very difficult. [10,11] Ilizarov technique is a popular method of management of open fracture of long bone. This method involves extensive debridement of the operative site, covering the fracture site as early as possible the stabilization of the fracture site with percutaneous wires and Ilizarov frame construct.[10,12,13] Ilizarov external fixator (IRF) allows compression, distraction, lengthening anddeformity correction. Immediate weight bearing is possible after IRF and joints mobilization of proximal and distal joints also possible. Corticotomy and bone transport is possible for bone defects. Extensive wound debridement helps to control the bone infection.[10,12,13,14] The aim of our study was to evaluate the outcome of management of open fracture shaft of tibia by Ilizarov technique.

Materials and methods

Study design& sites: The study was prospective observational study which was carried out at Bashundhara Ad-din Medical College Hospital, National Institute of Traumatology and Orthopaedic Rehabilitation (NITOR) and few private clinics in Dhaka between June 2018 and December 2022.

Study population

With open fracture shaft of tibia, a total number of 36 patients reported in outpatient department were included in the study. Both the infected and noninfected cases were included in the study.

Ethics statement

The study was approved by the ethics committee of the respective hospitals.

Patient management

Initially all patients were managed by thorough surgical toileting, wound debridement and broad-spectrum antibiotic was given. Immobilization was done by uniaxial external fixator or by long leg back slab. Open wounds were closed by split thickness skin grafting or flap coverage.

After primary management, adequate soft tissue coverage of exposed bones was done. For all cases surgery were done under spinal anaesthesia. Initially Ilizarov frame was built according to the radiological findings and per operative assessment. Taking best possible care of soft tissues incision was made in suitable site and all dead and devitalized tissues was removed extensively, freshening of fracture ends done and bleeding was seen from bone ends. Then the frame was fixed with 1.8mm Ilizarov wires and for more stability Olive wires were used when needed. After refreshing and debridement if gap at fracture site was found less than 4 cm primary docking was done and we started distraction of 1mm per day at corticotomy site from 7th postoperative day.[15] And if gap at fracture site was found more than 4 cm primary docking was not done and compression at fracture site and distraction at corticotomy site was started from 7th postoperative day. We did Corticotomy in all cases where shortening was more than 2cm.Upper tibial Corticotomy was done in 22 cases. With fracture of tibia, fibula was fractured in most of the cases. In some cases, fibula was fractured in such a way that it may complicate the tibial union. In those cases, partial Fibulectomy were done, otherwise they were left alone. distal neurovascular status was checked after surgery and limb was kept elevated during post-operative days. On the first postoperative day, a check x-ray was performed. Frame stability, pins sites were checked and dressing done on the first or second postoperative day. When pain permits, we advise the patient to do exercise at adjacent joints. With the help of axillary crutches, the patient started bear weight from the second postoperative day. Every patient was followed up by regular radiographs at appropriate times to access union, alignment of bone fragments and/or development of any angular deformity.[10] Pin track infection was the commonest complication. Other complications were loosening of the frame, breakage of Ilizarov wires, and poor quality of bone regenerate.

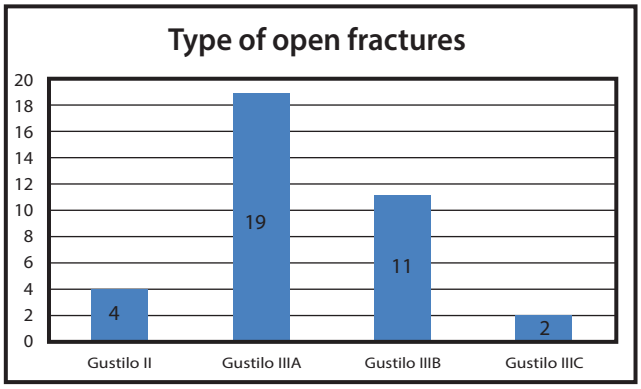

Figure 1. Distribution of patients according to types of open fractures

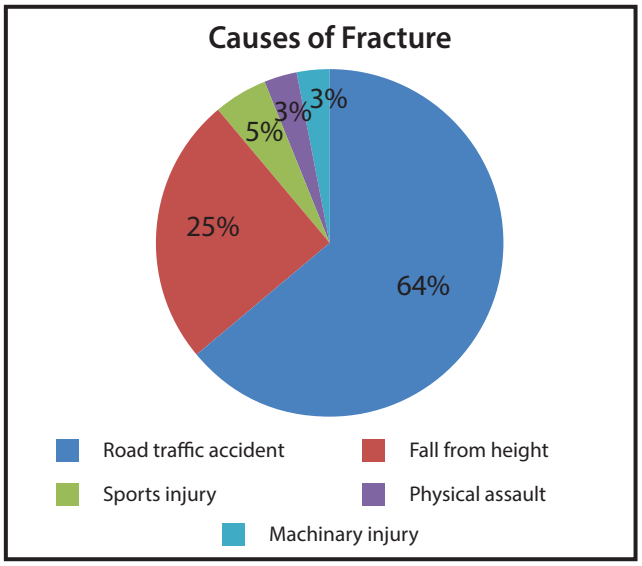

Figure 2. Distribution of patients according to cause of injury

Table 1: Association for the Study and Application of the Methods of Ilizarov scoring system

ASAMI scoring systems	Description	Score
Bone results		
Excellent	Union, no infection, deformity <7°, limb-length discrepancy <2.5 cm	29
Good	Union + any two of the following: Absence of infection, <7° deformity, and limb-length inequality of <2.5 cm	5
Fair	Union + any one of the following: Absence of infection, <7° deformity, and limb-length inequality of <2.5 cm	2
Poor	Nonunion/re-fracture/union + infection + deformity >7° + limb-length inequality >2.5 cm	0
Functional results		
Excellent	Active, no limp, minimum stiffness (loss of <15° knee extension/<15° dorsiflexion of ankle), no RSD, insignificant pain	29
Good	Active, no limp, minimum stiffness (loss of <15° knee extension/<15° dorsiflexion of ankle), no RSD, insignificant pain	5
Fair	Active, with three or all of the following limb, stiffness, RSD, significant pain	2
Poor	Inactive (unemployment or inability to return to daily activities because of injury)	0
Failures	Amputation	

ASAMI –Association for the Study and Application of Methods of Ilizarov; RSD – Reflex sympathetic dystrophy

Result:

Thirty-six patients with open fracture shaft of tibia were included. For follow-up patients came to the hospital regularly. The mean follow-up period was 22.5 months (6-37 months) Out of 36 patients, 21(58.3%) were male and 15(41.6%) were female. 26(72.2%) patients had age below 40 years, 8(22.2%) were between 40 to 60 years and 2(5.5%) were above 60 years of age. Out of 36 cases, right sided tibia fracture was on 25(69.4%) cases and left sided tibia fracture was on 11(30.5%) cases. Primarily all patients had open fracture of tibia, among them 4(11.1%) had Gustilo II, 19(52.7%) had Gustilo IIIA, 11(30.5%) had Gustilo IIIB and 2(5.5%) had Gustilo IIIC fractures, respectively (Fig 1).

Out of 36 patients, road traffic accident was 23(63.8%), 9(25%) had history of fall from height, 2(5.5%) had sports injury, 1(2.7%) had history of physical assault and 1(2.7%) had history of machinery injury (Fig 2).

Out of 36 patients, 25(69.4%) were initially managed by uniaxial external fixator and 11(30.5%) were managed by long leg back slab and then Ilizarov external fixator was applied. In our study, 17(47.2%) patients needed soft tissue coverage, among them split thickness skin grafting was done in 5(13.8%) cases and flap coverage was needed for 12(33.3%) cases. From starting, the primary management to application of IRF mean interval was 5 weeks. Fracture was united in every case. The mean time for successful union was 6.3 months (4-9 months). The average duration for frame removal was at 6 months (5-10 months).Clinical follow-up results were evaluated according to the ASAMI protocol. Bony unions were achieved in all cases of our study. Bone results were excellent in 29, good in 5, fair in 2 and no poor case. Functional results were excellent in 30 cases, good in 5, fair in 1 and no poor case (Table 1).

Discussion:

The incidence of open fracture shaft of tibia is increasing rapidly and now very common in outpatient and emergency departments. This injury in many cases are associated with soft tissue loss, shortening or deformity of the limb and most dangerously associated with infection.[16,17] For a trauma surgeon, management of a case of open fracture of shaft of tibia is still now very challenging.[18] Inter fixation gives very poor outcome in management of open fractures but IRF gives better outcome. That is why IRF has gained popularity. In cases of shortening of the limb, a unifocal or bifocal corticotomy can be done. After 7 days distraction started at the corticotomy site and compression was given at fracture site. This is called tension stress effect and this is the principle of Ilizarov ring fixation.[19]

In our study, the overall union rate was 100% which is similar to the union rates reported in four other series.[20-22] Bone results were excellent in 29(80.5%), good in 5(13.8%), fair in 2(5.5%) and no poor case. Functional results were excellent in 30(83.3%), good in 5(13.8%), fair in 1(2.7%) and no poor case.In every case we tried to preserve the blood supply of bone and meticulous soft tissue dissection was done always. These may be the causes of our higher union rate.[13} With IRF, fixator modification was possible anytime, if necessary. This may also help in fracture union. Various deformity, like angulation, shortening can be corrected during the course of treatment with IRF. In case of delayed union accordion maneuver can be applied which stimulates callus formation by cyclic compression-distraction. [23,24]

Open fracture of tibia has some serious complications, like osteomyelitis, amputation etc. In our study, we have shown that these major complications can be minimized with application of IRF. Other complications, like superficial infection, pin-site infection (23%) were minimum in our study which was comparable with other studies.[25] Oral antibiotics were given for this type of infection. Actually, this type infection should be considered as problem of open fracture, not a true complication.[26]

In every case, full weight bearing walking started from second or third postoperative day after removal of drain tube and check dressing. But still the Ilizarov frame have some troublesomeness,like as clothing difficulty and positioning problem during rest and sleeping. [26]

Conclusion:

Management of open fracture tibia has high complication risks because it is high energy trauma and often associated with severe soft tissue loss. To avoid infection wound debridement should be done very accurately and proper antibiotic should be given for optimum duration. During application of IRF principles of Ilizarov technique should be followed properly. Adequate tension should be given in wires and frame should be aligned properly with the leg to give adequate space for swelling. If corticotomy was done, distraction usually started after 7 days of corticotomy at the rate of 0.25 mm per 6 h. Patient should be under regular follow-up to find out any developing complication, so that management of complication will be less difficult. IRF is an outstanding treatment option for open fracture of tibia regarding the management of bone loss, control of infection, limb-length equalization and functional improvement of limb.

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Original article

Evaluation of Serum Interleukin-6 Level Among Hospital Admitted COVID-19 Patients and Correlation with Their Disease Severity in a Tertiary Care Hospital

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Abstract

Background:The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is the cause of Coronavirus Disease 2019 (COVID-19). Excessive and heightened release of pro-inflammatory cytokines (IL-6) is observed in COVID-19 patients, which can lead to several severe symptoms. Elevated levels of interleukin-6 in patients with COVID-19 indicate cytokine storm and are thought to be a significant factor in identifying the most severe forms of the illness. ***Objectives:*** The study is aimed to assess IL-6 levels among hospital-admitted COVID-19 patients and evaluate their relationship with disease severity. ***Materials and Methods:*** A total of 136 Rapid antigen test positive or RT-PCR positive COVID-19 patients from the Department of Medicine of Sylhet MAG Osmani Medical College and Shahid Shamsuddin Ahmed Hospital, Sylhet from January 2021 to December 2021 were enrolled in this study by convenient sampling technique. With all aseptic precautions 4ml of venous blood was collected from ante-cubital vein and the serum was separated by centrifuge process at 4000 rpm for 10 minutes and stored in the laboratory at -20ºC. Serum IL-6 level was assayed by solid-phase chemiluminescent immunoassay, according to the manufacturer's instructions. (ADVIA Centaur CP IL-6 immunoassay system, SIEMENS, Berlin, Germany, Lot no: 145032). ***Result:*** In this study among 136 patients, 66 (48.5%) were aged between 51-70 years and the mean age was 49.59 ± 18.03 years. There were 65 (47.8%) male and 71 (52.2%) female patients. Out of 136 patients, 76 (55.9%) were moderate cases, 43 (31.6%) were severe cases and 17 (12.5%) were critical cases. The mean age of moderate cases was 44.36 ± 18.53 years, severe cases were 52.36 ±14.34 years and critical cases were 64.65 ± 13.90 years. Interleukin-6 level was 6.06 ± 3.69 pg/mL in moderate cases, 44.71 ± 4.49 pg/mL in severe cases and 242.97± 21.48 pg/mL in critical cases and it was higher in critical cases than severe and moderate cases. ***Conclusion:*** This study showed that IL-6 level is significantly associated with the severity of illness. So, it can serve as an effective marker for the severity of the disease and can help physicians to correctly allocate the hospital-admitted COVID-19 patients at an early stage and to identify critically ill COVID-19 patients.

