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Nipah Virus Infection: Bangladesh Perspective

*Akhter M

Nipah virus (NiV) is a member of the genus *Henipavirus* in the family *Paramyxoviridae*. NiV infection is a fatal zoonotic disease transmitted to humans through infected animals (such as bats), or food (raw date palm sap, fruits) contaminated with saliva, urine, and excreta of infected animals. It can also be transmitted directly from person to person through close contacts.¹ Fruit bats (*Pteropus species*) are the natural hosts for NiV. The virus was first identified during an outbreak of encephalitis among pig farmers in Malaysia and Singapore in 1998 to 1999. Nipah virus was named after 'Kupmung Sungai Nipah' a village in Malaysia where the virus was isolated first. Since then sporadic outbreaks have been reported in South and Southeast Asia particularly in Bangladesh and India.^{2,3,4}

Bangladesh is one of the major endemic zone of NiV infections since the first outbreak in 2001. After that clusters of cases are found almost every year, especially during the winter months when raw date palm sap is widely available throughout the rural Bangladesh. We have noticed several hundred cases with a case fatality rate (CFR) exceeding 70%.⁵ Unlike the Malaysian outbreaks, which was associated with pigs as intermediate hosts, outbreaks in Bangladesh involve direct bat-to-human transmission and occasional person-to-person transmission, particularly in household and healthcare facilities.⁶

The incubation period of NiV infection is 3 to 14 days and in rare cases even up to 45 days. The clinical spectrum of NiV infection ranges from asymptomatic infection to rapidly progressive fatal disease and even death. Primarily there are nonspecific symptoms which include fever, headache, myalgia, dizziness, and vomiting. As

the disease progresses patients may develop altered consciousness, seizures, and neurological deficits (Acute Encephalitis). Respiratory manifestations such as cough, dyspnea, and acute respiratory distress syndrome are also common in the Bangladesh strain, making the clinical presentation somewhat different from previous Malaysian outbreaks.⁷ Many cases may deteriorate rapidly, progressing to coma within 1 to 2 days, and death often occurs within a week.⁸

Laboratory diagnosis of a patient with a clinical history of NiV infection is made by using a combination of tests. The main tests used are real-time polymerase chain reaction (RT-PCR) from body fluids and detection of IgM or IgG antibodies via ELISA. These tests are done during the acute and convalescent phases of the disease.⁹ Confirmation of diagnosis by laboratory tests is essential because the clinical features may mimic other viral encephalitis or severe respiratory infections.

There is no specific and effective antiviral therapy or licensed vaccine for NiV infection. Management is supportive which include intensive care, control of seizure and maintenance of airway and mechanical ventilation for respiratory failure. Some experimental therapies and monoclonal antibodies are being investigated, but they are not widely available for clinical use.⁸

The prognosis remains poor, with CFR ranging from 40% to 75%.¹⁰ Survivors may suffer from long-term neurological consequence, including persistent seizure or personality changes.¹¹

The epidemic potential of NiV remains a constant global health concern. NiV has been classified as a 'priority pathogen' by international health organizations because of its high mortality,

potential for human to human transmission, and absence of effective treatment or vaccine.^{9,10} Although sustained community transmission has not yet been observed, the possibility of viral evolution and wider spread cannot be ignored.

Preventive strategies are therefore important, particularly in endemic areas like Bangladesh. Public health measures include avoiding of raw date palm sap drinking, using physical barriers such as bamboo skirts and nets to prevent bats from contaminating sap collection pots, strengthening surveillance systems, and ensuring strict infection-control practices in healthcare settings.^{1,12} Community awareness campaigns, early case detection, and rapid outbreak response remain essential components of Niv disease control.

Nipah virus infection is a persistent public health challenge in Bangladesh. Continued vigilance and surveillance, public education, and patronizing research are essential to reduce the risk of future outbreaks and to develop effective therapeutic and preventive strategies against this notorious infection.

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Effect of Physical Activity on Anthropometric and Cardiovascular Parameters

*Ghosh E¹, Biswas P K², Sen S³, Shimi S⁴, Jeba J T⁵

Abstract

Background: Increasing sedentary lifestyle combined with the growing use of technology in daily life is causing higher levels of physical inactivity among persons of all ages, both in developed and developing countries. Therefore, this study was undertaken to analyze the differences in certain anthropometric and cardiovascular parameters in sedentary and non-sedentary workers. Cardiovascular disease (CVD) is one of the most common non communicable diseases which cause mortality and morbidity in both developed and developing countries. Although there are many other causes of cardiovascular diseases, sedentary life style is a notable cause of CVD. **Objectives:** The aim of the study was to assess the effect of physical activity on anthropometric parameters like weight, height, body mass index (BMI), waist circumference (WC), hip circumference (HC) and waist to hip ratio (WHR) as well as pulse, blood pressure among sedentary and non-sedentary workers. **Materials and Methods:** Total 60 healthy male subjects were selected randomly from the general population of Rajshahi city from January 2016 to December 2016. Out of which 30 were sedentary and 30 were non-sedentary subjects. Anthropometric parameters such as weight, height, BMI, WC and HC, WHR were assessed. Cardiovascular parameters such as pulse rate, blood pressure were also assessed. **Results:** In our study, there was statistically significant increase in anthropometric and cardiovascular parameters in sedentary subjects compared to non-sedentary subjects. **Conclusion:** This review provides clear evidence that low levels of physical activity are associated with an increased risk of weight gain and significant increase in blood pressure. Although our study is by no means exhaustive, it provides a glimpse into the variety of adaptations or alterations in anthropometric parameters that occurs due to sedentary life style, even in the absence of overt disease.

Keywords: Physical activity, Sedentary workers, Non-sedentary workers, Cardiovascular diseases.

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Introduction

Anthropometry is the study of the measurement of the human body in terms of the dimensions of bone, muscle, and adipose (fat) tissue. The lack of physical activity is a major underlying cause of death, disease, and disability. Preliminary data from a WHO study on risk factors suggest that inactivity, or sedentary lifestyle, is one of the 10 leading global causes of death and disability. More than two million deaths each year are attributable to physical inactivity. In countries around the world between 60% and 85% of adults are simply not active enough to benefit their health. Seden-

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tary lifestyles increase all causes of mortality, double the risk of cardiovascular diseases, diabetes, and obesity, and substantially increase the risks of colon cancer, high blood pressure, osteoporosis, depression and anxiety.¹ The etiology of overweight and obesity is clearly multifactorial, but ultimately it is determined by the long-term balance between energy intake and expenditure. This review will consider the effects on body weight and the risk of obesity of sedentary lifestyles, within the context of dietary habits.² A sedentary life style includes less than 150 minutes of moderate physical activity or less than 60 minutes of vigorous physical activity per week.³ Obesity can be defined as a state of excess adipose tissue mass.⁴ Currently over weight and obesity are classified by Body Mass Index (BMI) [weight in kilogram/square of the height in meter-kg/m²]. In adults, overweight is defined as BMI of 25.0 to 29.9 kg/m²; obesity is defined as BMI $\geq$ 30 kg/m².⁵ The results of extensive research programs lead to the conclusion that physical activity increases

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longevity, to a large extent protect against development of the major non communicable diseases such as coronary heart disease, hypertension, stroke, non-insulin dependent diabetes mellitus, osteoporosis and colon cancer.⁶ Hypertensive heart disease was present in light workers five times more frequent than in heavy workers and obese individuals tend to be more inactive physically.⁷ There is increased risk of metabolic complications for men with waist circumference ≥ 102 cm and women with a waist circumference ≥ 88 cm.⁸ Physical activity may be a critical target for the specific prevention of visceral fat accumulation and corresponding health risk in obese subjects.⁹ Visceral fat is more metabolically active than subcutaneous fat and hence may be more deleterious to health.¹⁰ Avoiding a sedentary lifestyle during adulthood not only prevents cardiovascular disease independent of other risk factors but also substantially expands the total life expectancy and the cardiovascular disease-free life expectancy for men and women.¹¹

Materials and Methods

The present study was conducted in the department of Physiology of Rajshahi Medical College, Rajshahi from January 2016 to December 2016. The study was undertaken to analyze the differences in certain parameters in healthy sedentary and non-sedentary male subjects. 30 sedentary subjects and 30 healthy non-sedentary subjects were selected from the general population of Rajshahi city randomly. All the subjects gave consent after explaining the procedure of the non-invasive technique to them. A brief personal history, childhood obesity, detailed history of exercise and a clinical examination of all the systems were done to exclude medical problems and to prevent confounding of results. Anthropometric parameters were measured in the morning session. Physical parameters such as weight in kilogram, height in centimeters, waist, hip and mid arm circumference in centimeters were measured.

The results were given as Mean $\pm$ Standard deviation. Comparisons were made between sedentary and non-sedentary subjects. Student's t-test (Unpaired) was used for comparisons between the groups. A p value ≤ 0.05 was considered as statistical significance. Subjects suffering from endocrinal disorders, hypertensive individuals, pregnant and lactating women, subjects with endovascular and cardiovascular diseases were excluded from the study.

The circumference technique measures body shape using a flexible plastic measuring tape, subjects were required to wear minimal thin cloth, the measurement is typically conducted in the morning before eating and after emptying the bladder. Subjects were measured in standing position and they were asked to breathe normally and gently which prevents the subject from contracting their abdominal muscles. Height was measured in centimeters with the standard position of the subject. It was measured by a plastic measuring tape after marking the subject to stand straight against an even wall. A sliding wooden head piece was used for accurate work.¹² Body weight of all the subjects was measured by using standardized weighing machine, which was calibrated in kilograms. The body mass index was derived by Quetlet's index from bodyweight (kg) / Height (m^2).¹³ Waist circumference was measured to the nearest centimeter with a plastic tape measure while the subjects were in the standing position at the end of gentle expiration. The following anatomical landmarks were used: laterally, midway between the lowest portion of the rib cage and iliac crest, and anteriorly midway

between the xiphoid process of the sternum and the umbilicus.¹⁴ Hip circumference was measured in centimeters (cm) in standing position with a plastic tape at the largest horizontal circumference around the buttocks.¹⁵ After completing the measurements, the waist circumference was divided by the hip circumference to determine the waist to hip ratio.¹⁶

Blood Pressure measurement (BP in mmHg) was recorded with a mercury sphygmomanometer, in supine position in the right upper limb by auscultatory method. Similarly, three readings were taken at an interval of 15 minutes each and an average of the three values calculated. Pulse rate/min was recorded by palpating the radial artery on the right side and counting was done for full one minute. The result for each parameter was compared between the sedentary and non-sedentary groups and was statistically analyzed.

Results

A total of 60 healthy adults male (30 sedentary workers and 30 non sedentary workers) were selected for the study.