Keywords: Serum interleukin-6 level, Moderate, severe and critical COVID-19 patients.

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Introduction:

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the cause of the worldwide health disaster known as the Corona Virus Disease of 2019 (COVID-19). The coronavirus, which is a member of the Coronoviridae family, was initially discovered and named in the 1960s. This virus is zoonotic, meaning it may infect both people and animals. The virus is encased and has a helical nucleocapsid.It has a non-segmented, single-stranded RNA genome. The spike protein (S), nucleocapsid protein (N), membrane protein (M), and envelope protein (E) are the four normal viral structural proteins.[1- 4]

The novel coronavirus (SARS-CoV-2) first emerged on December 31, 2019 in Wuhan, China. On January 30, 2020, the World Health Organization classified the COVID-19 outbreak as a worldwide public health emergency; the pandemic was announced on March 11, 2020. On March 8, 2020, the first case of patients suffering from COVID-19 disease caused by the SARS-CoV-2 virus was confirmed in Bangladesh. As of January 2022, there were 364,191,494 confirmed cases of COVID-19; including 5,631,457 deaths were reported to WHO. Among them, Bangladesh has confirmed a total of 1,798,833 cases and 28,394 deaths.[5]
Coronavirus initially undergoes viral replication in the respiratory tract and then spreads to other organs and tissues. Then it enters the pulmonary alveolar epithelial cells through angiotensin-converting enzyme receptor 2 (ACER-2). The main mechanism for inflammation and organ damage is cytokine storm, especially in pulmonary vascular endothelial cells with increased inflammatory cytokines such as interleukin-6 (IL-6), interleukin-10 (IL-10), and interferon- γ (IFN-γ). Exaggerated and excessive synthesized cytokines like IL-6, and IL-10 can lead to cytokine storm which is associated with the disease severity of COVID-19 patients.[6-10]

The clinical manifestations of COVID-19 are wide-ranging, from asymptomatic, mild, moderate to severe viral pneumonia such as acute respiratory distress syndrome (ARDS).COVID-19 patients with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) but no signs of severe pneumonia (SpO2≥ 90% on room air) are categorized as moderate case,adults with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) plus one of the following: severe respiratory distress, respiratory rate> 30 breaths/min or SpO2< 90% on room air categorized as severe case (Severe Pneumonia) and severe COVID-19 case with any of the following criteria: respiratory failure and requiring mechanical ventilation, sepsis, septic shock, ARDS, any organ failure that requires ICU care categorized asCritical cases (Cases requiring ICU care).[11] But some COVID-19 patients experience respiratory deterioration over a short period during their clinical course. Thus, it is essential to identify patients who are likely to develop severe conditions as early as possible.[12,13] Several blood markers could predict respiratory failure in COVID-19 patients. Some inflammatory cytokines could distinguish disease severity in COVID-19. In some patients, the general condition dramatically worsens within a couple of days with severe respiratory failure. Therefore, it is of high priority to identify reliable blood markers that could predict respiratory illness in the short term in clinical settings. In COVID-19 patients with cytokine release syndrome (CRS), interleukin-6 (IL-6), IL-10, and interferon (IFN)-γ are consistently elevated. In COVID-19 patients IL-6 contributes to many of the symptoms, such as the production of acute phase reactants by hepatocytes, activation of the extrinsic coagulation pathway, and production of vascular endothelial growth factor (VEGF), leading to endothelial inflammation. IL-6 plays a pivotal role in the pathophysiology of lung damage in COVID-19 patients.High levels of serum IL-6 have been observed in many patients with cytokine storm in severe COVID-19. COVID-19 patients with comorbidities with high IL-6 levels at admission are at increased risk of developing a severe form of the disease, requiring mechanical ventilation and ICU and progressing to respiratory distress syndrome and multiorgan failure.[14-18] In a meta-analysis including nine studies reported that mean IL-6 levels were more than three times higher in patients with complicated COVID-19 compared with mild or moderate diseases. The concentration of IL-6> 24pg/ml at initial assessment predicted the development of hypoxemia requiring hospitalization.[19] So, by this study we can evaluate serum IL-6 levels among hospital-admitted COVID-19 patients and can make an association of IL-6 with the severity of the diseases in hospital-admitted COVID-19 patients and can help in further management of patients.

Materials and Methods

From January to December 2021, the Department of Microbiology and Virology at Sylhet MAG Osmani Medical College and Shahid Shamsuddin Ahmed Hospital, Sylhet, collaborated with the Department of Medicine to undertake this cross-sectional observational study. After obtaining ethical clearance, a total of 136 Rapid antigen test-positive or RT-PCR-positive COVID-19 patients were enrolled in this study by convenient sampling technique. With all aseptic precautions 4ml of venous blood was collected from ante-cubital vein and the serum was separated by centrifuge process at 4000 rpm for 10 minutes and stored in the laboratory at -20ºC. Serum IL-6 level was assayed by solid-phase chemiluminescent immunoassay, according to the manufacturer's instructions. (ADVIA Centaur CP IL-6 immunoassay system, SIEMENS, Berlin, Germany, Lot no:145032). Data were recorded in a pre-designed structured data collection form. One-way ANOVA and unpaired t-test were applied to analyze the data by using SPSS version 26.

According to National Guidelines on Clinical Management of COVID-19, 9th edition:

Moderate case: Adult with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) but no signs of severe pneumonia (SpO2≥ 90% on room air).

Severe case (Severe Pneumonia): Adult with clinical signs of pneumonia (fever, cough, dyspnoea, fast breathing) plus one of the following: severe respiratory distress, respiratory rate> 30 breaths/min or SpO2< 90% on room air.

Critical cases (Cases requiring ICU care): Severe COVID-19 case meeting any of the following criteria: respiratory failure and requiring mechanical ventilation, Sepsis, Septic shock, ARDS or any organ failure that requires ICU care.

Results

The patients in this study were between the ages of 18 and 90. The patient's average age was 49.59 (SD± 18.03) years.

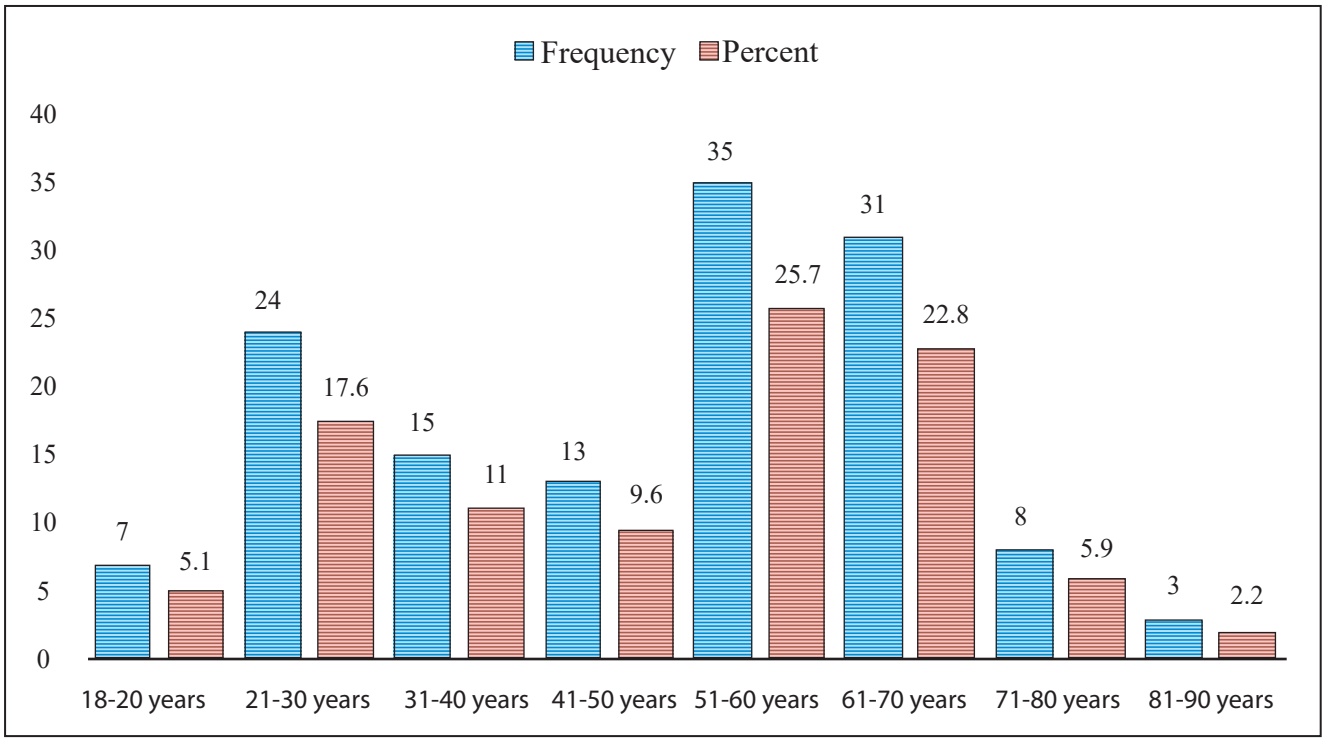

Figure 1: Distribution of the patients according to age group (n=136)

Figure 1: Shows out of 136 patients, the majority 35(25.7%) were aged between 51-60 years followed by 31 (22.8%) were in the age group of 61-70years.

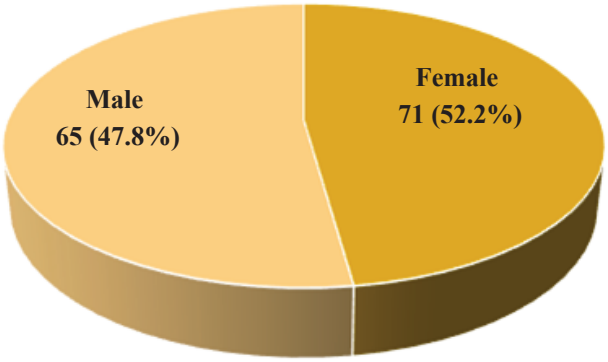

Figure 2: Distribution of the patients according to gender (n=136)

Figure 2: Shows out of 136 COVID-19 patients, male patients were 65 (47.8%) and female patients were 71 (52.2%).

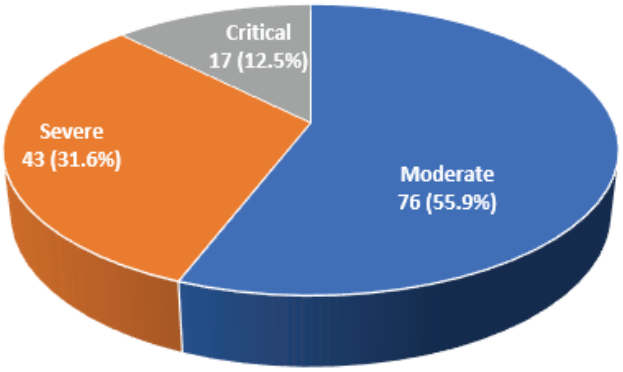

Figure 3: Distribution of the COVID-19 patients according to disease severity

Figure 3: Shows out of 136 patients, moderate cases were 76 (55.9%), severe cases were 43 (31.6%) and critical cases were 17 (12.5%).