Table 1: Distribution of basic health parameters

Parameters	Sedentary workers (mean±SD)	Non sedentary workers (mean±SD)	p value
Age (Years)	50.73 ± 6.05	50.63 ± 5.95	>0.005
Weight (Kg)	75.77 ± 6.13	59.23 ± 3.83	<0.005
Height (Meter)	1.67 ± 0.039	1.66 ± 0.032	>0.005
<i>p</i> ≤ 0.05 indicates level of significance (Unpaired Student's "t" test), SD; standard deviation.			

Table 1 show the distribution of different parameters of healthy adults, age expressed in year, weight in Kg and height in meters. Values of basic characteristics were expressed as mean ± SD (standard deviation). "*p*" value at or below 0.05 are representing statistically significant result.

Table 2: Distribution of anthropometric parameters

Parameters	Sedentary workers (mean±SD)	Non sedentary workers (mean±SD)	p value
BMI (Kg/m ²)	27.29 ± 2.27	21.38 ± 1.28	<0.005
WC (Inch)	38.63 ± 2.44	30.57 ± 2.45	<0.005
HC (Inch)	39.70 ± 2.86	33.07 ± 2.61	<0.005
WHR	0.97 ± 0.04	0.92 ± 0.03	<0.005
BMI; body mass index, WC; waist circumference, HC; hip circumference, WHR; waist-hip ratio, <i>p</i> ≤ 0.05 indicates level of statistical significance (Unpaired Student's "t" test), SD; standard deviation.			

Table 2 shows that the BMI of sedentary workers (27.29 ± 2.27) is more than of the non-sedentary workers (21.38 ± 1.28). So are the waist circumference (inch) in sedentary workers (38.63 ± 2.44) than in non-sedentary workers (30.57 ± 2.45), hip circumference (inch) in sedentary workers (39.70 ± 2.86) than in non-sedentary workers (33.07 ± 2.61) and waist-hip ratio in sedentary workers (0.97 ± 0.04) than in non-sedentary workers (0.92 ± 0.03). Hence these features are found to be significantly higher (*p* ≤ 0.05) in sedentary workers than in non-sedentary workers.

Table 3: Distribution of additional parameters

Parameters	Sedentary workers (mean±SD)	Non sedentary workers (mean±SD)	p value
Pulse (bpm)	82.37 ± 6.63	69.07 ± 4.47	<0.005
SBP(mmHg)	131.00 ± 10.61	110.67 ± 8.27	<0.005
DBP (mmHg)	85.17 ± 4.99	72.83 ± 6.90	<0.005
bpm; beats per minute, SBP; systolic blood pressure, DBP; diastolic blood pressure, <i>p</i> ≤ 0.05 indicates level of statistical significance (Unpaired Student's "t" test), SD; standard deviation.			

Table 3 shows the distribution of additional parameters. The pulse of sedentary workers (82.37 ± 6.63) is higher than of non-sedentary workers (69.07 ± 4.47). The systolic BP (mmHg) of sedentary workers (131.00 ± 10.61) is higher than of non-sedentary workers (110.67 ± 8.27). The diastolic BP (mmHg) of sedentary workers (85.17 ± 4.99) is higher than of non-sedentary workers (72.83 ± 6.90). Hence showing that, these parameters are found to be significantly higher (*p* ≤ 0.05) in sedentary workers than in non-sedentary workers.

Discussion

Many studies have shown the health burden of a sedentary Lifestyle. This is an analysis and discussion of the anthropometric and cardiovascular parameters assessed in sedentary and non-sedentary workers. In our study BMI was increased in sedentary subjects when compared to non-sedentary subjects and it was statistically

significant. Recent studies in adolescents and adults have demonstrate significant relationship between physical activity and other adverse health practices, such as consumption of less-healthy foods or increased fat intake. Inactive individuals tend to consume more quantities of dietary fat. These data suggest that inactivity tends to cluster with other health behaviors that have adverse effect on the quantity and location of body fat deposition which results in obesity.¹⁷ Modern life style associated with easy access to food, lack of exercise, sedentary life style, calories dense food, and excessive television and cell phone viewing is among the identified contributes to the obesity epidemics.¹⁸ In our study the mean WHR in sedentary subjects was increased by 0.04 which was statistically significant this may be due to lack of physical exercise leading to an increased deposition of fat in the abdomen in sedentary subjects. Abdominal obesity is defined as waist circumference >102 cm in men and > 88 cm in women. Physical inactivity decreases the production of Nitric Oxide (NO) by the abnormal endothelium, which leads to changes in vessel diameter leads to vascular structural changes which result in hypertension.¹⁹ The reason for the association between increased body weight and elevated blood pressure is unclear. One possibility is that obesity is associated with higher circulating levels of insulin (a consequence of insulin resistance) and consequently with enhanced renal retention of sodium, resulting in increased blood pressure.²⁰ Sedentary normotensive have 20–50% higher risk of developing hypertension than individuals who are undertaken physical exercise regularly.²¹ Systolic blood pressure increased by 6 mmHg and diastolic blood pressure by 4 mmHg for a 10% gain in body fat. Weight loss of 1 kg produced a 20% decrease in both systolic and diastolic blood pressure in hypertensive patients even when the sodium intake was kept constant. The most characteristic lipid disorder in obesity was elevated total cholesterol and triglycerides, high

LDL cholesterol and low HDL cholesterol. For every 1 kg of weight loss, there was corresponding reduction by about 1% in total cholesterol and LDL, a rise by 1% in HDL and a reduction by 3% in triglycerides.²² Another study (Tatiana Y. et al., 2010) shows that physical inactivity has become a major public health concern because it is the second leading single cause of death in the United States.²³ Visceral fat although influenced by calorie intake to a certain extent, visceral fat accumulation is a mechanism which is determined also by estrogen deficiency (post-menopausal hypertension) or enhanced corticoid influences. It is hypothesized that excess catecholamine triggers various adverse processes which, if persist, can lead or aggravate hypertension and insulin resistance. Visceral fat but not peripheral fat mass was correlated with atherogenic effect.²⁴ Overweight and obesity is strongly related to major cardiovascular risk factors such as raised blood pressure, glucose intolerance, type-2 diabetes and dyslipidemia. In our study that anthropometric parameters were increased in sedentary subjects which was statistically significant increase. Maintaining a healthy lifestyle, including exercise, will result in increased energy levels throughout working period. So, the benefits of regular physical activity are numerous, people who exercise live longer and healthier.

Conclusion

Sedentary life style was associated with increase in anthropometric parameters such as BMI, WC, WHR and it was also noticed in our study that cardiovascular parameters such as pulse and blood pressure was increased in sedentary subjects and was statistically significant. A better understanding of ethnic/racial differences in the development and progression of various complications in sedentary lifestyle is needed. Hormonal assay and lipid profile estimation along with fat parameters would have given a better understanding about sedentary life style and its consequences. We need to evaluate the strategies

and efficacy of physical activity in various diseases. Those who are sedentary, an exercise program are an excellent way to significantly improve their health. Maintaining a healthy lifestyle, including exercise, will result in increased energy levels throughout working period.

Conflict of Interest: Authors declare no conflict of interests.

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Spectrum of Headache Disorder: Patient Attending Eye OPD in A Tertiary Teaching Hospital

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Abstract

Background: Headache disorders are one of the most common reasons for patients seeking medical attention, with varying underlying causes that can significantly affect a person's quality of life. This study aimed to assess the spectrum of headache disorders to alert ophthalmologists and general physicians to distinguish urgent headaches (those that may be life as well as vision-threatening) from less urgent headaches. **Materials and Methods:** This was a cross-sectional observational study conducted at the Eye Outpatient Department (OPD) in Ad-din Sakina Medical College Hospital, Pulerhat, Jashore, from December 2023 to November 2024, to assess the spectrum of headache disorders among patients attending the Eye OPD. **Results:** The results of this study revealed that among 500 patients attending the Eye OPD, the highest incidence of headaches was observed in the 21-30 years age group (30%), with a notable female predominance (80%). The most common headache condition was migraine (30%), followed by anxiety and tension headaches (20%), and eye strain due to mobile and laptop use (15%). Urgent symptoms, such as headaches related to intracranial space-occupying lesions (ICSOL), were rare, affecting only 0.5% of patients. **Conclusion:** This study highlights the diverse spectrum of headache disorders with a predominance of migraines, anxiety-related headaches, and eye strain due to prolonged screen time. The findings emphasize the critical importance of distinguishing between urgent and non-urgent headache conditions among patients attending the Eye OPD. Although the urgent conditions, such as headaches related to intracranial space-occupying lesions (ICSOL) are rare (0.5%), necessitating immediate attention from ophthalmologists and general physicians.

Keywords: Ophthalmology, Migraine, Tension headache, Eye strain

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Introduction

Headache is a common cause of suffering. Although headaches are painful and annoying, the vast majority do not indicate a serious disorder. Its nature is said to be dull, aching, boring, superficial, deep-seated, or migrainous; its time of occurrence is constant, intermittent, related or unrelated to the use of eyes. In the majority of patients, the cause is trivial and reversible. Headache may present as serious in intracranial diseases and it must be distinguished from those of more common forms of headache.¹ The relationship between headaches and ocular health is multifaceted, as various headache types, including primary and secondary headaches, can manifest in conjunction with visual symptoms. Primary headache disorders, such as migraine and tension-type headaches (TTH), are frequently reported among individuals who seek care in Eye OPDs. Migraine, characterized by recurrent, severe, and often

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unilateral pain, is commonly associated with visual symptoms like aura, photophobia, and blurred vision. The connection between migraines and visual disturbances is well-documented, as migraines can lead to cortical spreading depression, which disrupts visual processing pathways in the brain.²

Additionally, patients with migraines often report increased sensitivity to light, making them more likely to visit an Eye OPD for evaluation and symptom management.³ In this context, the role of the ophthalmologist is crucial, as they can help differentiate between visual aura associated with migraines and other ophthalmological conditions that may present with similar symptoms. Tension-type headaches, which are typically bilateral and characterized by a dull, pressing pain, also commonly affect individuals with Eye OPDs. These headaches are often linked to stress, anxiety, and poor posture, which may contribute to visual strain. Prolonged screen use, poor lighting, and uncorrected refractive errors can exacerbate the

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symptoms of TTH. Therefore, patients with TTH frequently seek care in Eye OPDs, not only for headache management but also for evaluation of their visual function. The interplay between visual factors, such as uncorrected refractive errors, and headache development is a critical area of investigation for clinicians working in these settings.⁴ Secondary headaches, particularly those related to ocular conditions, are another prominent aspect of the headache spectrum in Eye OPDs. Glaucoma, a condition characterized by elevated intraocular pressure, is often associated with headache symptoms. Patients with glaucoma may experience a dull, aching headache that worsens with prolonged visual activity or at night.

Furthermore, ocular inflammation, such as uveitis or retrobulbar neuritis, can present with headache as a secondary symptom, often accompanied by pain and discomfort in the eye.⁵ These conditions can present challenges for both diagnosis and management, as headaches may be perceived as a primary symptom by patients, while the underlying ophthalmological condition requires prompt treatment to prevent further complications. Moreover, visual strain from uncorrected refractive errors, such as myopia, hypermetropia, and astigmatism, can trigger or exacerbate headache symptoms. Patients with undiagnosed refractive errors may experience persistent headaches due to eye strain, particularly with prolonged reading or screen use. In Eye OPDs, refractive errors are frequently identified as a contributing factor to headaches, particularly in younger patients or those who engage in extensive visual tasks.⁶ Corrective measures, such as prescribing glasses or contact lenses, often provide significant relief, making the role of the ophthalmologist vital in these cases. In addition to primary and secondary causes, environmental factors, such as prolonged exposure to screens and poor lighting conditions, contribute to the development of headaches in patients attending the Eye OPD. The widespread use of digital devices has led to a surge in cases of

digital eye strain, characterized by symptoms like dry eyes, blurred vision, and headaches. These symptoms are particularly prevalent among younger individuals and those working in occupations that require extensive screen time. The management of digital eye strain is an emerging area of focus in the Eye OPD, with recommendations emphasizing proper ergonomics, frequent breaks, and the use of anti-reflective lenses to reduce strain on the eyes.⁷ Psychosocial factors, such as stress and anxiety, are also significant contributors to headache disorders. Tension headaches, which are closely linked to psychological stress, are often present in patients who report anxiety or emotional distress.

Moreover, emotional factors may exacerbate visual strain, leading to an increased frequency and intensity of headaches. Addressing these psychosocial aspects in a comprehensive treatment plan is important for improving both headache management and overall patient well-being.⁸ Sleep disturbances and irregular sleep patterns are also frequently reported among patients with headaches. A lack of restorative sleep or poor sleep quality can act as a trigger for both migraines and tension-type headaches. Studies have shown that individuals with chronic insomnia are more likely to experience frequent and severe headaches, which may be compounded by visual strain due to extended screen time or inadequate lighting. Thus, managing sleep hygiene and addressing underlying sleep disorders is a crucial part of a comprehensive headache management plan.⁹ The association between headache disorders and visual disturbances in Eye OPDs underscores the need for a multidisciplinary approach to treatment. Ophthalmologists, neurologists, and mental health professionals must collaborate to address the complex interplay of visual, neurological, and psychosocial factors in patients with headaches. Additionally, patient education plays a crucial role in managing headache disorders, as individuals need to be aware of the potential

triggers, including visual strain, stress, and sleep disturbances. Prevention strategies, such as the regular use of corrective eyewear, practicing stress reduction techniques, and adopting good sleep hygiene, can help to reduce the frequency and severity of headache episodes. This study aimed to assess the spectrum of headache disorders among patients attending eye OPD, to alert ophthalmologists and general physicians to distinguish urgent headaches (those that may be life as well as vision-threatening) from less urgent headaches.

Materials and Methods

This was a cross-sectional observational study conducted at the Eye Outpatient Department (OPD) in Ad-din Sakina Medical College Hospital, Pulerhat, Jashore, from December 2023 to November 2024. The study aimed to assess the spectrum of headache disorders among patients attending the Eye OPD, focusing on demographic characteristics, headache conditions, duration, and common triggers. A total of 500 consecutive patients who presented with headaches and sought care were included in the study. The patients were selected based on their willingness to participate and the presence of headache symptoms. Patients of all ages, both male and female who consented to participate were included in the study. Patients with pre-existing neurological disorders or systemic conditions affecting the head and neck region, patients with a history of trauma or surgery affecting the head or eyes and unable to provide informed consent were not included in this study.

Data was collected using a structured questionnaire administered to each patient during their OPD visit. The questionnaire captured demographic information (age, sex), headache characteristics (duration, type, and frequency), associated ocular conditions (e.g. refractive errors, eye diseases), and common triggers (including stress, screen time, and sleep disturbances). Information

was gathered through patient interviews, clinical examination, and a review of medical records. Data were analyzed using descriptive statistics. The demographic characteristics, headache conditions, duration, and triggers were summarized as frequencies and percentages. Age and gender distributions were examined to identify trends and the prevalence of various headache conditions was calculated. Data analysis was performed using IBM SPSS 25. Ethical approval for the study was obtained from the institutional review board, and informed consent was obtained from all participants before data collection.

Results

Table 1: Distribution of patients according to age group (n=500)

Age (years)	Number	Percentage
0-10	55	11.0
11-20	125	25.0
21-30	150	30.0
31-40	100	20.0
41-50	40	8.0
51-60	20	4.0
61-70	10	2.0

In Table 1 the age distribution of 500 patients attending the Eye OPD with headaches shows that the highest number of cases (30%) were in the 21–30 years age group (150 patients), followed by the 11–20 years group, which accounted for 25% (125 patients). The 31–40 years age group comprised 20% (100 patients). Younger children aged 0–10 years represented 11% (55 patients). The frequency of headaches decreased with age, with 8% (40 patients) in the 41–50 years group, 4% (20 patients) in the 51–60 years group, and only 2% (10 patients) in the 61–70 years category.

Table 2: Distribution of patients according to gender (n=500)

Gender	Number	Percentage
Male	100	20.0
Female	400	80.0

Table 2 presents the gender distribution of 500 patients attending the Eye OPD with headaches, showing a significant female predominance. Out of the total patients, 80% (400 patients) were female, while only 20% (100 patients) were male.

Table 3: Distribution of Patients According to Different Conditions (n=500)

Condition	Number	Percentage
Migraine	150	30.0
Anxiety & Tension Headache (HAS, Student)	100	20.0
Eye Strain (Mobile & Laptop)	75	15.0
Uncorrected Refractive Errors	50	10.0
Diseases of the Eyes (Glaucoma, Iritis, Retrobulbar neuritis)	48	9.5
Insomnia & Depression	35	7.0
Sinus headache	25	5.0
Weak accommodation & convergence	15	3.0
Urgent headache (ICSOL)	2	0.5

HAS (Husband Abroad Syndrome)

Table 3 presents the distribution of patients according to different headache-related conditions among 500 individuals attending the Eye OPD. Migraine was the most common condition, affecting 30% (150 patients), followed by anxiety and tension headaches, including HAS (Husband Abroad Syndrome) and student-related stress, which accounted for 20% (100 patients). Eye strain due to mobile and laptop use was reported in 15% (75 patients), while uncorrected refractive errors contributed to 10% (50 patients). Various eye diseases, such as glaucoma, corneal disorders, iritis, and retrobulbar neuritis, were present in 9.5% (48 patients). Insomnia and depression were linked to 7% (35 patients) of cases, while sinus headaches accounted for 5% (25 patients). Weak accommodation and convergence issues were observed in 3% (15 patients). The least common but most urgent cases were intracranial space-occupying lesions (ICSOL), reported in 0.5% (2 patients).

Table 4: Headache with Duration (n=500)

Duration of Headache	Number	Percentage
< 1 month	120	24.0
1 – 6 months	180	36.0
7 – 12 months	90	18.0
> 1 year	110	22.0

Table 4 presents the distribution of patients according to the duration of their headache symptoms among 500 individuals attending the Eye OPD. The majority of patients experienced headaches for 1 to 6 months (36%, 180 patients), followed by those with headaches lasting for less than 1 month (24%, 120 patients). Headaches lasting between 7 to 12 months were reported by 18% (90 patients), while 22% (110 patients) of patients had been suffering from headaches for more than 1 year.

Table 5: Common Triggers of Headache Among Patients (n=500)

Trigger Factor	Number	Percentage
Stress & Anxiety	140	28.0
Prolonged screen time	120	24.0
Sleep disturbances	90	18.0
Bright light exposure	70	14.0
Noise sensitivity	50	10.0
Dietary factors (Caffeine, Skipping meals)	30	6.0

Table 5 presents the common triggers of headaches among 500 patients attending the Eye OPD. The most frequently reported trigger was stress and anxiety, affecting 28% (140 patients), followed by prolonged screen time, which contributed to 24% (120 patients) of cases. Sleep disturbances were identified as a trigger in 18% (90 patients), while bright light exposure triggered headaches in 14% (70 patients). Noise sensitivity was reported by 10% (50 patients) of patients, and dietary factors, such as caffeine consumption and skipping meals, triggered headaches in 6% (30 patients).

Table 6: Urgent and Non-Urgent Prevalent Eye Symptoms (n=500)

Symptom Category	Symptom	Number	Percentage
▪ Urgent symptoms	Urgent headache (ICSOL)	2	0.5
▪ Non-urgent symptoms	Migraine	150	30.0
	Anxiety & Tension headache	100	20.0
	Eye strain (Mobile & laptop)	75	15.0
	Uncorrected refractive errors	50	10.0
	Diseases of the eyes (Glaucoma, Cornea, Iritis, Retrobulbar neuritis)	48	9.5
	Insomnia & Depression	35	7.0
	Sinus headache	25	5.0
	Weak accommodation & Convergence	15	3.0

Urgent symptoms, such as headaches related to intracranial space-occupying lesions (ICSOL), were rare, affecting only 0.5% of patients, but they require immediate attention due to their potential threat to life and vision. In contrast, the majority of patients (99.5%) presented with non-urgent conditions (Table 6), including migraines (30%), anxiety and tension headaches (20%), and eye strain from prolonged screen use (15%). Other non-urgent conditions included uncorrected refractive errors, eye diseases like glaucoma and iritis, and issues such as insomnia and depression.

Discussion

In this study, the highest prevalence of headaches was found in the 21–30 years age group, which accounted for 30% (150 patients) of the total sample. This finding aligns with previous research, which indicates that headaches, particularly migraines, are most common in young adults, especially those in their productive years.¹⁰ The 11–20 years age group also represented a significant portion (25%, 125 patients), likely reflecting the stressors of adolescence, including academic pressure and the increasing use of digital devices, both of which can contribute to headache development.¹¹ The study also revealed a female predominance, with 80% of the patients being female. This gender disparity is consistent with previous studies, which have consistently reported that headaches, particularly migraines and

tension-type headaches, are more prevalent in women compared to men. The hormonal influences, including estrogen fluctuations during menstruation, pregnancy, and menopause, are thought to contribute to this increased vulnerability in females.¹² Migraine was the most common headache condition in this study, affecting 30% (150 patients) of the sample.