Table 1: Distribution of age of the COVID-19 patients according to disease severity

Age(year)	Severity			*p -value
	Moderate	Severe	Critical	
Mean	44.36	52.36	64.65	p<0.001
± SD	± 18.53	±14.34	± 13.90	

*One-way ANOVA was applied to analyze the data

Table 1: Shows the mean age of moderate cases was 44.36 ± 18.53 years, severe cases was 52.36 ±14.34 years and critical cases were 64.65 ± 13.90 years and the difference in the age of the patients of the different groups was statistically significant (F=11.360; p<0.001).

Table 2: Distribution of COVID-19 patients according to co-morbidity

Co -morbidity	**Frequency**	**Percentage**
Hypertension	20	14.7
Diabetes mellitus	15	11.0
Bronchial asthma	5	3.7
Chronic kidney disease	4	2.9

Table 2: Out of 136 participants, 14.7% were hypertensive, 11.0% were diabetic, 3.7% were asthmatic and 2.9% with chronic kidney disease.

Table 3: Comparison of IL-6 levels among moderate, severe and critical COVID-19 patients:

Interleukin-6 level (pg/mL)	Severity			*p -value
	Moderate(76)	Severe(43)	Critical(17)	
Mean	6.06	44.71	242.97	p<0.001
± SD	± 3.69	±4.49	± 21.48	

*One-way ANOVA was applied to analyze the data

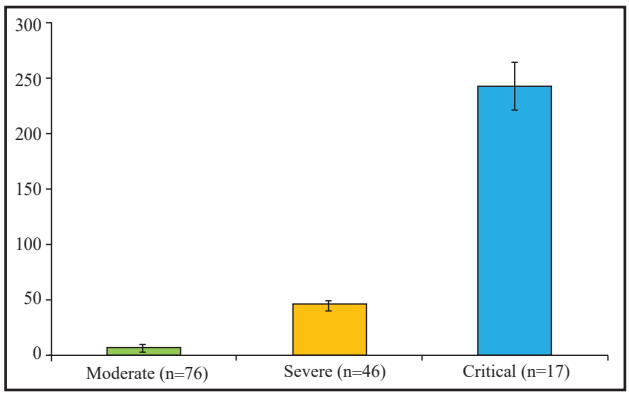

Figure 4: Comparison of interleukin-6 level among severity of COVID-19 patients

Table 3 and Figure 4: Show IL-6 levels were 6.06 (SD± 3.69) pg/mL, 44.71 (SD± 4.49) pg/mL, and 242.97(SD± 21.48) pg/mL in moderate, severe and critical cases of COVID 19 patients respectively. IL-6 level significantly differed among the severity of cases (F=318.641; p<0.001) and the difference was statistically significant.

Discussion:

The present study was undertaken for the assessment of IL-6 levels of moderate, severe and critical COVID-19 patients. In this study the age of the patients ranged from 18 years to 90 years and the mean age was 49.59± 18.03 (SD) years. In Cruz et al. mean age was 45.24±13.97 (SD) years and in Vultaggio et al.[20] The mean age was 63±15 (SD) years. The majority 66(48.5%) of the patients were aged between 51-70 years.

There were 65 (47.8%) male and 71 (52.2%) female cases with a ratio of male and female 1:1.1 in this study. Gender distribution was reported by Liu et al. in China, where 46.98% were male and 53.02% female and in Cruz et al. where male patients were 52%, and female patients were 48%.The gender ratio of the study subjects was almost the same. Men and women had the same prevalence of COVID-19 in Jiang et al.

In the present study out of 136 patients, 76 (55.9%) were moderate cases, 43 (31.6%) were severe cases and 17 (12.5%) were critical cases of COVID-19. (Patient's categorization: moderate, severe, and critical case according to national guideline 9th edition: pages 1 & 2). The case distribution was reported by Azmy et al., 2021 in New Haven, USA where the moderate case was 48%, the severe case was 30% and the critical case was 22%.

In this study the mean age of moderate cases was 44.36 ± 18.53 years, severe cases were 52.36 ±14.34 years and critical cases was 64.65 ± 13.90 years. A similar observation was recorded in China where the mean age of moderate cases was 45.29± 13.08 years, severe cases was 60.41 ± 9.80 years and critical cases was 65.88 ± 13.61 years. So, the mean age of patients was higher in critical cases, than in severe and moderate cases.

In this study out of 136 patients, 20 (14.7%) patients had hypertension, 15 (11.0%) patients had diabetes mellitus, 5 (3.7%) patients had bronchial asthma and 4 (2.9%) patients had chronic kidney disease. A total of 32.3% of patients had at least one comorbidity. A study conducted in China by Liu et al., 2019 also showed a total of 36.23% of COVID-19 patients had at least one comorbidity.

In the present study interleukin-6 level was 6.06 ± 3.69 pg/mL in moderate cases, 44.71 ± 4.49 pg/mL in severe cases and 242.97± 21.48 pg/mL in critical cases of COVID-19 patients respectively. The mean value of IL-6 was higher in critical cases than in severe and moderate cases. A study by Vultaggio[21] in China showed the mean interleukin-6 level was 15.7 ± 15.60 pg/mL in moderate cases and 53.63± 63.8 pg/mL in severe and critical cases, 27 ± 40.9 pg/mL in all cases of COVID 19 patients respectively. Another study in New Haven, USA showed interleukin-6 level was 19.5 pg/mL in moderate cases and 21.20 pg/mL in severe cases of COVID-19 patients respectively. IL-6 value in critically ill COVID-19 patients admitted to the ICU was 336 pg/mL in Gorham, California. So, IL-6 level was significantly higher in critical cases compared to moderate and severe cases.

Conclusion:

COVID-19 was caused by a new strain of beta coronavirus, SARS-CoV-2. Worldwide it is emerging as a huge threat to human health and the mortality rate is higher among severe and critical cases. Cytokine storm causes systemic inflammation, ARDS and multi-organ dysfunction in COVID-19 patients. The current study indicates that high IL-6 levels suggest a cytokine storm which may play a major role in the pathophysiology of this disease and are considered as a relevant parameter in predicting most severe cases of disease. In this study, IL-6 level was significantly higher in critical cases compared to moderate and severe cases who needed more intensive care and treatment due to severe lung damage. IL-6 levels significantly differed among the moderate, severe and critical COVID-19 patients. Therefore, IL-6 could be a potential marker for disease monitoring in hospital-admitted COVID-19 patients. Anti-cytokine therapies that target IL-6 may be useful in treating inflammatory cytokine storms as the illness worsens. Gaining further insight into the role of IL-6 in the pathophysiology of COVID-19, particularly in severe instances, might potentially improve disease management.

Limitations of the study

1. This study did not examine correlations or associations with other inflammatory markers.

2. To eliminate this uncertainty, IL-6 and its upstream and downstream characteristics should be recorded in greater detail.

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Original article

Water Quality of the Rivers and Water-bodies around Dhaka city: a Public Health Concern

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Abstract

Background: The deteriorating water quality in Dhaka city has emerged as a critical concern. The rivers and lakes in the vicinity of Dhaka city have experienced significant pollution due to the discharge of effluents, rendering them unfit for use. This contamination has also adversely impacted the quality of groundwater. ***Methodology:*** This research aimed to scrutinize the water quality of Dhaka city during the specific period of 2008 and 2009. Water samples were systematically collected from designated locations and subjected to thorough analysis at the BCSIR (Bangladesh Council of Scientific and Industrial Research) Laboratories in Dhaka, Bangladesh, focusing on specific parameters.Various physical, chemical, and biological properties of water were meticulously examined. ***Results:*** The study revealed that the water quality of Buriganga is notably worse than that of Tongi Khal, Turag river, and Balu river. Regions affected by this pollution emit unpleasant odors, attributed to fecal and chemical wastes, presenting environmental challenges. The dissolved oxygen concentration in Buriganga river, Tongi Khal, Balu, and Turag river was alarmingly below the standard value (4-6 mg/l). Chemical oxygen demand and biological oxygen demand in these rivers exhibited unfavorable conditions, with coliform bacteria exceeding acceptable limits. During the study period of 2008 and 2009, prevalent health issues included skin diseases and diarrhea, along with instances of Cholera, Typhoid, and Dysentery. Notably, water pollution was observed to have psychological effects on the residents. The water in Dhaka city, characterized by its fully black color and disagreeable odor, is unsuitable for recreational purposes. Fish populations dwindled, local fish species faced endangerment, and many were no longer observable in the four rivers surrounding Dhaka city. ***Conclusion:*** Polluted water detrimentally affects terrestrial plants and animals. As polluted water traverses over land surfaces, it contaminates soil, posing threats to plants and animals. Aquatic flora and fauna are also adversely affected by polluted water. Consequently, water pollution casts negative implications across every facet of the environment. The environmental predicament in Dhaka city, particularly pertaining to water quality, exerts direct and indirect influences on daily, personal, and social life.

Keywords: water pollution, environmental health, water quality, river, lake, canal, water bodies, pollution, impurities, Dhaka city.

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Introduction:

Water quality emerges as a critical environmental concern due to its fundamental role in supplying water for drinking, domestic use, irrigation, and supporting aquatic life, including fisheries. The degradation of river water quality in Dhaka is primarily attributed to human activities, unplanned urbanization, stormwater and sewerage systems, household waste, oil spills, sedimentation, and industrial effluent discharge into rivers, alongside encroachment on riverbanks.[1]

The repercussions of compromised water quality extend beyond immediate concerns, affecting climate, soil, plants, animals, and human life. Rivers in and around Dhaka have deviated from their natural state, exhibiting alterations in physical, chemical, and biological properties, rendering them unsuitable for any beneficial use. Contaminated river water is characterized by its black color, unpleasant taste, offensive odors, diminished aquatic life, and increased weed growth, transforming these water bodies into receptacles for a myriad of solid, liquid, and chemical wastes.[2]

Dhaka, the capital city of Bangladesh, holds paramount significance as the focal point of socio-economic and political activities in the country. This rapidly expanding metropolis is encompassed by vital rivers, serving as indispensable resources for its sustenance. These rivers play a pivotal role in providing drinking water, drainage systems, various fish species, and water routes for transportation.[3]

The severity of pollution has reached a point where water treatment becomes practically impossible. The Water Supply and Sewerage Authority (WASA) resorts to supplying water treated with chlorine and ammonia sulfate, resulting in an undesirable odor. River narrowing due to waste dumping and encroachments further impedes communication and forces residents near rivers to use polluted water, often unaware of the associated health risks. This, in turn, contributes to the spread of waterborne diseases.[3]

The discharge of solid wastes and effluents into rivers renders the water unfit for the survival of fish and other subaqueous organisms. Elevated levels of Biological Oxygen Demand (BOD) caused by waste influx lead to an oxygen crisis for aquatic life, posing a threat to biodiversity in and around the rivers. River pollution in Bangladesh mirrors a global issue, prompting the formulation of government rules, regulations, policies, and strategies to safeguard these invaluable water resources from further deterioration.[4]

Materials and Methods

Water samples were collected from three sampling stations of four rivers in and around Dhaka city and two lakes, Dhanmondi and Gulshan Lake, for surface water and from eight stations for groundwater quality analysis. Sampling stations were selected purposively. Water was collected through fieldwork during four seasons: winter, pre-monsoon, monsoon, and post-monsoon. Water parameters were categorized into three groups: physical, chemical, and biological parameters.Physical parameters included: Temperature, Turbidity (NTU), Electric conductivity (EC), Total dissolved solids (TDS), Odor and Color. Chemical parameters included: pH, DO, BOD, COD. Trace elements such as Total Alkalinity (Alk), Chloride (Cl), Phosphate (PO4), Nitrate (NO3), Cadmium (Cd), Chromium (Cr), and Zinc (Zn). Biological parameters included: Coliform bacteria (Fc), Hydrophytes, Fish.