Migraines are known to be highly prevalent, particularly in younger adults, and are often accompanied by other symptoms, such as nausea, photophobia, and phonophobia. The association of migraine with visual symptoms, such as visual aura, photophobia, and blurred vision, makes these patients frequent attendees at Eye OPDs.¹³ Anxiety and tension headaches, including those associated with "Husband Abroad Syndrome" (HAS) and student-related stress, accounted for 20% (100 patients) of the cases. Tension-type headaches are the most common primary headache disorder, often linked to emotional stress, anxiety, and poor posture. This finding highlights the increasing role of psychosocial factors, particularly stress and anxiety, in the development of headaches. The prevalence of stress-induced headaches in the younger population, including students and individuals in stressful work environments, further reinforces the importance of addressing psychosocial triggers in headache management.¹⁴ Eye strain due to prolonged use of

digital devices, such as mobile phones and laptops, was reported by 15% (75 patients) of the study population which was somewhat similar to another study.¹⁵ This finding is increasingly relevant in the modern era of digitalization, where excessive screen time is linked to a variety of visual and neurological symptoms, including headaches. The symptoms of digital eye strain, including blurred vision, dry eyes, and headaches, are commonly observed in individuals who spend long hours on screens. The increasing use of digital devices for work and leisure is likely to exacerbate this condition, making it an important factor in the management of headaches, especially in younger adults.¹⁶

Other conditions contributing to headaches included uncorrected refractive errors (10%, 50 patients) and ocular diseases such as glaucoma, corneal disorders, and uveitis (9.5%, 48 patients). These findings were similar to the findings of Serenje M et al.¹⁷ Refractive errors, such as myopia, hypermetropia, and astigmatism, are known to cause visual strain, which can result in headache symptoms. The majority of patients in this study reported experiencing headaches for 1 to 6 months (36%, 180 patients), followed by those with headaches lasting for less than 1 month (24%, 120 patients). The duration of headaches is an important factor in determining the underlying cause and guiding treatment strategies. Chronic headaches, particularly those lasting for more than a year, require careful evaluation for secondary causes, such as ocular diseases, neurological conditions, or systemic disorders.¹⁸ The fact that 22% (110 patients) of individuals reported headaches lasting more than 1 year suggests the chronic nature of some headache disorders, emphasizing the need for long-term management and monitoring in these patients. The most common triggers for headaches in this study were stress and anxiety (28%, 140 patients), followed by prolonged screen time (24%, 120 patients). How-

ever, a study conducted by Lampl et al. showed that the prevalence of stress and anxiety to cause headaches ranged from 5 to 10%.¹⁹ Sleep disturbances were also reported as a common trigger (18%, 90 patients), reinforcing the well-established relationship between poor sleep quality and increased headache frequency.²⁰ Bright light exposure (14%, 70 patients) and noise sensitivity (10%, 50 patients) were also reported as common triggers. These environmental factors can significantly exacerbate the symptoms of migraines and other headache types, highlighting the need for environmental modifications and patient education on managing these triggers.²¹

This study highlights the rarity of urgent cases and the predominance of non-urgent conditions. A similar study by Shenoy found that although most headache cases are benign, urgent conditions like intracranial space-occupying lesions (ICSOL) and severe ocular pathologies can present with headache as a primary symptom, requiring immediate intervention to prevent life-threatening complications.²² Our study similarly identified ICSOL as an urgent condition, but it was rare, accounting for just 0.5% of patients. This aligns with findings from the study of Wolf et al, who noted that severe and life-threatening headaches are less common but must be differentiated early in the clinical evaluation process to avoid delays in treatment.²³

Conflict of Interest: The authors declare no conflict of interest.

Conclusion

This study highlights the diverse spectrum of headache disorders among patients attending the Eye OPD, with a predominance of migraines, anxiety-related headaches, and eye strain due to prolonged screen time. The findings emphasize the critical importance of distinguishing between urgent and non-urgent headache conditions among patients. While urgent conditions, such as headaches related to intracranial space-occupying

lesions (ICSOL), are rare (0.5%), they pose significant risks to both life and vision, necessitating immediate attention from ophthalmologists and general physicians.

Recommendation

In light of the study's findings, it is recommended that ophthalmologists play a pivotal role in the management of headache disorders, particularly in identifying and addressing ocular-related causes such as uncorrected refractive errors and eye diseases, which contribute significantly to headache symptoms. Ophthalmologists should incorporate routine screenings for refractive errors, glaucoma, and other eye conditions that may manifest as headaches, ensuring timely interventions to alleviate symptoms.

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The Role of Arginase-1 and Glypican-3 Immune Markers in Differentiating Hepatocellular Carcinoma from Metastatic Carcinoma in Liver Using Cell Block Preparation

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Abstract

Background: Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of liver. Accurate diagnosis and differentiation of HCC by FNAC is often a problem particularly in case of poorly differentiated carcinoma and if the material is insufficient. The cell block method yielded more cellularity and provided better morphological details. Immunohistochemistry (IHC) with the use of Arginase-1 and Glypican-3 immune marker in cell blocks prepared from aspirated material can help to differentiate HCC from metastatic tumor. **Materials and Methods:** This cross-sectional study was carried out in Pathology department of Bangabandhu Sheikh Mujib Medical University from March 2017 to December 2018 with the aim to study the role of Arginase-1 and Glypican-3 IHC markers in differentiating HCC from metastatic carcinoma in liver space-occupying lesion (SOL). **Results:** In this study, mean age was 52.3± 15.1 years. After final diagnosis, 23 (92%) cases were confirmed as HCC, one (4%) as metastatic carcinoma and one (4%) as hepatocellular dysplasia. Arginase-1 was positive in 26 cases. Out of these, 25 cases were finally diagnosed as positive for HCC (true positive) and one case as negative for HCC (false positive). Arginase-1 was negative in 14 cases (true negative). Glypican-3 was positive in 24 HCC cases (true positive). Glypican-3 was negative in 16 cases. Out of these 16 cases, one case was finally diagnosed as positive for HCC (false negative) and 15 cases were finally diagnosed as negative for HCC (true negative). **Conclusion:** Use of cell block and IHC using Arginase-1 and Glypican-3 to differentiate HCC from metastatic tumor will enrich our knowledge and improve patient welfare.

Keywords: Liver, Hepatocellular carcinoma, Cell block, Immunohistochemistry.

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Introduction

Liver cancer was the sixth most commonly diagnosed cancer and the fourth leading cause of cancer death worldwide in 2018, with about 841,000 new cases and 782,000 deaths annually. The highest rate of incidence of liver cancer is found in Mongolia.¹ Although the highest liver cancer rates are found in East and Southeast Asia and sub-Saharan Africa, the incidence is increasing in other areas because of the increased incidence

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of chronic hepatitis C infection and steatohepatitis attributed to the obesity epidemic.²

Primary liver cancer includes hepatocellular carcinoma (HCC) (comprising 75%-85% of cases) and intrahepatic cholangiocarcinoma (comprising 10%-15% of cases) as well as other rare types. The main risk factors for HCC are chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV), aflatoxin-contaminated foodstuffs, heavy alcohol intake, obesity, smoking, and type 2 diabetes. The major risk factors vary from region to region. In most highrisk HCC areas (China, Eastern Africa), the key determinants are chronic HBV infection and aflatoxin exposure, whereas in other countries (Japan, Egypt), HCV infection is likely the predominant cause. In Mongolia, HBV and HCV virus and coinfections of HBV carriers with HCV or hepatitis D (Delta) virus, as well as alcohol abuse, all contribute to the high burden. The rising obesity prevalence is considered a contributory factor to the observed increasing incidence of HCC in

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low-risk HCC areas.¹

Bangladesh lies in the intermediate zone regarding the prevalence of HBV. The Prevalence of HCC is 35% among all liver diseases in Bangladesh. The prevalence of HBsAg and HCV positivity are 5.4% and 0.8% respectively in healthy population.³ The incidence rate of liver cancer in Bangladesh is 2.39% and the number of new cases and deaths annually are 3128 and 2841 respectively. Among all cancers, liver cancer is in the 11th position.⁴

Liver is also a common site for metastatic tumors, accounting for 25% of all metastases to solid organs. Common primary sites from which metastasis occurs are stomach, colon, lung, breast, thyroid and kidney. The comparative morphologic evaluation of HCC and their mimics is often a challenging issue for definitive diagnosis. Some of these diagnostic challenges are: a) the variety of neoplasms that can arise from the hepatic cells; b) metastasis that can mimic variants of primary HCC; c) the diagnostic limitations of serum alpha-fetoprotein (AFP); and d) the distinction of poorly differentiated HCC from cholangiocarcinoma and metastatic carcinoma.⁵

Fine-needle aspiration cytology (FNAC) of the liver under ultrasound or computed tomography guidance has been used in the routine diagnosis of masses of the liver to establish a diagnosis. Because it is a less-invasive method with relatively low cost, rapid turnaround time and complication rate lower than that of core needle biopsy.⁶ It has high sensitivity (100%) but low specificity (63.3%).⁷ Although 80% of malignant lesions of the liver can be correctly diagnosed through cytomorphological analysis and good clinico-radiologic correlation, around 20% can pose differential diagnostic problems.⁸ This diagnostic pitfall exist in the morphologic distinction of HCC from metastatic carcinoma, particularly in small biopsy material with inadequate sampling and in poorly differentiated tumors. A clear distinction between HCC and metastatic tumors in the liver is clinically important as it carries a significant impact on subse-

quent prognosis and therapeutic management.⁹

Cell block prepared from residue of fine needle aspiration can be useful adjunct to smears for establishing a more definitive cytopathologic diagnosis. It can be particularly useful for categorization of tumor with the help of histochemical stain and immunohistochemistry (IHC) which may not be possible from smears.¹⁰ The sensitivity of cell block varies from 60%- 86% depending on sampling type, type of specimens and aspiration techniques.¹¹ If conventional smear and cell block are combinedly used, the diagnostic and subtyping accuracy is significantly improved.¹²

The application of adjunct diagnostic tools, such as immunocytochemical staining, which can be selected on the basis of suspected diagnoses, is sometimes essential for a definitive diagnosis.⁹ A number of diagnostically useful immunohistochemical (IHC) markers for identification of HCC in routine surgical pathology practice remains limited to hepatocyte paraffin antigen (HepPar-1), polyclonal carcinoembryonic antigen, CD10, and alpha-fetoprotein. However, the utility of each of these markers is limited either by suboptimal sensitivity or by difficulty in interpretation.¹³

Arginase-1 (Arg-1) is a newly described marker in some studies. It is a key urea cycle metalloenzyme and is involved in the hydrolysis of arginine to ornithine and urea. It is recently recognized as a sensitive and specific marker for hepatocytes, both in benign and malignant hepatocytes.¹⁴ In sections of normal liver and HCC, anti Arg-1 produce strong, diffuse cytoplasmic and nuclear reactivity in all hepatocytes throughout the lobule. There is no reactivity in bile duct epithelial cells, sinusoidal endothelial cells, Kupffer cells, or vascular endothelial cells.¹⁵

Glypican-3 (GPC3) is a member of the heparan sulfate proteoglycan family. It is an oncofetal protein that is expressed in the embryo and involved in morphogenesis and growth control during development. It produces cytoplasmic,

membranous, and canalicular staining. GPC3 is a specific immunomarker for HCC that can be used to distinguish HCC from benign hepatocellular mass lesions, particularly hepatocellular adenoma.¹⁶ Immunocytochemical staining for GPC3 in cell block material is a highly sensitive and specific method capable of distinguishing HCC from the vast majority of metastatic carcinomas of the liver. Poorly differentiated cases showed the highest GPC3 sensitivity (100%), followed by moderately differentiated cases (96.5%), while well differentiated cases expressed GPC3 in 90% of cases. 83.3% of metastatic carcinomas are negative for GPC3.⁸

Several recent studies have identified that GPC3, when used as a single IHC marker, or as part of a panel with other markers, may be very helpful in differentiating HCC from metastatic neoplasms to the liver and also in differentiating benign from malignant hepatic lesion. Arginase-1 demonstrates superior sensitivity compared with GPC-3 and HepPar-1 in the diagnosis of HCC, whereas GPC-3 demonstrates superior specificity, as staining is not observed in benign hepatocellular lesions. Hence, use of Arginase-1 with HepPar-1 and GPC-3 can aid in the diagnosis of HCC and separating from metastatic carcinoma.²

Materials and Methods

Methodology: This was a cross-sectional observational study carried out at the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh; from March 2017 to December 2018. Permission for the study was taken from the concerned departments and Institutional Review Board (I.R.B.) of BSMMU, Dhaka. The sample size was 40 and non-random purposive sampling method was followed.