To determine the geographical locations, MAGELLAN GPS 310 was used. All the parameters of water samples were analyzed in the laboratory with standard methods (APHA, 1995) except alkalinity (Trivedy and Goel, 1986). The following instruments were used for the analysis of water samples: pH meter Model HANNA, TDS meter, EC meter, DO meter, Shimadzu UV Visible Spectrophotometer Model UV mini-1240 Japan for colorimetric detections, Atomic Absorption Spectrometric methods to determine metals, Perkin-Elmer Model 3110 Atomic Absorption Spectrophotometer to detect Zn and other trace elements, BINDER Microbiological Incubator Model BD-53 Germany for analysis of Coliform bacteria.

Results

Analysis of Water Quality by Pearson's Correlation Matrix: The variations of water pollution in different seasons were identified and verified by comparing physical, chemical and biological data with Pearson's correlation two-tailed matrix. In Buriganga, DO had negative impacts on other parameters. In Turag, only two parameters, such as DO and turbidity had negative correlations and other parameters had positive correlations. The Tongi Khal and Balu had positive correlations with maximum parameters.

Analysis of water quality by ANOVA test:Seasonal variation of different parameters was determined by analyzing two years of data with the help of the ANOVA test in SPSS and the result was tabulated with List Significance Difference Duncan Multiple Range Test at 5% level. From the ANOVA test, it was found that there was a significant variation of physicochemical and biological parameters concentration in river water among four seasons. In Buriganga, TDS and TC had more significant values than other parameters. In Turag, only one most significant value was in NO3 with DO. Tongi Khal had significant value in four parameters: BOD, COD, Alkalinity and Cl. In Balu River significant values were with four parameters: TDS, BOD, Cl and NO_3.

Table 1: The water quality parameters of different stations of the four studied rivers

River	Buriganga			Turag			TongiKhal			Balu		
Stations→	BG1	BG2	BG3	T1	T2	T3	Tk1	Tk2	Tk3	BL1	BL2	BL3
Parameters↓	Mean ± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean± SD	Mean ± SD	Mean± SD	Mean± SD	Mean± SD
NTUppm	6.5± 0.85	6.73± 1.36	8.94± 2.68	6.19± 0.26	6.24± 0.26	6.13± 0.23	6.75± 0.97	6.34± 0.31	6.28± 0.25	7.56± 1.21	7.48± 1.06	7.88± 1.79
EC µs/cm	579.0 ± 416.8	501.6 ± 299.5	505.9 ± 279.5	737.4± 736.1	299.9± 114.5	574.9± 389.3	607.0± 426.6	503.6± 259.5	1612 ± 896.3	556.5± 229.4	490.9± 196.0	521.5± 232.1
TDS ppm	380.8 ± 179.5	458.4± 220.5	379.5± 175.9	1127± 256.8	465.8± 101.8	347.6± 220.1	451.1± 222.6	379.1± 278.2	710.8 ± 333.6	933.4± 350.5	705.1± 124.9	698.6± 134.2
pH	7.13± 0.18	7.32± 0.16	7.18± 0.16	7.78± 0.37	7.41± 0.30	7.35± 0.21	7.41± 0.09	7.36± 0.13	7.31± 0.15	7.29± 0.35	7.30± 0.27	7.10± 0.43
DO ppm	3.06± 2.52	2.21± 2.15	2.85± 2.37	2.36± 2.36	5.05± 1.75	1.76± 2.07	2.19± 2.10	2.29± 2.23	2.29± 2.02	2.73± 2.11	3.50± 0.95	3.19± 1.29
BOD ppm	15.29 ± 9.6	24.6±15. 66	17.2± 9.30	15.3± 11.89	11.9± 8.48	13.8± 8.68	20.0± 12.12	15.1± 8.33	16.4± 7.68	15.4± 9.62	14.3± 10.27	15.78±8. 86
COD ppm	48.44 ± 8.1	72.38±25.6	54.2± 14.4	54..6± 8.07	49.5± 6.59	51.6± 5.21	53.0± 13.5	52.4± 9.52	49.3± 10.9	55.6± 5.95	54.1± 6.06	53.4± 7.41
Alk ppm	174.1 ± 90.8	201.5±10 4	155.1± 87.6	131.0± 83.8	123.4±83 .9	125.3± 81.4	173.5±70.7	153± 80.1	170± 72.9	125.1± 57.9	127.1± 55.4	128.4± 57.9
Cl ppm	17.89 ± 7.6	28.63±10.6	18.1±10.09	15.44±8.8	14.8± 8.12	17.5± 12.9	19.1± 7.95	15.4± 6.78	18.14 ± 7.55	18.81±7.66	17.7± 8.79	17.9±9.31
PO_4 ppm	11.9± 6.15	14.21±7. 61	13.85±5. 93	11.0± 8.69	3.78±1.7 9	1.66± 0.74	2.83± 0.45	10.3± 10.3	6.29± 5.15	11.8± 3.71	8.76± 3.54	9.65± 3.98
NO_3 ppm	15.3± 4.26	21.25±7. 27	18.7± 4.87	8.85±6.7 5	3.23± 1.54	1.57± 0.43	2.48± 0.81	11.4± 8.33	8.03± 2.14	5.63± 4.09	3.53± 1.0	3.44± 2.23
Cd ppm	2.18± 0.54	4.04± 1.62	1.26± 1.25	2.63± 2.59	1.03± 0.98	1.11± 0.57	1.29± 1.63	3.29± 5.23	2.36± 3.34	2.85± 0.64	1.59± 1.43	1.83± 1.67
Cr ppm	8.34± 7.01	8.06± 9.31	7.60± 8.67	4.21± 4.84	0.58± 0.18	0.45± 0.24	0.54± 0.23	6.10±6.48	4.95± 4.72	2.79± 0.67	2.49±1.0 2	2.36± 0.77
Zn ppm	1.52± 0.55	1.64± 0.61	1.53± 0.57	1.34± 0.73	0.65± 0.51	0.33±0.0 7	0.53± 0.09	0.89± 042	1.0± 0.48	0.80± 0.21	0.69± 0.18	0.60± 0.18
TC mpn	1965 ± 511	1943± 408	2263± 431	2115± 397	2215±28 5	2010±44 6	2447±27 8	3700±12 16	4188 ± 1723	1833± 482	1901± 544	1874±551

All average values are in ppm except EC (µs/cm), pH, TC (mpn) and FC (mpn).

Table-2: Seasonal variation of different parameters of river water (2008-09)

Parameters	Seasons			
	Winter	Pre-monsoon	Monsoon	Post-monsoon
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
NTUppm	7.65±1.83	6.58±1.66	6.60±0.97	7.03±1.19
EC µs/cm	1139.33±664.08	624.83±301.07	292.29±185.20	474.21±335.15
TDS ppm	771.38±275.12	555.29±293.04	497.92±336.23	538.25±322.54
pH	7.47±0.36	7.38±0.19	7.14±0.30	7.30±0.21
DO ppm	0.87±1.45	1.98±1.77	4.66±1.03	3.61±1.67
BOD ppm	27.88±5.10	20.15±7.96	5.74±3.85	10.73±4.41
COD ppm	65.90±13.91	54.63±10.79	45.21±4.41	50.0±5.41
Alk ppm	237.46±31.72	161.54±57.74	89.21±63.86	107.08±51.62
Cl ppm	30.31±5.46	18.21±5.97	10.68±4.75	13.59±3.90
PO4 ppm	13.47±7.79	6.29±4.0	5.81±4.04	10.52±6.86
NO3 ppm	12.22±10.11	6.78±5.94	6.81±5.19	8.58±8.11
Cd ppm	4.64±2.81	1.49±1.17	0.85±0.80	1.45±1.48
Cr ppm	9.22±8.50	1.44±0.94	1.78±1.24	3.62±2.82
Zn ppm	1.39±0.88	0.69±0.32	0.80±0.33	0.97±0.39
TC mpn	2594±995	2352±1027	1978±952	2568±988

All average values are in ppm except EC (µs/cm), pH, TC and FC (mpn).

Table-3: Different parameters of Lakes water in study period 2008 and 2009

Lakes	year	NTU	EC	TDS	pH	DO	BOD	COD	TC
Dhanmondi	2008	11.38	27.33	172.67	7.16	0.50	2.17	31.43	500.00
	2009	8.37	27.73	175.67	7.21	0.57	2.70	32.00	523.33
Average	2008	9.76	32.83	201.33	7.15	0.48	13.58	52.72	751.67
Gulshan	2008	8.13	38.33	230.00	7.13	0.47	25.00	74.00	1003.33
	2009	8.90	40.33	156.67	7.30	0.40	30.00	79.67	1023.33
Average	2009	8.63	34.03	166.17	7.25	0.48	16.35	55.83	773.33

All average values are in ppm except EC (µs/cm), pH, TC and FC (mpn).

Table 5: Comparative Water Quality of different sources with BDWS and WHO Standards

Para-meters	Sources							
	Buriganga River	**Turag River**	**Tongi Khal**	**Balu River**	**Lakes Water**	**Ground Water**	**BD**	**WHO**
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Standard	GVS
NTUppm	**7.56 ± 2.30**	**6.20±0.25**	**6.48±0.61**	**7.64±1.34**	**9.20±3.73**	**1.7±1.81**	**5**	**≤ 5**
EC µs/cm	**535.79± 333.29**	**526.61±491.52**	**934.54±756.24**	**522.96± 211.81**	**33.43 ±6.63**	**272.81±82.15**	-	-
TDS pm	**402.9± 186**	**640.0 ±393.70**	**534.25±300.35**	**779.04± 245.08**	**183.75±39.29**	**151.56 ±34.23**	**165-500**	**≤ 100**
pH	**7.21± 0.18**	**7.51±0.34**	**7.34± 0.12**	**7.23 ± .36**	**7.20±0.22**	**7.3±0.19**	**6.5-8.5**	**6.5- 8.5**
DO ppm	**2.71 ± 2.28**	**2.98 ±2.44**	**2.22 ±1.99**	**3.14 ±1.50**	**0.48±0.09**	-	**6**	-
BODppm	**18.83 ±11.94**	**13.38 ±9.38**	**16.84±9.76**	**15.16 ±9.19**	**14.97±3.56**	-	**0.2**	-
CODppm	**57.77±19.34**	**51.87±6.64**	**51.67±10.93**	**54.38±6.29**	**59.28±23.92**	-	**4**	-
Alk ppm	**176.21±91.94**	**124.0±78.73**	**167.67±71.70**	**126.88±54.56**	-	**106.81±12.06**	**100**	**100**
Cl ppm	**21.20±10.26**	**15.66 ± 9.63**	**17.57±7.30**	**18.15±8.25**	-	**47.44 ±16.78**	**150- 600**	**250**
PO_4 ppm	**13.33±6.40**	**6.16 ± 6.27**	**6.55 ±7.06**	**10.06±3.81**	-	-	**6**	-
NO_3 ppm	**18.61±6.16**	**4.46 ± 4.89**	**7.04 ±5.97**	**4.20 ±2.82**	-	-	**10**	**50**
Cd ppm	**2.45 ± 1.64**	**1.57±1.70**	**2.31 ±3.64**	**2.09±1.38**	-	-	**0.005**	**0.003**
Cr ppm	**7.95± 7.93**	**1.74±3.15**	**3.82 ±5.06**	**2.54± 1.82**	-	-	**0.05**	**0.05**
Fe ppm	-	-	-	-	-	**0.42±0.3**	**0.3-1.0**	**0.3**
Mn ppm	-	-	-	-	-	**0.3±0.23**	**0.1**	**0.5**
Zn ppm	**1.56 ±0.55**	**0.77±0.64**	**0.82 ±0.41**	**0.70± 0.20**	-	-	**5**	**3**
FC mpn	-	-	-	-	-	**3.19±0.9**	**0**	**0**
TC mpn	**2069±466**	**2131±378**	**3440±1398**	**1869±504**	**763±305**	**0**	**0**	**0**

All average values are in ppm except EC (µs/cm), pH, TC (mpn), and FC (mpn).

Discussion:

The public health status of a nation stands as a pivotal indicator of socio-economic development. Furthermore, the cornerstone of public health primarily hinges upon the quality of drinking water. Human activities have significantly altered the natural environment to fulfill societal needs. Consequently, both soil and water are experiencing contamination, leading to adverse effects on various species such as fish, amphibians, reptiles, birds, and mammals, attributable to these developmental interventions.[1]

Dhaka City, classified as a mega city with a population exceeding ten million, is experiencing unprecedented urban growth. The demand for water in the city is escalating steadily, resulting in recurrent and severe water shortages, both in terms of quality and quantity. This study addresses the prospective requirements for water, encompassing considerations for both its quality and the requisite volume to meet the needs of the city's residents. Currently, the rivers in and around Dhaka City pose a significant environmental challenge, yielding direct and indirect consequences on various aspects of daily, personal, and social life. Notably, the detrimental effects of water pollution manifest prominently in human health, with direct repercussions on fishery resources. The adverse impact on aquatic flora and fauna is particularly pronounced, given their dependence on water bodies for crucial stages in their life cycles.

The Buriganga River exhibits an exceptionally high level of water pollution compared to other rivers. Within Dhaka City, 49% of various wastes are indiscriminately dumped into the Buriganga River. The water of this river contains toxic chemicals such as lead, arsenic, chromium, and cadmium, as reported by the Department of Environment in 1990.[2] Notably, the dissolved oxygen (DO) levels in the river vary significantly, with a maximum of 6.10 mg/l observed at the Bangladesh-China Friendship Bridge station during the post-monsoon season of 2009 and a minimum of 0.5 mg/l in winter. The river is considered biologically dead, and its water, with a maximum biological oxygen demand (BOD) of 45 mg/l (exceeding the Bangladesh standard of 30 mg/l), is unsuitable for various purposes including fish breeding, drinking, cooking, swimming, and other domestic uses. The critical DO level for the survival of aquatic life is 6 mg/L, and alarming readings of 0 mg/L were noted from Kamrangirchar to Pagla and at the Bangladesh-China Friendship Bridge point. In the Turag River and Tongi Khal, where numerous industries are concentrated in the Export Processing Zone (EPZ) area, effluents are discharged directly through numerous canals without any treatment. The rivers' water is tainted black and foul-smelling due to sewerage and domestic waste from approximately 800,000 inhabitants in the Tongi area. The rivers, resembling dead khals, witness the flow of substantial effluents, adversely impacting rice production.

The Balu River reports DO levels consistently below the desirable limit of 4 mg/l. Rahman and Hadiuzzaman (2005) identified Narai Khal as the primary source of ammonia in the Balu River system, characterized by very low DO and high Coliform values.[5] Hazardous levels of pollution were notably observed at Hazaribagh and Tongi Railway Bridge stations. During both lean (April-May) and high flow (July-August) periods, the DO levels were below acceptable critical levels, rendering the river water unsuitable for sustaining aquatic life. The winter season witnessed exceedances of permissible limits for DO, BOD, COD, and Coliform bacteria. Reduced DO levels led to elevated BOD and COD readings. The dry season (November-April) saw heightened concentrations of ammonia and algae in water intake, alongside concerns about high organic loading and the presence of toxic metals. Dhanmondi Lake, a water body within the city, faces pollution from sewerage and household garbage, with a bacterial count reaching 1200/ml. The Tejgaon washing and dyeing company discharge around 12,000 cubic liters of highly toxic wastewater into the lake, further contributing to its deterioration.[3] Phosphate pollution in rivers and lakes triggers algal blooms (eutrophication), diminishing water DO and disrupting the natural food chain. The water in Dhanmondi Lake has acquired a green hue.

Gulshan Lake, another water body in the city, is contaminated with garbage and sewage from surrounding areas like Gulshan, Baridhara, Kalachandpur, Nadda, and Shaorabazar. Consequently, the lake water emits a foul odor and exhibits a bluish color. The DO values range from 0.2 to 0.6, falling below standard values and rendering the water unsuitable for aquaculture.

Groundwater, traditionally considered a reliable source of drinking water, faces contamination risks from polluted surface water leaching.[6] Abandoned landfill sites, often in proximity to residential areas, contribute to this risk.[4] Chemical agents and contaminants leach into surface and groundwater, particularly from inorganic waste decomposition, agricultural pesticide residues, and industrial discharges.[7] The infiltration of residual fertilizers into soil with irrigation or rainwater exacerbates nitrogen pollution.[8] In Dhaka City, groundwater contamination by fecal coliform results from leaching from polluted surface water, poor tube well design, faulty construction, and management. The deteriorating capacity of groundwater to sustain both human and ecosystem needs is a significant concern.[9] The seepage of chemicals into shallow aquifers renders the water unfit for human consumption.[10] Of the 24 samples analyzed, 17 exceeded the World Health

Organization's recommended value for drinking water (0.01 mg/l), and two exceeded the Bangladesh guideline of 0.05 mg/l. Although we have identified certain similarities in our findings with those of other researchers, a more comprehensive examination underscores the unmistakable trend of ongoing deterioration in the water quality of the water bodies situated in and around Dhaka city. This concerning pattern suggests a pressing need for thorough investigation and targeted interventions to address the root causes of this decline and implement effective measures for water quality improvement.

Conclusion:

Water holds a central position in the fabric of human existence, representing the single most crucial natural resource essential for the sustenance and well-being of humanity. Recognizing the paramount significance of ensuring access to safe drinking water and safeguarding water reservoirs from contamination, the United Nations General Assembly, on 22nd March, designated this day as 'World Water Day.' Furthermore, the UN, acknowledging the imperative nature of addressing water-related challenges, declared the period from 2005 to 2015 as the "Water for Life" decade.

On the 4th of October 2009, the Ministry of Environment and Forest took a significant step by publishing a Gazette, officially declaring four rivers in the vicinity of Dhaka city as ecologically vulnerable. The pervasive issue of water pollution in Dhaka city accentuates the need for strategic interventions. The anticipated outcomes of ongoing research endeavors aim to provide valuable insights for the establishment of water-processing plants and comprehensive water management strategies.

Looking ahead, the projections indicate that the expansion of urban and rural areas, intensified land utilization, aquaculture practices, and the burgeoning population will necessitate increased consumption of water resources. Notably, during the dry season, water scarcity emerges as a substantial challenge in urban areas for domestic purposes and in rural areas for irrigation needs. The escalating extraction of groundwater contributes to the decline in aquifer water levels and the infiltration of contaminated groundwater from surface water sources.

Consequently, addressing the water-related challenges in Dhaka city requires a meticulous spatial assessment of surface water quality along the peripheral rivers. This assessment is indispensable for identifying viable solutions based on the judicious conjunctive use of both surface water and groundwater resources, ensuring the optimal utilization of available water reservoirs.

Recommendations

1. To improve the water quality of Dhaka city, it is essential to regularly clean solid and liquid wastes. Dumping wastes into rivers and lakes should be stopped, and waste dumping places should be replaced with suitable locations far away from water bodies that could not seepage water level.

2. To prevent the serious degradation of surface water quality and stabilize an effective ecosystem, waste effluent treatment should be established to remove pollutants for utilization and recycling of waste as much as possible.

3. To develop a state of knowledge and capability that will enable the country to design future water resource management, environment-friendly chemicals and machineries should be used in those industries, where as much as possible.

4. An effective plan is needed for river-side industrialization, urbanization, and slums.

5. The water qualities of the four studied rivers are deteriorating rapidly, which will not be suitable for potable water supply even after treatment. Therefore, the rivers Jamuna, Meghna, and Padma may be the potential sources for the water treatment plants to supply water.

6. Proper application of existing laws in protecting point sources of pollution should be ensured. In the case of non-point sources of water pollution, chemicals used in crops should be limited as little as possible by formulating and adopting legal measures and motivating city dwellers. Information technology, media, and mass communication should be improved to take effective measures for public awareness. Major agencies with activities in the water sector and those that have environmental cells and EIA should work cooperatively for large effective projects.

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Review article

The Interplay of Helicobacter pylori Virulence Factors and Host Immune Responses: Implications for Disease Management

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Abstract

Helicobacter pylori (H. pylori), which was first discovered in 1982 and is found all over the world, has been linked to gastritis, gastric ulcers, and duodenal ulcers. The prevalence of H. pylori infection exhibits significant global variation. This review meticulously explores the pathogenesis of *H. pylori* infection, unravelling the bacterium's strategies, including adherence to the gastric epithelium, mucosal colonization, disruption of intercellular junctions, and evasion of host immune defences. Virulence factors such as urease, phospholipases, adhesins, and the Cag pathogenicity island are scrutinized for their pivotal roles. It is looked at how the bacteria can colonize the stomach lining with the help of a group of unipolar flagella and how well they can break down mucin and affect the protection of the local lining. Virulence factors like urease, phospholipases, and adhesins are studied to see what role they play in infection. The *Cag* pathogenicity island *(CagPAI)* causes inflammation by releasing interleukin-8, which can lead to chronic gastritis, peptic ulcers, and a higher risk of gastric cancer. Host factors, encompassing immune responses and genetic polymorphisms, are explored for their influence on clinical outcomes. Notably, environmental factors like low socio-economic status, poor sanitation, and inadequate water supply contribute to *H. pylori* prevalence. Antioxidants, particularly vitamin C, are identified as protective, while tobacco smoking emerges as an additional etiological factor. In conclusion,this comprehensive analysis consolidates the most recent advancements in the examination of *H. pylori* virulence determinants, with a specific emphasis on their interactions with host responses and environmental variables. The insights provided are pivotal for advancing therapeutic strategies, to mitigate the global health impact of *H. pylori*-related diseases.

Keywords: Helicobacter pylori; Vaculating Cytotoxin A (VacA); Cag Pathogenicity Island, Host immune response

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1. Introduction

Helicobacter pylori, a Gram-negative bacterium characterized by its spiral shape with and a genome of around 1.65 Mb, that affects approximately 50% of the global human population. The cultivation of this bacteria was first conducted in 1982, leading to the later discovery of its role as the etiological factor in gastritis, as well as gastric and duodenal ulcers.[1] The prevalence of *Helicobacter pylori (H. pylori)* infection exhibits significant variationacross worldwide. Notably, Latin American have a high prevalence range of 75 to 83% whereas Japan and the United States exhibit comparatively

lower prevalence rates of 39.6% and 17.1%, respectively.[2] There is a well-established correlation between *Helicobacter pylori* infection and several gastrointestinal disorders, such as gastritis, peptic ulcer, duodenal ulcer, and gastric cancer. The complicated interaction between bacterial, host, and environmental variables mediates various illness and its consequences. The elucidation of the precise contribution of bacterial virulence factors to the pathogenesis of *Helicobacter pylori* will greatly enhance the development of vaccines and other therapeutic approaches.[3] This review is designed to clarify current advancements in the understanding of Helicobacter pylori virulence factors and their role in the etiology of related diseases.