Inclusion criteria:

- Patients with suspected malignant hepatic mass detected by radiology and imaging and physically fit to sustain FNA procedure.

Exclusion criteria:

- Cases which are benign both in cytologic smear and cell block.
- Inadequate material.
- Patients having bleeding disorders.
- Non-cooperative patient.
- Cases not accompanied by complete clinical information.

Selections of patients: A total of 40 cases having suspected malignant hepatic space-occupying lesion (SOL) with good coagulation profile was selected for USG guided FNAC. Informed written consent was taken from the patients or from their guardians in all cases. According to standard protocol, FNAC was done by an expert physician under local anesthesia in the Hepatology and Radiology department, BSMMU and also in the Radiology department, DMCH.

Clinical information: Relevant clinical information was taken. These include- Age and sex of the patients, Relevant history, Clinical features, Laboratory data and Radiological & imaging findings. The data were obtained either directly from the patients or from history sheets in a pre-designed proforma before doing aspiration. All the necessary and relevant data were recorded methodically in a prepared proforma.

Sample collection: Under ultrasonography guidance, FNAC was performed by an expert pathologist, radiologist or hepatologist. Cytology smears were made from the aspirated material and immediately fixed in 95% ethanol. Remainder of the aspirate was submitted for cell block and kept in 10% neutral buffered formalin.

Sample processing: Several cytological smears were prepared and fixed immediately in 95% alcohol. The smears were left in alcohol for at least 30 minutes at room temperature before staining. Cell blocks prepared from the residual aspirated material were fixed in 10% neutral buffered formalin and later processed to prepare paraffin embedded blocks according to the standard protocol followed at the department of pathology, BSMMU.

Cytological examination:

1. **Staining:** Smears prepared from FNA were stained by Papanicolaou and hematoxylin & eosin stain according to the standard staining procedure followed at the department of pathology, BSMMU.
2. **Cytopathological examination:** The stained slides were examined on the next day. Satisfactory smears containing adequate number of representative cells were examined to evaluate the nature of the lesions and presence of malignant cells. Cases were diagnosed according to well established morphologic criteria related to the pattern of cellular arrangement, cellular and nuclear details, and background characteristics.

Examination of cell block: Serial sections of paraffin embedded cell blocks were prepared for routine histopathological examination. The sections were cut at 4-6 micro meter thickness and stained with hematoxylin and eosin (H&E) stain by following its' staining protocol. Sections of cell blocks were examined to assess the cellularity and arrangement of the cells. Histopathological assessment of the slides were done according to WHO morphologic criteria.¹⁷

Immunohistochemical staining of Arginase-1 and Glypican-3: Only cases with adequate cellular material on the cell block sections were selected for immunohistochemistry. For each case, one hematoxylin and eosin stained slide from each block was prepared for evaluation of adequacy. Additional two sections were prepared on commercially available Poly-L-Lysine coated glass slides for immunohistochemical stain. Immunohistochemistry was done using the DAKO EnVision TM FLEX, (Low pH Detection system, peroxidase/DAB+, Mouse). In this system primary antibody, anti- Liver Arginase mouse monoclonal antibody (ARG1/1125) and anti-Glypican-3, Cell Marque (clone 1G12) were used. Secondary antibody conjugated to an enzyme (horseradish peroxidase)

labeled polymer (dextran) chain was used subsequently. All slides were counterstained with hematoxylin. Appropriate positive control (histologic sections of known case of HCC for Arg-1 and GPC3) was used. The whole staining procedure was performed in the IHC lab of BSMMU following its standard staining protocol. Immunostained sections were examined by light microscopy under 10x, 20-40x objective lenses.¹⁸

The staining intensity was graded as strong (clearly seen with x4 or x10 objective lens) or weak (visible at x20 objective or higher) and distribution was recorded as negative (<5% of tumor cells stained), focal (5% -50% staining) and diffuse (>50% staining).¹⁹

Analysis of result: Statistical analysis of the results was obtained by using window-based computer software device with statistical Packages for Social Sciences (SPSS-20). Categorical data were expressed in percentage or number. Parametric data were expressed in mean $\pm$ SD. The results were calculated by using statistical formulas and presented in tables, figures and diagrams.

Results

This cross-sectional study was carried out in Pathology department of Bangabandhu Sheikh Mujib Medical University from March 2017 to December 2018 with the aim of study the role of Arginase-1 and Glypican-3 immuno markers in differentiating hepatocellular carcinoma from metastatic carcinoma in liver SOL. Out of total 40 cases, most of the study patients (45%) were in age between 31-50 years. The mean age was 52.3 ± 15.1 years with age ranging from 26 to 85 years (Figure 01). In this study, 32 (80%) cases were male with a male to female ratio of 4:1 (Figure 02).

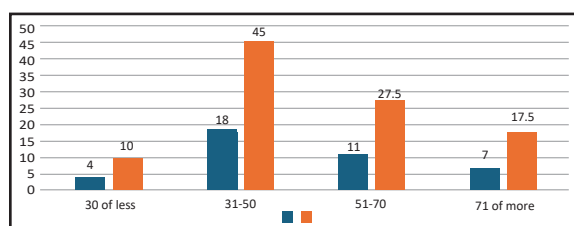


Figure- 01: Age of the study cases, (n=40)

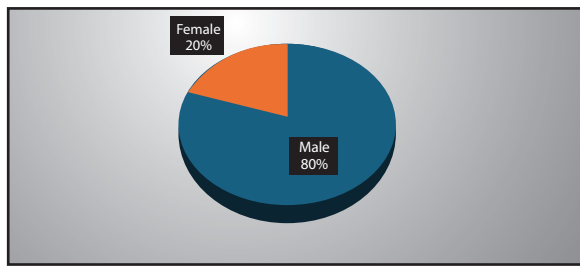


Figure- 02: Sex of the study cases (n=40)

The detailed cytomorphology of HCC and metastatic carcinoma and expression pattern of Arginase-1 and Glypican-3 immune markers in cell blocks were also assessed. A total of 40 patients having radiologically suspected malignant hepatic mass and physically fit to sustain the FNAC procedure and fulfilling the other selection criteria was included in this study. Cases which are benign both in cytologic smear and cell block sections, inadequate material, patients having bleeding disorders, and not accompanied by complete clinical information were excluded from this study. Among the 40 study cases, 25 (62.5%) cases were diagnosed as hepatocellular carcinoma, 9 (22.5%) were metastatic carcinoma, 1 (2.5%) was hepatocellular dysplasia and 1 (2.5%) was negative for malignancy. Four (10%) cases were positive but not categorized as HCC or metastatic carcinoma. These were diagnosed as adenocarcinoma either primary or metastatic (Figure 03). After preparation of smear from the fine needle aspirates on the glass slides, the residual material was routinely processed as cell block. On cell block examinations, 26 (65%) cases were diagnosed as hepatocellular carcinoma and remaining 14 (35%) were diagnosed as metastatic carcinoma (Figure 04).

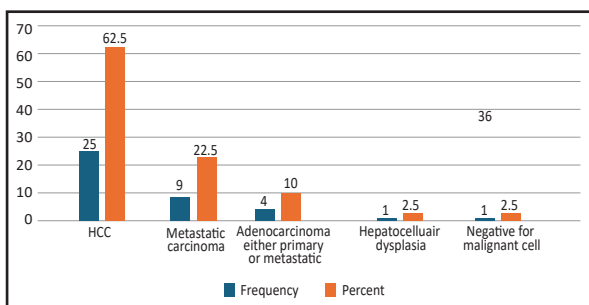


Figure - 03: FNAC diagnosis of the study patients (n=40)

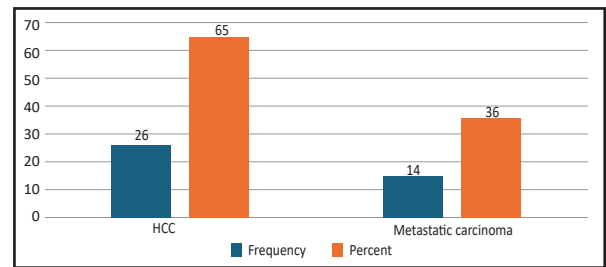


Figure 04: Cell block diagnosis of the study patients (n=40)

Subsequently, immunohistochemistry was performed on cell block sections using Arginase-1 and Glypican-3 immuno markers to classify and confirm the diagnosis. Among the total 40 cases, 25 (62.5%) cases were finally diagnosed as hepatocellular carcinoma, 14 (35%) cases as metastatic carcinoma and one (2.5%) case was diagnosed as hepatocellular dysplasia. Arginase-1 immuno stain was positive in all the finally diagnosed 25 (100%) HCC cases and also in case of hepatocellular dysplasia. It was negative in all the 14 (100%) metastatic carcinoma cases. Among the 25 cases of HCC, 24 (96%) was positive for GPC3 and one (4%) was negative. All the 14 (100%) metastatic carcinoma cases and the hepatocellular dysplasia case were negative for GPC3 stain (Table 1) 22 (88%) of the positive cases were diffusely stained with Arg-1 and 17 (68%) were with GPC3. Intensity was strong in 23 (92%) cases for Arg-1 and 17 (68%) cases for GPC3. The staining intensity was graded as strong (clearly seen with x4 or x10 objective lens) or weak (visible at x20 objective or higher). Distribution was recorded as negative (<5% of tumor cells stained), focal (5% -50% staining) and diffuse (>50% staining) (Table 2).

Out of 26 cases positive for HCC by cell block diagnosis, 25 were finally diagnosed as positive for HCC (true positive) and one case as negative for HCC (false positive). Out of 14 negatives for HCC cases in cell block, all were finally diagnosed as negative for HCC (true negative) (Table 3). Arginase-1 was positive in 26 cases. Out of these 26, 25 cases were finally diagnosed as positive for HCC (true positive) and one case as negative for HCC

Table 1: Interpretation of Arginase-1 and Glypican-3 immuno-stain in HCC, metastatic carcinoma and hepatocellular dysplasia, n=40.

Immuno marker	Hepatocellular carcinoma (%)	Metastatic carcinoma (%)	Hepatocellular dysplasia (%)	Total
Arginase-1				
Positive	25 (100)	0 (0)	1 (100)	26
Negative	0 (0)	14 (100)	0	14
Glypican-3				
Positive	24 (96)	0 (0)	0	24
Negative	1 (4)	14 (100)	1 (100)	16
Total	25	14	1	40

Table 2: Distribution and intensity of Arg-1 and GPC-3 immunomarker in HCC (n=25)

		Arginase-1 n (%)	Glypican-3 n (%)
Distribution	Focal	3 (12)	7 (28)
	Diffuse	22 (88)	17 (68)
Intensity	Weak	2 (8)	7 (28)
	Strong	23 (92)	17 (68)
Total		25	24

(false positive). Arginase-1 was negative in 14 cases. All of these were finally diagnosed as negative for HCC (true negative) (Table 4).

Table 3: Comparison between cell block diagnosis with final diagnosis for HCC; HCC, hepatocellular carcinoma

Cell block diagnosis	Final diagnosis	
	Positive for HCC (n=25)	Negative for HCC (n=15)
Positive for HCC (n=26)	25 (True positive)	1 (False positive)
Negative for HCC (n=14)	0 (False negative)	14 (True negative)

Table 4: Comparison between cell block IHC diagnosis by Arginase-1 with final diagnosis, n=40.

Cell block IHC diagnosis	Final diagnosis	
	Positive for HCC (n=25)	Negative for HCC (n=15)
Positive for Arginase-1 (n=26)	25 (True positive)	1 (False positive)
Negative for Arginase-1 (n=14)	0 (False negative)	14 (True negative)

Glypican-3 was positive in 24 cases, all of which were finally diagnosed as positive for HCC (true positive). Glypican-3 was negative in 16 cases. Out of these 16 cases, one case was finally diagnosed as positive for HCC (false negative) and 15 cases were finally diagnosed as negative for HCC (true negative) (Table 5). The validity of Arginase-1 in the diagnosis of HCC was measured by calculating sensitivity (100.0%), specificity (93.3%), accuracy (97.5%), positive predictive value (96.15%) and negative predictive value (100.0%). The validity of Glypican-3 in the diagnosis of HCC was measured by calculating sensitivity (96.0%), specificity (100.0%), accuracy (97.5%), positive predictive value (100.0%) and negative predictive value (93.75%) (Table 6).

Table 5: Comparison between cell block IHC diagnosis by Glypican-3 with final diagnosis, n=40

Cell block IHC diagnosis	Final diagnosis	
	Positive for HCC (n=25)	Negative for HCC (n=15)
Positive for Glypican-3 (n=24)	24 (True positive)	0 (False positive)
Negative for Glypican-3 (n=16)	1 (False negative)	15 (True negative)

Table 6: Sensitivity, specificity, accuracy, positive and negative predictive values of Arginase-1 and Glypican-3 immuno marker in differentiation of HCC from metastatic carcinoma.

Test of validity	Percentage	
	Arginase-1	Glypican-3
Sensitivity	100.0	96.0
Specificity	93.33	100.0
Accuracy	97.5	97.5
Positive predictive value	96.15	100.0
Negative predictive value	100.0	93.75

Discussion

This cross-sectional study was carried out in Department of Pathology, Bangabandhu Sheikh Mujib Medical University from March 2017 to December 2018 with an aim to study the role of Arginase-1 and Glypican-3 immuno markers in differentiating hepatocellular carcinoma from metastatic carcinoma in liver using cell block preparation. In this study, the detailed cytomorphology of HCC and metastatic carcinoma and the expression pattern of Arginase-1 and Glypican-3 immune markers in cell blocks were assessed.

A total number of 40 patients having radiologically suspected malignant hepatic mass, physically fit to sustain the FNAC procedure and fulfilling the other selection criteria were included in this study. More than three fourths (80%) of the patients were male and 20% were female. Male to female ratio was 4:1. This indicates that hepatocellular carcinoma is predominant in male patients.

In this study, it was observed that 45% of the patients were in 4th decade and above. The mean age was 52.3 ± 15.1 years with age ranging from 26 to 85 years. In case of HCC patients, the majority of patients (40%) belonged to age group 31-50 years and the lowest (12%) patients belonged to age $\leq$ 30 years.

In the current study, according to cytomorphological features, 25 (62.5%) cases were diagnosed as hepatocellular carcinoma, 9 (22.5%) cases as metastatic carcinoma, 1 (2.5%) as hepatocellular dysplasia and 1 (2.5%) case as negative for malignancy. In four (10%) cases the features were overlapping between HCC and metastatic tumor. These indeterminate cases could not be categorized. These were diagnosed as adenocarcinoma either primary or metastatic. Few authors showed in their study, 70% of lesions were diagnosed as HCC, representing the majority of the cases and 30% cases were diagnosed as metastatic carcinoma.⁸ Similarly, some authors reported that 58.5% of

malignant hepatic tumors were HCC and 41.5% were metastatic origin. In the current study, the cell block diagnosis was HCC in 26 (65%) cases and metastatic carcinoma in 14 (35%) cases.²⁰

The final diagnosis was made on the basis of cytomorphology of neoplastic cells, clinico-radiological correlation, serum tumour markers, cell block assessment and immunohistochemistry with Arginase-1 and Glypican-3 immuno marker on cell block sections. Same diagnostic criteria were also followed by a group of authors.⁸ According to the final diagnosis, the distribution of the patients in this current study was HCC in 25 (62.5%) cases, metastatic carcinoma in 14 (35%) cases and hepatocellular dysplasia in one (2.5%) case.

In this study, Arginase-1 immuno stain was positive in all the 25(100%) HCC cases and also in a single case of hepatocellular dysplasia. It was negative in all the 14 (100%) cases of metastatic carcinoma. Tahoun & Ezzat, (2013) showed that 96.1% cases of HCC were positive for Arginase-1. Among the 25 cases of HCC, 24 (96%) cases were positive for Glypican-3 and one (4%) case was negative. All the metastatic carcinoma 14 (100%) cases and hepatocellular dysplasia case were negative for Glypican-3 immunostain.⁹ Zaakook et al. (2013) showed in their study that Glypican-3 was positive in 95.2% of HCC.⁸

In this study, the arginase-1 immunostain was positive in 25 cases. The staining was cytoplasmic or cytoplasmic and nuclear in hepatocytes. Three (12%) cases were focally and 22 (88%) were diffusely positive. The intensity was weak in two (8%) and strong in 23 (92%) cases. No Arginase-1 staining was found in 14 cases. Glypican-3 immunostain was positive in 24 cases. The staining was cytoplasmic and membranous in neoplastic hepatocytes. Seven (28%) of these were focally and 17 (68%) were diffusely positive. The intensity was weak in seven (28%) cases and strong in 17 (68%) cases. No Glypican-3 staining was seen in 16 cases.

In the present study, out of total 40 cases, cytological diagnosis was HCC in 25 patients. After final diagnosis, out of these 25 HCC cases, 23 (92%) cases were confirmed as HCC, one (4%) as metastatic carcinoma and one (4%) as hepatocellular dysplasia. Nine patients were cytologically diagnosed as metastatic carcinoma, out of which one (11.1%) case was confirmed as HCC and remaining eight (88.9%) cases were confirmed as metastatic carcinoma. Single case of hepatocellular dysplasia was finally confirmed as HCC. Single case which was negative for malignancy in cytology was confirmed as metastatic carcinoma. Four cases cytologically were positive but not conclusive and diagnosed as adenocarcinoma either primary or metastatic. These four cases were finally diagnosed as metastatic carcinoma.

The study showed that cytologically diagnosed hepatocellular carcinoma were true positive in 23 (92%) cases, false positive in 2 (8%) cases, false negative in 2 (8%) cases and true negative in 13 (52%) cases according to final diagnosis. In this study cytologically diagnosed metastatic carcinoma were true positive in eight (57.1%) cases, false positive in one (7.14%) case, false negative in six (42.85%) cases and true negative in 25 (96.15%) cases according to final diagnosis. So, the overall sensitivity and specificity of cytological diagnosis was 92.0 % and 86.67% respectively for the diagnosis of HCC. And for the diagnosis of metastatic carcinoma, the sensitivity and specificity were 57.1% and 96.1% respectively. Various studies have reported sensitivity varying from 67% to 99.5% and specificity 87% to 100%. Swamy et al. 2011 and Zaakook et al. (2013) found that 80% of HCC can be correctly diagnosed by cytomorphological analysis and good clinico-radiological correlation.^{8,21}

In this study, Arginase-1 was positive in 26 cases. Out of these 26 cases, 25 cases were finally diagnosed as positive for HCC (true positive) and one case as negative for HCC (false positive). Argin-

ase-1 was negative in 14 cases. Out of these 14 cases, all were finally diagnosed as negative for HCC (true negative). So, regarding the validity of Arginase-1 immunostaining in differentiating HCC from metastatic carcinoma with sensitivity, specificity, positive predictive value, negative predictive value and total accuracy were 100%, 93.33%, 96.15%, 100% and 97.5% respectively. Few study showed, Arginase-1 achieved a specificity ranging from 87 to 100%.^{2, 14, 22, 23} The specificity for Arginase-1 detected in the current work (93.33%) agreed with the values observed in previous studies. In the present study, Glypican-3 was positive in 24 cases, all of which were finally diagnosed as positive for HCC (true positive). Out of 16 negative Glypican-3 cases, one case was finally diagnosed as positive for HCC (false negative) and 15 cases were finally diagnosed as negative for HCC (true negative). So, regarding the validity of Glypican-3 immunostaining in differentiating HCC from metastatic carcinoma with sensitivity, specificity, positive predictive value, negative predictive value and total accuracy were 96.0%, 100.0%, 100.15%, 93.75% and 97.5% respectively. Other studies showed sensitivity ranging from 72% to 95% and specificity between 96% to 100%.^{8,16,20} The current study showed that both Arginase-1 and glypican-3 have higher sensitivity and specificity in differentiating hepatocellular carcinoma from metastatic carcinoma.

Limitations of the study:

Sample size was small. Study population was selected from tertiary care hospital, so it may not reflect the exact picture of the country. Follow up biopsy was not available.