2. Pathogenesis of *H. pylori infection*

H. pylori exhibits its pathogenic traits by (i) adhering to the gastric epithelium; (ii) colonizing the mucous gel layer, thereby enhancing permeability to hydrogen ions and pepsin; (iii) infiltrating and disrupting intercellular junctions; (iv) invading gastric glands and canaliculi of parietal cells; (v) evading the host's immune defenses; and (vi) releasing enzymes and generating cytotoxins.[4]

2.1 Colonization of the gastric mucosa

H. pylori usually colonizeas commensal in the stomach.[5] The mucosal barrier lining the stomach protects it from the harmful effects of gastric secretions and other substances, protecting the stomach's epithelial and it's deeper layers.[6] Tight cellular junction and the presence of a protective mucus layer preserve the integrity of the mucosal layer. Prostaglandin is a chemical messenger that protects the stomach lining by promoting mucus production, enhancing bicarbonate secretion, and improving blood flow.[7] *H. pylori* is an infectious agent that usually adheres only to the mucus-secreting cells of the stomach (except in Barrett's oesophagus and duodenal ulcers, where gastric mucosa replaces the epithelial layer), thrives in the acid environment of the stomach, and disrupts the mucosal barrier.[5,8] Motility of *H.pylori* within the gastric mucosa is aided by a bundle of unipolar flagella that possess a sheath to prevent depolymerization in an acidic environment. The organism remains mainly in the mucus, while a subpopulation adheres to specific receptors of gastric epithelial cells.[5,9] *H. pylori* degrades mucin and has the capacity to interfere with the local protection of the mucosa against gastric acid. It also may produce toxins that directly damage the mucosa and produce ulceration in other ways.[10] *H. pylori* has evolved several mechanisms to evade primary host defenses such as acidity and peristalsis causes persistent infection within the stomach.[11] This organism elaborates a number of enzymes such as urease, catalase, oxidase, hydrogenase etc. Catalase helps the organism to survive in the host by preventing the formation of oxygen metabolites from hydrogen peroxide in neutrophils.[8,12]

2.2 Virulence factors

a) Urease

Urease is an important virulence factor for *H. pylori* which metabolizes urea producing ammonia. Thus, the pathogen can successfully survive in the gastric lumen (pH 1-2) for a short period time and it penetrates into the mucus layer of stomach with bicarbonate-buffered, its real habitat.[5] This enzyme may assimilate organic nitrogen due to its cytoplasmatic urease activity. Ammonia may affect stomach mucosa and epithelial permeability. Urease also activates mononuclear phagocytes and produces inflammatory cytokines.[13,14]

b) Phospholipases

H. pylori phospholipases induce generation of productssuch as lysolecithin which disrupt the protective phospholipid rich layer on the apical membrane of mucus cells.[13]

c) Neutrophil activating protein

*H. pylor*i activate neutrophils and increase their adhesion to endothelial cells by expressing a 150 kDa activating protein (Hp-Nap) with 10 identical subunits from the *napA* gene.The purified protein activates neutrophils dose-dependently to produce oxygen free radicals and attach to endothelium cells, becoming Hp-Nap pathogenic. Additional information is required before Hp-Nap is considered an important bacterial virulence component.[15]

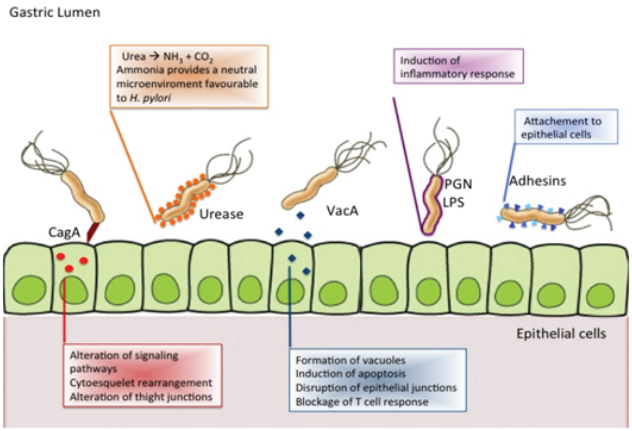

Figure 1: Virulence factors of *H. pylori*[16]

d) Adhesins

*H. pylor*i adheres to stomach epithelial receptors using adhesins. Several specific receptors are also involved including lipids, gangliosides and sulfated carbohydrates, and other different types of adhesins such as SabA, OlpA, A1pA, and A1pB, including the BabA2; outer membrane protein, which is encoded by the bab (blood group antigen binding) genes[17,18]

e) IceA (induced by contact with epithelium)

The gene encoding *IceA* has been identified in isolates from patients with peptic ulcer, independently of the *vacA* and *cagA* genotype.[19] The expression *ofIceA* is induced by adherence of *H. pylori* to gastric epithelium. DNA sequencing has revealed the presence of two families: *IceA*1 and *IceA*2. Strains with the *IceA1* gene are most frequently associated with peptic ulceration and increase the production of IL-8 .[20]

f) Vaculating Cytotoxin A (VacA)

VacA is an oligomeric toxin with 87 kDa active subunits from low pH treatment. Antiserum against these proteins neutralizes the protein's cytotoxicity. It causes vacuolar degeneration of target cells by interfering with intracellular membrane fusion.[21] The vacuolation mechanism involves the stimulation of adenosine tri phosphate dependent proton pump and of a small GTPase. vacA induces an mitochondrial damage, leading to impairment of the gastric epithelial cells cycle.[22] Mosaicism in vacA alleles is expressed by vacA subtypes of which three concern signal sequence regions *(s1a, s1b and s2)* and two middle region motifs *(m1 and m2)*.[23,24] The s1a strains produce higher levels of cytotoxin with more severe gastric inflammation and duodenal ulceration than the other two allelic sub types. The *m1* middle region allele is more frequently associated with a higher level of gastric damage as compared with the *m2* form. Virulence may be assessed by vacuolating cytotoxin activity, *VacA* serology, and genotyping. A non-invasive test like *vacA* serology would be better, *vacA* signal sequence type better indicates peptic ulceration. Infection with *vacA sla* strains causes peptic ulceration more than *sib* strains. For determination of *vacA* genotyping requires stomach biopsy, that's why *vacA* genotyping cannot be used in non-invasive screening strategies. It is currently regarded as a best research tool.[25]

g) Cag Pathogenicity Island

Helicobacter pylori *(H. pylori)* strains from gastric epithelium are classified as type I and type II. Type I is associated with severe diseases due to a specific genomic locus, the *Cag* pathogenicity island *(CagPAI)*, inducing inflammation.[26,27] The *CagPAI*, containing 31 genes, triggers the release of inflammatory chemokine (IL-8), leading to neutrophil infiltration. Type I strains, with the *CagPAI*, are linked to chronic gastritis, peptic ulcers, and an increased risk of gastric cancer.[25,28] The *Cag*A gene, located in *CagPAI*, encodes a 120 kDa immunodominant antigen associated with cytotoxin expression. *Cag*A-positive *H. pylori* infections have been linked to food allergies. The presence of both *Cag*-positive and *Cag*-negative strains in a patient suggests a dynamic balance affecting disease expression.[4] *Cag*A serology, indicating *Cag*A presence, is a useful virulence marker. However, treating only *Cag*A-positive individuals may lead to unnecessary treatment, considering the common occurrence of CagA-positive strains. Treating all *H. pylori* infections could be an alternative, but it poses challenges like expense, side effects, and antibiotic resistance concerns.[25]

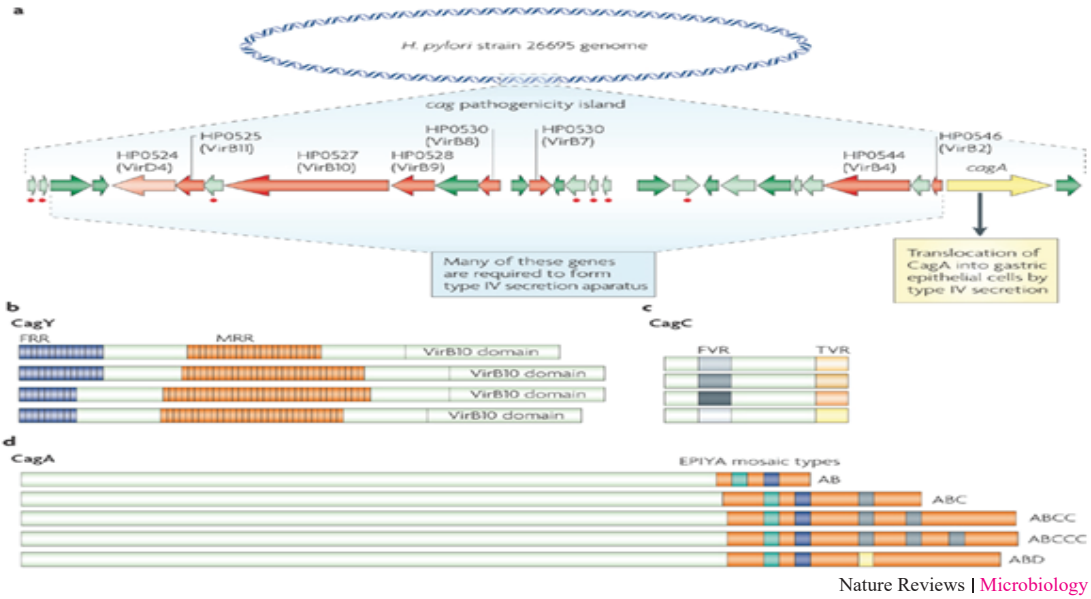

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Figure 2:Arrangement of cag PAI genes in *H. pylori* strain 26695[29]

[Most of the cag genes may be involved in the type IV secretion pathway that transports cagA into gastric epithelial cell cytoplasm. Seven red genes resemble type IV secretion system components. The island encodes proteins that induce gastric epithelial cells to produce IL-8 and translocate *cagA* from the bacteria to host cells. All genes with arrows in dark red and green are necessary for IL-8 induction, whereas those with lighter colors are not. The non-marked genes are important for *cagA* translocation, but the red-dotted arrows are not. b–d]

h) Direct neutrophil activation by H. pylori

H. pylori not only trigger the production of the cytokine IL-8 in epithelial cells but also directly activate neutrophils. There are two types of *H. pylori* based on the strength of this activation. Approximately 50% of strains induce a quick and robust oxidative burst in neutrophils more prevalent in patients with peptic ulcer, while the remaining 50% induce a slower and weaker burst.[30]

i) Duodenal ulcer promoting gene (dupA)

Duodenal Ulcer Promoting Gene (*dup*A) in the *H. pylori* genome's plasticity zone may indicate virulence marker. Lu et al. (2005)[31]found that *dup*A-positive strains increased duodenal ulcer risk and decreased stomach cancer risk in Colombia, South Korea, and Japan. Later studies in Belgium, South Africa, China, and the US revealed no relationship between *dup*A expression and duodenal ulcers but did uncover a link to stomach cancer.[32] Japanese and Swedish strains had no association between *dup*A expression and stomach cancer or duodenal ulcers, but Chinese strains did.[33] *dup*A may cause duodenal ulceration and stomach cancer in some people but not all.