Conclusion

Both Hepatocellular and metastatic carcinoma are common in liver. A clear distinction between HCC and metastatic carcinoma is important because it carries a significant impact on subsequent prognosis and therapeutic management. The distinction between these tumors is often difficult by

cytology alone. The current study showed that, cell block prepared from aspirated material and stained with Arginase-1 and Glypican-3 immunomarkers has high sensitivity and specificity in differentiation of HCC from metastatic carcinoma. So, it can be concluded that cell block with immunohistochemistry using Arginase-1 and Glypican-3 immuno-panel in conjunction to cytology is a very useful diagnostic modality in differentiating HCC from metastatic carcinoma in suspicious cases.

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Length of Inferior Division of Splenic Artery in Different Age & Sex

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Abstract

Background: Splenic arterial interventions are being used more frequently to manage a range of medical conditions. When appropriate, these procedures can serve as an alternative to surgery. For instance, embolization is commonly used in cases of splenic injury following trauma and to enhance hematological parameters, such as in patients with pancytopenia, thrombocytopenia, leukopenia, or anemia. This is particularly beneficial for individuals with hypersplenism, as well as for those who need high-dose chemotherapy or immunosuppressive therapy but would otherwise be unable to receive it. Additionally, these interventions can be performed to isolate splenic artery aneurysms from the parent vessel to prevent rupture, and to lower portal pressure, thereby reducing complications associated with portal hypertension. **Objectives:** Knowledge of intraparenchymal division of splenic artery is of great importance in surgical practice. It is for the branches of its terminal division or for its polar branches that the surgeon will turn when making a particular area for devascularization in order to perform a partial splenectomy. **Materials and Method:** This cross sectional descriptive study was done to measure length of inferior division of splenic artery in different age & sex group. Using a purposive sampling technique, 80 human spleen samples were collected from autopsy laboratory of the department of Forensic Medicine and later allocated into three separate groups. **Results:** The mean ($\pm$ SD) length of inferior division of splenic artery was 1.45 ± 0.54 cm in Group A, 2.08 ± 0.68 cm in Group B and 2.18 ± 0.69 cm in Group C. **Conclusion:** The mean length of inferior division of splenic artery was increased with age.

Keywords: Spleen, Age, Sex, Splenic artery, Bangladeshi cadaver.

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Introduction

The spleen is a large, encapsulated, complex mass of vascular and lymphoid tissues situated in the upper left quadrant of the abdominal cavity between the fundus of the stomach and the diaphragm. The size of the spleen roughly corresponds to the cupped hand or fist of the subject. Including the diaphragmatic and visceral surfaces, it has superior and inferior borders and anterior and posterior ends or poles.¹ Splenic parenchyma consists of white and red pulp that is surrounded by serosa and a collagenous capsule with smooth muscle fibers, trabecular dense connective tissue rich in collagen and elastic fibers. These with the reticular framework, support the cells of the spleen and surround the vessels in the

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splenic pulp. Spleen is an elastic, controllable reservoir that is important in adjusting the volume of the circulating blood. In life the spleen undergoes both rhythmic and passive contractions. In part this activity is attributed to the smooth muscle in its framework.^{2,3} The spleen filters blood and reacts immunologically to blood-borne antigens. It has both morphologic and immunologic filtering functions. Aged, damaged, or abnormal red blood cells are trapped by macrophages associated with unusual vascular sinuses in the red pulp. These macrophages break down the red cells, begin the metabolic breakdown of hemoglobin and retrieve and store the iron from the heme for reutilization in the formation of new red blood cells in the bone marrow.⁴ The spleen is supplied exclusively from the splenic artery. This is the largest branches of the celiac trunk and its course is among the most tortuous in the body. It vascularizes the spleen, a unique organ from the others in this region of the body given its hematopoietic function. Its blood flow is abundant and variable. It is a spongy organ with considerable capacity of retention and

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excretion of blood where the splenic artery possesses a wall which may respond at any time to the needs of the organ. Thus, this artery undergoes strong fluctuations in blood flow and its arterial wall is constantly being reorganized.⁵ Near the splenic hilum, the artery usually divides into terminal and polar arteries. The superior terminal branches are usually longer than the inferior terminal branches and provide the major splenic arterial supply. As these branches enter the hilum they divide further into four or five segmental arteries that each supplies a segmental vessel often leads to infarction of part of the spleen.^{6,7} There is typically a superior polar artery, which sometimes communicates with the short gastric arteries, superior, middle, and inferior terminal arteries and an inferior polar artery. Knowing these variable distributions is necessary when performing resections, specially a spleen preserving procedure. Because of the variable nature of the splenic artery, one must be cautious when operating near this vessel and its tributaries.⁸ Splenic sinuses are filled with blood instead of lymph. It is the most vascular organ in the human body, which is involved in the regulation of circulating blood volume. Approximately 350 L of blood passes through it per day. The spleen receives approximately 5% of the cardiac output, and 40% source of the blood in the portal circulation. The spleen contains about one unit of blood at a given time, 25% of total lymphocytes in the body, 30 to 40 ml of mature RBC and one-fourth of the circulating platelets.^{9,10} Therefore, it is necessary to know the vascular distribution, as well as its probable location for increasing the knowledge about the gross anatomy and histology of the spleen in Bangladeshi people of different age and sex group. Moreover, to establish a normal standard which is basic and essential for reliable understanding of any disorders of spleen in Bangladeshi people.

Materials and Methods

The study was conducted in the department of

Anatomy at Mymensingh Medical College. It took place over a period from June 2013 to July 2014. The study protocol was approved by Ethical Review Committee of Mymensingh Medical College. A total of 80 human spleens were collected using a purposive sampling technique from October 2013 to April 2014. Among the samples, 47 were from males and 33 were from females. All specimens were obtained from Bangladeshi cadavers in the autopsy laboratory of the Department of Forensic Medicine at Mymensingh Medical College. Samples were collected from individuals who had died within the previous 12 hours. After collection, the specimens were kept to get fixed for 48-72 hours and preserved in 10% formol-saline solution. To facilitate the comparison of the length of inferior division of splenic artery in relation to age and sex, the samples were categorized into three groups: Group A (upto 20 years), Group B (21 to 40 years) & Group C (41 to 60 years). Each of these groups was further subdivided into male & female categories. For statistical analysis, differences among age groups were assessed using a one-way ANOVA test, while difference between sexes were evaluated using an unpaired 't' test. All data were recorded in a pre-designed data sheet, analyzed using the SPSS program, and compared with findings from national and international studies as well as standard text books. The aim of this study was to determine variations in the length of inferior division of splenic artery in Bangladeshi population and to compare the results with those reported by various established researchers in this field.

Results

From the Table I (a) and I (b) it was evident that the maximum length of inferior division of splenic artery was 2.50, 4.00 and 4.00 cm in Group A, B and C respectively. The minimum length of inferior division of splenic artery was 0.50 cm in group A, 0.80 cm in group B and 0.50 cm in group C. The mean length of inferior division of splenic artery

was maximum in group B, 2.21 cm in male and in group C 2.26 cm in female and minimum in group A, 1.47 cm in male and 1.42 cm in female. The mean ($\pm$ SD) length of inferior division of splenic artery was 1.45 ± 0.54 cm in Group A, 2.08 ± 0.68 cm in Group B and 2.18 ± 0.69 cm in Group C. It was evident from figure A that the mean length of inferior division of splenic artery increased with age.

Table I (a): Mean Length of Inferior Division of Splenic Artery in Different Age Groups		
Age Group	Number of specimen	Length of Inferior Division of Splenic Artery (cm) Mean $\pm$ SD (Minimum – Maximum)
A (0-20 years)	28	1.45 ± 0.54 (0.50- 2.50)
B (21 – 40 years)	31	2.08 ± 0.68 (0.80- 4.00)
C (41 to 60 years)	21	2.18 ± 0.69 (0.50- 4.00)

Table I (b): Comparison of Length of Inferior Division of Splenic Artery among the Age Groups					
Comparison Between Variables		Mean Difference	Std. Error	p	Level of significance
A	B	0.43	0.26	0.114	NS
B	C	1.19	0.49	0.043	S
C	A	0.76	0.42	0.1	NS
Comparison between 3 groups were done by ANOVA test. p value > 0.05, not-significant at 5% confidence interval (CI) level. NS; not significant. S; significant					

The mean difference of length of inferior division of splenic artery between group A & B and C & A was statistically not significant ($p > 0.05$) and B & C was statistically significant ($p < 0.05$).

Table II (a): Mean Length of Inferior Division of Splenic Artery in Different Sex Groups				
Age Group	Sex of the person	Number of specimen	Mean Length of Inferior Division of Splenic Artery in cm	$\pm$ SD
A (0-20 years)	Male	18	1.47	0.54
	Female	10	1.42	0.58
B (21 – 40 years)	Male	13	2.21	0.75
	Female	18	1.99	0.63
C (41 to 60 years)	Male	16	2.16	0.75
	Female	5	2.26	0.51

Table II (b): Comparison of Length of Inferior Division of Splenic Artery between Sexes					
Age group	Mean Difference between sex	Std. Error Difference	t	p	Level of significance
A	0.34	0.498	0.683	0.525	NS
B	0.175	0.327	0.535	0.61	NS
C	1.25	0.907	1.299	0.521	NS
Comparison between sexes of 3 groups were done by ANOVA test. p value > 0.05, not-significant at 5% confidence interval (CI) level. NS; not significant. S; significant					

Table- II (a) depicts that the mean ($\pm$ SD) length of inferior division of splenic artery was higher in male (1.47 ± 0.54) cm in Group A and (2.21 ± 0.75) cm in group B than that of female (1.42 ± 0.58) cm and (1.99 ± 0.63) cm in Group A and B respectively, but lower in Group C (2.16 ± 0.75) cm where mean length of inferior division of female splenic artery was (2.26 ± 0.51) cm in Group C. Variance analysis shows that there was no significant difference among sexes of the age groups ($p > 0.05$).

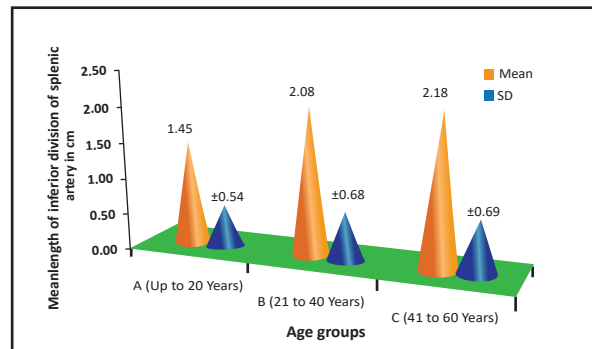


Figure A: Bar diagram representing the mean length of inferior division of splenic artery in different age groups.

Discussion

In our study, the maximum length of inferior division of splenic artery were 2.50, 4.00 and 4.00 cm in Group A, B and C respectively. The minimum length of inferior division of splenic artery was 0.50 cm in group A, 0.80 cm in group B and 0.50 cm in group C. The mean length of inferior division of splenic artery was maximum in group B, 2.21 cm in male and in group C 2.26 cm in female and minimum in group A, 1.47cm in male and 1.42 cm in female. The mean ($\pm$ SD) length of inferior division

of splenic artery was 1.45 ± 0.54 cm in Group A, 2.08 ± 0.68 cm in Group B and 2.18 ± 0.69 cm in Group C. Moreover, it was evident that the mean length of inferior division of splenic artery was increased with age. The mean difference of length of inferior division of splenic artery between group A & B and C & A was statistically not significant but B & C was statistically significant. The mean ($\pm$ SD) length of inferior division of splenic artery was higher in male (1.47 ± 0.54 cm) in Group A and (2.21 ± 0.75 cm) in group B than that of female (1.42 ± 0.58 cm) and (1.99 ± 0.63 cm) in Group A and B respectively, but lower in Group C (2.16 ± 0.75 cm) where mean length of inferior division of female splenic artery was (2.26 ± 0.51 cm) in Group C. Variance analysis shows that there was no significant difference between male and female among the age groups

In 2008 Chawdhury studied 120 spleens of Bangladeshi cadaver and found the maximum mean length of inferior divisional artery was in above 60 years age group (male was 2.21 cm and 2.30 cm of female). The minimum length was in up to 15 years age group (male 1.40 cm and 1.45 cm of female). The maximum and minimum mean length of present study in relation to different age and sex group was similar to the findings of Chawdhury (2008).¹⁰

The mean length of inferior divisional artery described by Rayhan (2006) was maximum 3.45 cm in 40-48 years age group and minimum 2.99 cm in 0-19 year's age group. The mean length of present study was lower than Rayhan because most of the values were close to the lower limit but the range was similar to the 0.50- 4.00 cm of present study.¹¹ In the present study the length of inferior divisional artery was 0.50- 4.00 cm which was within the range described by Michel's as 1-12cm in 1942 and common length 4 cm.¹² The terminal splitting pattern is bifurcated in 95.47% of individuals, and the presence of an inferior polar artery is directly correlated with a shorter distance to the hilum.¹³ Different authors have different inclusion

criteria and the results are stated in different ways, e.g. different age grouping, mean length or ranges, making it difficult to comparison with each other.

Conflict of Interest: The authors declare no conflict of interest.

Conclusion

From the present study, it was concluded that the mean length of inferior division of splenic artery was maximum in group B, 2.21cm in male and in group C 2.26cm in female and minimum in group A, 1.47cm in male and 1.42cm in female. The mean ($\pm$ SD) length of inferior division of splenic artery was 1.45 ± 0.54 cm in Group A, 2.08 ± 0.68 cm in Group B and 2.18 ± 0.69 cm in Group C. It was evident from the findings that mean length of inferior division of splenic artery was increased with age. It was also observed that the mean difference in length of inferior divisional artery was insignificant in between sexes of different age groups.

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A Case Report on Gangrenous Cholecystitis Operated Laparoscopically in Bangladesh

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Abstract

Gangrenous cholecystitis (GC) is a severe complication of acute cholecystitis, commonly affecting elderly patients with comorbidities such as diabetes and coronary artery disease. This case report presented a 75 years old diabetic male from Bangladesh with right upper quadrant abdominal pain, nausea, and vomiting. Ultrasound revealed gallstones, a thickened gallbladder wall, and pericholecystic fluid. Despite initial conservative management, the patient's condition deteriorated, necessitating laparoscopic cholecystectomy. Intraoperative findings included omental wrapping, subhepatic bile collection, and gangrenous patches on the gallbladder. The critical view of safety was achieved, and surgery was completed without major complications. Histopathology confirmed GC. Postoperative recovery was uneventful, with the patient discharged on the third day. This case highlights the importance of timely surgical intervention for GC, even in high-risk patients, to achieve favourable outcome. Laparoscopic cholecystectomy, when performed by an experienced team, remains the standard of care for such cases.

Keywords: Gangrenous cholecystitis, Acute cholecystitis, Laparoscopic cholecystectomy, Gallstones

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Introduction

Gangrenous cholecystitis (GC) is a serious complication of acute cholecystitis.^{1,2} GC is seen in 10%–40% of patients with acute cholecystitis.^{3–5} GC has been described since 1894, and figures vary depending on the percentage of acute cholecystitis (AC) that GC makes up. GC patients tend to be sicker than the average AC patients, and their surgical procedure tends to be more challenging and is associated with an increased risk of morbidity and mortality when compared to all causes of AC.⁶ GC risk factors include diabetes mellitus, males, older age, elevated white blood cells, and coronary heart disease.^{2,3} GC is the progression of AC, which is commonly due to obstruction of the cystic duct due to an impacted stone, which commonly dislodges on its own and does not progress to GC. If the stone does not dislodge, then there will be vascular compromise and epithelial injury leading to ischemia locally and necrosis of that region's gallbladder wall. These patients will require early interventions to

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decrease morbidity and mortality.^{7,8} The incidence of GC is more common in older patients and is associated with a higher white blood cell count (WBC) and diabetes.⁹

Case Presentation

A 75-years-old diabetic, normotensive male patient presented with a history of right upper quadrant abdominal pain, nausea, and occasional vomiting for 3 episodes over 5 days. He was admitted under a gastroenterologist at a tertiary care hospital in Bangladesh. USG showed a gall bladder containing multiple calculi and a thickened wall. Liver function tests were SGPT U/L, ALP U/L, S. Bilirubin, total count 16000/mm³. Initially, he was treated conservatively with broad-spectrum antibiotics, but as the condition was not improving clinically, he was referred to a consultant surgeon.

Laparoscopic cholecystectomy was planned. Intraoperatively, the gall bladder was wrapped with omentum. Bile was found in sub-hepatic space. GB was inflamed; part of the fundus and body were found with gangrenous patch and a clear demarcation line. Calot's triangle was oedematous and friable, but a critical view of

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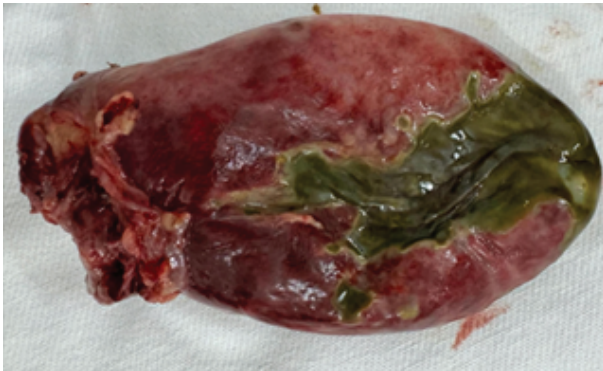


Photo 1: Perforated gangrenous Gall bladder

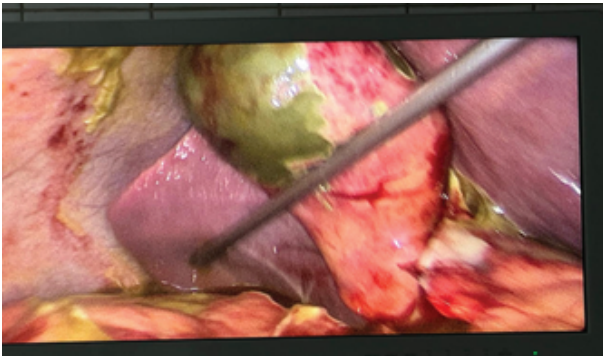


Photo 2: Exudation and bile under the right sub hepatic space

safety was established. Cystic duct was divided between clips. The cystic artery was divided using bipolar diathermy. Sub hepatic space collection was sucked out, irrigated with copious amount of normal saline. Gallbladder was retrieved through umbilical port by increasing the previous longitudinal incision generously.



Photo 3: There was adhesion among the gall bladder, gut and omentum

Postoperative period was uneventful. Minimal peritoneal collection was found at the subhepatic drain tube. Patient was kept on intravenous antibiotics and analgesics for 3 days and then discharged to home. The histopathological report of the gallbladder showed gangrenous cholecystitis. On follow up at 10 th post-operative day, the patient was good-looking with no wound complications.

Discussion

Acute calculus cholecystitis in the patients having gall stone may suffer from varieties of physical illness and may develop Gangrenous cholecystitis in around 2-29.6% of patients.^{10,11} There is an increased risk of GC from AC if the patient has diabetes, coronary artery disease, elevated white blood cells count and/or is elderly.² Gangrenous cholecystitis is defined as cholecystitis involving necrosis and the bleeding of tissues after oedematous alterations.¹²

The initial impaction of calculus at the infundibulum or cystic duct causes noticeable distension of gall bladder, which raises the GB wall's tension. Ischemic necrosis of the wall is caused by concomitant inflammation, and may be followed by secondary bacterial infection which may or may not be accompanied by cystic artery thrombosis.³ The obstruction of the cystic duct leads to prolonged increase in intraluminal pressure to the point where this pressure exceeds the arterial inflow causing ischemia.¹³

Diabetic patients due to neuropathy have less feeling of pain or may be asymptomatic were later found to have GC during surgery^{11,14} around 10% of such cases GB may found perforated.^{3,11} Biliary fluid may accumulate around the liver and grow to the area between the Glisson's capsule and the liver itself, known as the subcapsular space.¹⁵

Traditionally the first imaging modality used in patients with clinically suspected acute cholecystitis is ultrasonography, which showed the presence of thickening of gall bladder wall and pericholecystic fluid collections.¹⁶

The mainstay of the treatment is surgical intervention. However, due to chronic inflammation and adhesions, it can be challenging to visualize Calot's triangle.¹⁷ The first surgical option is laparoscopic cholecystectomy (LC) for such cases.¹⁸ However it has been reported in randomized controlled trial and meta-analysis that LC has an equivalent treatment outcome with open cholecystectomy along with a shorter postoperative admission duration, lower incidence of complications, and superiority in mortality and morbidity.¹³

Although achieving the anatomical critical view of safety is challenging, skilled teams and centres can accomplish it with minimal complications. In Bangladesh a study of 172 laparoscopic cholecystectomy procedure reveals less than 2% of major complications such as bowel & bile duct injuries which included 30 acute cases.¹⁹ In our case we have observed excellent post-operative outcomes and patient satisfaction with no per operative major injuries.

Conclusion

We experienced an elderly case of acute cholecystitis that later during cholecystectomy was found to be gangrenous gall bladder and spilled out bile at sub hepatic region. Definitive diagnosis prior to surgery is very difficult to be made in such cases. But if an appropriate treatment method can be carried out an elderly comorbid patient can be discharged to home with smiley faces without any adverse events.

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