2.3 Host factors responsible for *H. pylori* infection

Host-related factors play a crucial role in determining the clinical outcome of *H. pylori* infections, particularly those influencing the growth of the bacterium in the stomach. One significant factor is the variation in gastric acid production among individuals, which can impact the growth and localization of *H. pylori* in the stomach mucosa, as well as influence the growth of metaplastic gastric tissue in the duodenum, which provides additional niche for *H. pylori*. Additionally, polymorphisms in the receptors on gastric epithelial cells to which *H. pylori* adheres, or variations in the composition of gastric mucous, may also be key determinants. Gender is another factor, with *H. pylori* infection being more prevalent in men aged 20 to 39 years compared to women, suggesting a gender-specific influence on infection rates.[34,35]

a) Host immune response

The immune response to *H. pylori* is crucial due to concerns about potential damage to host tissues from leukocyte products. Additionally, there is interest in exploring immunization possibilities against *H. pylori.* The ideal host response should effectively clear the infection without causing excessive inflammation. However, evidence, including studies by Suarez et al. (2006)[36] and Tanih et al. (2010)[37], indicates that the immune response may contribute to *H. pylori* pathogenesis. Instead of eliminating the bacteria, the immune response can lead to the destruction of epithelial cells and thinning of the mucosal lining, increasing mucosal contact with luminal acid.[38,39]

b) Innate immune response to *H. pylori*

The immune-pathogenesis of *H. pylori* involves the up-regulation of various genes associated with the innate immune system, including Toll-like receptors (TLRs), complement factor C3, lactoferrin, and bactericidal/ permeability-increasing protein.[9] TLRs, particularly TLR4, play a crucial role in recognizing bacterial antigenic molecules and are expressed by various cell types in the gastrointestinal tract. Activation of TLRs, often through bacterial lipopolysaccharide (LPS) signaling pathways, leads to NF-KB activation and the expression of pro-inflammatory genes. This process is evident in antigen-presenting cells like monocytes and dendritic cells. Contact with *H. pylori* induces the secretion of pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-8, which act as local chemo-attractants and promoting granulocytic infiltration.[39]

c) Adaptive immunity

i) Cellular immune response

After innate immune responses failed to remove *H. pylori,* adaptive immune responses evolved. Polymorph nuclear leukocytes (PMN), T lymphocytes, macrophages, and plasma cells infiltrate the stomach during *H. pylori* inflammation. *Helicobacter pylori* attaches to stomach mucosal cells and releases chemicals that change their function.[40,41] Chronic active gastritis increases the CD4/CD8 T-cell ratio and accumulates CD4+ T-helper cells in the lamina propia.[42] Numerous studies reveal a polarized T helper cell response to *H. pylori,* with CD4+ T cells in infected patients producing Th1 cytokines (lL-12, IFN-γ, and TNF) but not Th2 cytokines (IL-4).[43,44] Gastric epithelium and activated macrophages generate cytokines, especially IL-8. While presenting *H. pylori* antigens to particular T cells in the gastric antrurn, antigen presentation cells also produce cytokines such IL-I, lL-6, TNF-α, and IL-12, which significantly impact the developing T-cell response.[43] Th17 CD4+ T cells, which cause infections and inflammation, also invade the stomach mucosa. Th17 are generated in gastric stroma during *H. pylori* infection and gastric cancer, suggesting an association between inflammation and carcinogenesis.[45]

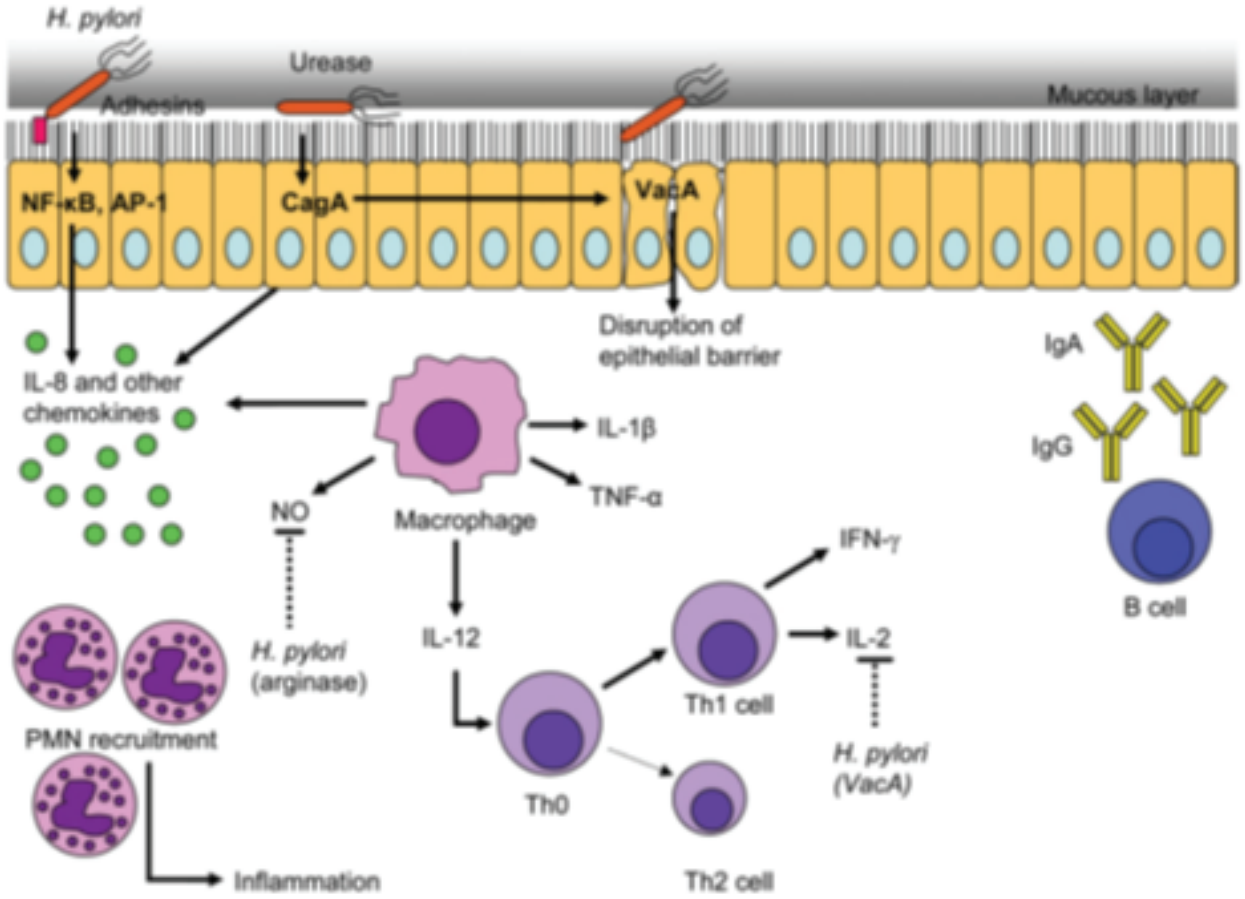

Figure 3: *H. pylori* pathogenesis, the inflammatory immune response and some of escape mechanisms[46]

During *H. pylori* infection gastric B cells detected which are often auto-reactive that indicate it leads to the development of gastric autoimmunity.In uncomplicated chronic gastritis and gastric MALTomas most of gastric *H. pylori*-specific T cells showed combined secretion of both Th1- and Th2-type cytokines.[47]

Some infection with *H. pylori* elicits Th2 instead of Th1 immune responses, which could possibly change the *H.pylori*-induced protective immune response to the gastric mucosa.Reports have indicated that Th2 response may provide protection against gastric cancer.[46]

ii) Humoral response

In response to H. pylori infection, individuals develop a robust antibody response. Both systemic and local antibodies are produced, including IgA and IgG. IgA, produced by plasma cells in the gastric mucosa, plays a crucial role in neutralizing urease and *VacA*, inhibiting *H. pylori* adherence to the gastric mucosa.[48–50] IgG antibodies enhance phagocytosis of *H. pylori,* and their binding triggers complement activation through classical or alternative pathways. This multifaceted antibody response is vital in the immune defense against *H. pylori* infection.[51]

iii) Role of host cytokine gene polymorphism in H. pylori induced gastric pathology

Host genetic factors, particularly cytokine gene polymorphisms, play a significant role in the pathology of gastro-duodenal diseases. These polymorphisms can affect the secretion of cytokines, influencing the magnitude of the immune response and contributing to individual clinical outcomes. The interaction between *H. pylori* and host cells induces genetic alterations, promoting gastric carcinoma development.[52]

Deoxyribonucleic acid (DNA) sequences of the human genome reveal that many genes are polymorphic. In the coding or non-coding regions of specific genes, there may be either a single base pair substitution or a variable number of tandem repeats (VNTR) of a short repetitive DNA sequence. These variations or polymorphisms may influence the rate of gene transcription, the stability of the messenger ribonucleic acid (mRNA) or the quantity and activity of the resulting protein. Thus, the susceptibility or

severities of a number of disorders are influenced by possession of specific alleles of polymorphic genes.[53]
Cytokine gene polymorphisms have recently attracted considerable interest since it has been discovered that different alleles of cytokine genes are associated with different immunomodulatory diseases. It directly influence interindividual variation in the magnitude of cytokine response, and this clearly contributes to an individual's ultimate clinical outcome.[54] Genes encoding cytokines and related molecules harbor polymorphic regions, which are considered to alter gene transcription and thereby influence inflammatory processes in response to infectious disease.[53]

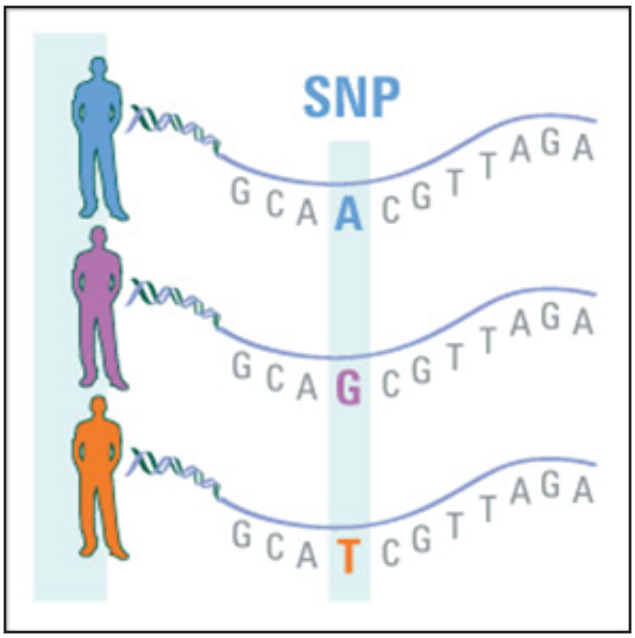

Figure 4: Single Nucleotide polymorphism

In several circumstances, chemokines may indicate blood cell migration. The main mediators of granulocyte accumulation are C-X-C chemokines. In *H. pylori*-associated gastritis stomach biopsy samples, the chemokine interleukin-8 (IL-8) is increased.[55] IL-8 has a key function in *H. pylori*-induced diseases. Neutrophils and lymphocytes are strongly attracted by it. It affects cell proliferation, migration, and tumor angiogenesis.[56] Transepithelial signal transmission is thought to initiate the inflammatory response in *H. pylori*-associated gastritis since the bacterium is noninvasive. Chemokines may induce second signals when bacteria are attached to the epithelium, among the various cytokines that may be generated by bacterial infection and cause pathologic alterations in inflammation. Thus, it is not unexpected that multiple studies have identified IL-8 as a key modulator of *H. pylori*-associated gastritis..[57,58] High IL-8 levels are related to *H. pylori*-associated gastritis. A particular IL-8 gene polymorphism (-251 T/A) increases IL-8 production, severe inflammation, and precancerous stomach anomalies. The polymorphism is associated to *H. pylori*, peptic ulcer disease, and chronic gastritis. Some studies relate IL-8 polymorphisms to stomach cancer susceptibility, but outcomes vary by demographic and geography. In conclusion, host genetic variables, notably cytokine gene polymorphisms, aggravate *H. pylori* infection and gastroduodenal disorders.[59]

2.5.4 Environmental factors

Low-income people with poor sanitation, congested living circumstances, and limited water supply often have *H. pylori* infection. An essential antioxidant, ascorbic acid scavenges reactive oxygen species and suppresses N nitrosation. Infection incidence decreases with antioxidant micronutrient consumption, especially vitamin C.Consuming more fruits and vegetables reduces duodenal ulcer risk. Smoking and having a family history increase the incidence of peptic ulcers, especially in males.[60] Low-income nations with unhygienic lifestyle had greater sero-conversion rates and higher reinfection rates following *H. pylori* eradication.[61]

Conclusion

Helicobacter pylori, a globally prevalent Gram-negative bacterium, plays a pivotal role in various gastrointestinal disorders. This review provides the pathogenesis of *H. pylori* infection, emphasizing the significance of virulence factors, host immune responses, and environmental factors. The interplay of bacterial adherence, colonization, immune activation, and genetic polymorphisms underscores the complexity of disease outcomes. Understanding these multifaceted interactions is crucial for advancing therapeutic strategies, including vaccine development, to mitigate the impact of *H.pylori*-related diseases globally.

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Case report

Revealing Weak D: A Unique Exploration at a Hospital in Bangladesh

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Abstract

The weak D phenotype is a variant of the Rh blood group system, specifically related to the D antigen. Individuals with the weak D phenotype exhibit reduced expression of the D antigen on their red blood cells, making it challenging to detect using standard blood typing methods. This case report brings attention to an extraordinary instance involving a 45-year-old male who was serendipitously identified as possessing the weak D phenotype, a condition not only uncommon in Bangladesh but also exceptionally rare on a global scale.

Key word: Weak D, partial D, Rh type

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Introduction

The Rh blood group system is a critical component in transfusion medicine and clinical assessment that has gained increased attention due to its intricacies, especially the phenomenon of weak D variants. In 1946, the weakly reacting D antigen (DU antigen) was delineated by Stratton.[1] These cells possess the D antigen but have fewer D antigen sites per red cell than normal Rh-positive cells.[2] This distinct feature makes the weak D antigen less immunogenic, presenting difficulties in its identification.[3] Groundbreaking work in 1948 by Race et al. and in 1950 by Renton and Stratton discovered that DU red cells did not agglutinate directly with anti-Rh (D) serum. Instead, the presence of this antigen was revealed only after the subsequent addition of anti-globulin.[4] The prevalence of this weak D antigen varies between 0.2% and 1% among Caucasians,[5] while in India, the estimated occurrence of weak D ranges from 0.0075% to 0.2% within diverse geographic donor populations.6 A multicenter study reported that the prevalence of Weak D among the Bangladeshi population is 0.19%.[7] Despite its rarity, the significance of Weak D lies in its potential ramifications for blood transfusions, urging careful consideration in clinical practices.

Case Summary

Admitted to our hospital, Mr. Ahsan (pseudonym), a 45-year-old gentleman, expressed concerns about fever and generalized body aches persisting over a 4-5 day period. The fever manifested suddenly, characterized by a continuous pattern. His medical history notably revealed him as a documented case of type 2 diabetes mellitus being managed with medication (Tab. Metformin 500 mg once daily). He was free from symptoms like diarrhea, vomiting, rash, or any other complications. Moreover, he didn't have hypertension or bronchial asthma. During the general examination, the patient exhibited a heightened body temperature of 102°F. Furthermore, moderate anemia, mild

dehydration, joint tenderness, and subtle flushing of the face were noted. Systemic examination revealed no evident abnormalities.

His Dengue NS1 antigen tested positive. His platelet count registered at 202,000/uL, only to precipitously drop to a mere 23,000/uL after two days. Recognizing the urgency, we recommended a comprehensive blood grouping and Rh typing test.

In managing Mr. Ahsan, a patient of admirable cooperation, a discrepancy emerged in his blood grouping and Rh typing report, initially identified as B-negative, conflicting with his documented B-positive status on his Saudi Arabian driving license. Upon retesting and further consultation with the Department of Transfusion Medicine, an unexpected and rare weak D phenotype was unveiled.

ABO grouping of the patient's sample (slide method):

Cell grouping		Serum Grouping		Interpretation
Anti A	Anti B	A cell	B cell	
-	4+	3+	-	B

RhD grouping of the patient's sample (slide method):

Anti D (Tulip)	Anti D (Biorex)	Interpretation
-	-	RhD Negative

So initially, the patient's blood group was reported as B negative.

Due to the patient's historical RhD positivity, we conducted further investigations. Immediate centrifugation with one drop of anti-D (Tulip) mixed with a 5% suspension of the patient's red cells in a test tube resulted in a 'w+' reaction (barely visible agglutination, turbid background). Subsequently, the same test tube was incubated at 37°C for 15 minutes. After incubation, the red cells were washed three times with normal saline. Following the last wash, 2 drops of Anti-Human Globulin (Atlas) were added, mixed gently, and centrifuged at 1000 rpm for 30 seconds. Gentle resuspension of the cell button revealed 2+ agglutination. The ultimate interpretation regarding RhD status was B Positive (Weak D).

Discussion

The Rhesus blood group system encompasses more than 50 antigens.[8] 'D' stands out as the most immunogenic antigen among these.[9] The presence of this antigen on red blood cells is denoted as 'Rh(D) positive' or simply 'Positive'. This antigen is mainly a transmembrane protein, containing 417 amino acids.[10] The entire RhD protein must be present for the D antigen to be expressed serologically, otherwise it is termed weak D.[11] This phenotype can manifest through distinct mechanisms. Through genetic inheritance, an individual acquires the RHD gene that codes for the weak expression, resulting in a mutation. This mutation impacts the amino acids within the transmembrane or intracellular regions of the RhD protein. So these changes in the protein occur only 'inside' the cell membrane rather than externally. Consequently, the number of expressed D antigens on the red blood cell surface is diminished, although the D antigen is usually complete. This genetic alteration gives rise to the Weak D phenotype.There is anothermechanism called "position effect" or "gene interaction effect"where the allele carrying RhD is in the 'trans' (opposite haplotype) position to the allele carrying C. (Such as in Dce/dCe haplotype). Despite the Rh antigen appearing normal, this arrangement impedes the proper expression of the D antigen on the cell membrane.[12,13] Partial D is another aspect. Typically, RhD encompasses more than 30 epitopes, and for an individual to be RhD-positive, all these epitopes must be expressed. The alteration or absence of some epitopes on the D antigen gives rise to the partial D phenotype.[14] Unlike weak D, these changes occur in extracellular regions.[15]

Observational studies from central Europe reported that individuals with weak D types 1, 2, 3, and 4.1 are not prone to developing anti-D and can be safely regarded as RhD-positive, making them suitable for transfusion with RhD-positive blood.[16,17] However, alloimmunization has been noted when RhD-positive units are transfused in specific weak D types, such as weak D types 4.2, 11, and 15 but it is rare.[17,18] Additionally, there have been several reports of RhD-positive individuals presumed to have a partial D phenotype forming anti-D antibodies.[19,20]

The preceding discussion highlights that when weak D results from a genetic mechanism or position effect, the alterations are confined to the interior of the red blood cell (RBC). This primarily impacts the quantity, not the quality, of RhD. Consequently, the likelihood of developing anti-D antibodies is low, allowing the individual to safely receive D-positive blood in most cases. Conversely, with partial D, the changes occur externally to the RBC, influencing the quality rather than the quantity.[21] This poses a risk of producing anti-D antibodies, potentially leading to complications such as Hemolytic Disease of the Fetus & and the Newborn or transfusion reactions.[12,13] Therefore, individuals with partial D shouldn't receive D-positive blood. Unfortunately, the challenge lies in the absence of a serological procedure to differentiate between weak D and partial D variants. This situation raises a critical question about the approach to blood transfusion for individuals with D variants, particularly when the distinction between weak D and partial D cannot be made. It is advisable to transfuse individuals with a D variant with RhD-negative blood for safety.[22] While these individuals have the 'D' antigen in their red blood cells, caution is recommended to prevent them from donating blood to RhD-negative individuals.[23] In the

laboratory, the 'D' antigen is identified using anti-D reagents. Typically, we expect a robust 3+ or 4+ reactions visible to the naked eye.[24] But in instances of weak D expression, the red blood cell agglutination is milder (≤2+) than anticipated for RhD typing or no reaction at all with potent anti-D reagents.[7] Approximate identification of the D antigen is achieved through moderate or strong agglutination observed in the indirect anti-globulin test (D^U test) after incubation with anti-D[25]. Despite the passage of numerous years since the discovery of the weak D antigen, its clinical significance, immunogenicity, and associated guidelines remain controversial.[26,27] RHD genotyping offers a more comprehensive characterization of these D variants.[28] As genotyping is unavailable in our center; we rely on the antihuman globulin test to label a sample as weak D. And to ensure patient safety, we still follow the policy of considering all weak D-positive donors as RhD-positive.

Conclusion

The discovery of a rare Weak D blood group emphasizes the imperative for accuracy in blood grouping tests, highlighting the risk of diagnostic oversights. Meticulous lab practices and rigorous protocols are essential for accurate clinical diagnoses. Specifically, taking extra caution in suspecting Weak D is crucial, as misdiagnosis is imminent without careful consideration.

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Information for the Contributors/Authors

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2. Review articles
3. Case reports

After receiving the manuscript, it will be **checked for plagiarism**. If the manuscript contains >10% plagiarized content, it will be sent back to theauthor for correction. We shall contact the author by email. After passing the plagiarism check, we shall send it to our experts for **peer review**. Reviewers will review it and contact the author for correction/revision if required. After completion of the review, it will be **accepted** for **publication**. Submitted articles will be published usually within 9 months of submission.Both online version and hard copies will be published. Authors will be informed soon after the publication.

Typing rules:

Language	: English
Font	: Times New Roman
Font size	: 10
Line spacing	: 1.5
Paper size	: A4
Margins	: 1 inch at all sides

SStandard **abbreviations** may be used. Avoid abbreviations in the title and abstract. However, the full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Statistical procedure should be described in the material and methods section and supported by reference to enable a knowledgeable reader with access to the original data to verify the reported results.

Tables and illustrations should be separate from the text of the paper. Tables should be simple and should not duplicate the text of the article. Do not use internal horizontal and vertical rules. The uses of too many tables are not encouraged.

References must be in the **Vancouver** style and must be verified by the author(s) against the original documents before submission. They should be numbered consecutively in the order in which they are first mentioned in the text.

The Editor reserves the customary right to **style** and if necessary shortens the material accepted for publication and to determine the priority and time of publication. Editor assumes that work based on honest observations. It is not a task of the editor to investigate scientific fraud paper. For any information about Journal mail to **article.admj@gmail.com**

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The following limits are applicable for the submitted articles:

Original articles	Abstract	Up to 300 words. Consisting of 4 subheadings: 1. Background & objectives 2. Methodology 3. Result 4. Conclusion
	Main text	Up to 4000 words (including tables and figures)
	Figures and tables	Up to 6
	References	Up to 60
Review articles	Abstract	Up to 300 words (1 paragraph with no subheading)
	Main text	Up to 7000 words
	Figures and tables	Up to6
	References	Up to 100
Case reports	Abstract	Up to 250 words (1 paragraph with no subheading)
	Introduction, case summery, discussion, conclusion &references	Up to 2000 words
	Figures and tables	Up to6
Editorial	Several paragraphs with no subheadings	Up to 1500 words Up to30 references 1 figure or table allowed

Ad-din Medical Journal
January 2024, 2 (1):51

Sample of forwarding letter

To
The Executive Editor
Ad-din Medical Journal
Bashundhara Ad-din Medical College, Dhaka

Subject: Formal Manuscript Submission for Ad-din Medical Journal

Dear Sir,

We submit our manuscript for potential publication in the upcoming Ad-din Medical Journal issue. We affirm its exclusivity and confirm thorough author approval. Each author takes responsibility for accuracy, showing commitment to financial and legal implications.

We assure the Editorial Panel of prompt provision of research data upon request for thorough evaluation by the reviewer panel or editorial team. The undersigned authors, listed below, declare authorship adherence to specified criteria. We guarantee no contestation from individuals not listed.

Thank you for your consideration.

Sincerely,

Sequence	**Name of the author**	**Signature**	**Authorship criteria** (please encircle)
1			*A B C D*
2			
3			
4			
5			
6			
7			
8			

Authorship criteria:

A	Substantial contribution to conception or design of the work, or acquisition, analysis or interpretation of data for the work.
B	Drafting the work or revising it critically for important intellectual content.
C	Final approval of the version to be published.
D	Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Title of the article:			
Type:	□ Original article	□ Review article	□ Case report