ISSN 1813-0437
Key title: Sir Salimullah Medical College journal
Abbreviated key title: Sir Salimullah Med. Coll. j.

Sir Salimullah Medical College Journal

Volume 24 Number 2 July 2016

CONTENTS

An Official Journal of the Sir Salimullah Medical College Teachers' Association, Dhaka, Bangladesh

SIR SALIMULLAH MEDICAL COLLEGE JOURNAL

VOL. 24, NO. 2, JULY 2016

An Official Journal
of
Sir Salimullah Medical College Teachers' Association

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Date of Publication: August 2017

Jointly Published by
Dr. Sunirmal Roy
Publication Secretary
On behalf of Sir Salimullah Medical College Teachers' Association.

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Review and Action

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Editorial

Use of Immunohistochemistry in Surgical Pathology

Immunohistochemistry is a technique that microscopically detects cellular constituents via specific antibodies and is being used in the search for cell or tissue antigens ranging from amino acids and proteins to infectious agents and specific cellular populations. By the utilization of monoclonal and polyclonal antibodies for the detection of specific antigens in tissue sections, it is an extraordinarily powerful tool for the diagnostic surgical pathologist[1,2,3].

In 1941, this technique was first reported by Coons and his colleagues when they used fluorescein isothiocyanate (FITC)-labelled antibodies with a fluorescent dye to localize pneumococcal antigens in infected tissues. Later on different enzyme labels have been introduced, such as peroxidase and alkaline phosphatase,[4,5,6.7]

Regarding application of IHC, it is very useful to diagnose tumors of uncertain origin in both primary and metastatic from unknown primary. A panel of antibodies is used in such cases. The selection of antibodies depends on clinical history, morphological features and result of relevant investigations. Immunohistochemical stains for intermediate filaments are expressed by tumor cells (keratin, desmin, vimentin, neurofilaments, and glial fibrillary acidic proteins).[3,8]

It is commonly used to predict therapy and prognosis in tumors of breast. ER, PR. and Her-2/neu is being done in Bangladesh now.This method is now being used as prognostic markers of tumors by identification of enzymes, tumor specific antigens, oncogenes, tumor suppressor genes, and tumour cell proliferations markers.

This method is being used to confirm infectious agents in tissue such as Hepatitis B and C, Cytomegalovirus etc. This technique can also be used to detect organisms in cytological preparations including fluids, sputum sample, and FNAC (fine needle aspiration cytology) material. This method can also be helpfull in detecting pneumocystis from sputum of immuno-compromised patients[3].

This method is being used to determine the function of specific gene products, such as p53 [3]. It is also used to diagnose muscle diseases[9,] It is frequently being used to diagnose CNS tumors, haematolymphoid malignancy, anaplastic tumours, neuroendocrine malignancy and small round cell tumours. Most of the oral tumours needed immunohistochemistry for diagnosis in a study performed in Malaysia[10].

This valuable and essential technique though started earlier (1999) in Armed Forces Institute of Pathology of Bangladesh but still is being practiced only in a few large private hospitals and BSMMU. It is also available In a few private laboratories of Dhaka and Chittagong city[11]. However, it is still not available in Government Medical Colleges of Bangladesh. Introduction of this technique in medical college hospitals and specialized hospitals is urgently needed for proper diagnosis and treatment of patients in Bangladesh.

Postgraduate courses in Pathology (MD and FCPS) are being taught in BSMMU, BIRDEM and in a few large Government Medical colleges only .Moreover, research is an integral part of MD and FCPS degree. So for conduction of research of post graduate students in Pathology in Bangladesh, introduction of this technique in those hospitals is essential.

(Sir Salimullah Med Coll J 2016; 24: 38-39)

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Original Articles

Structural Variations of Nose and Paranasal Sinuses in Various Sinonasal Pathologies-Tomographic Study of 50 Cases in Bangladeshi People

Manilal Aich Litu, Sharfuddin Mahmud, Md. Monwarul Abedin Khan, Md. Asadur Rahman, DGM Akaiduzzam, Mohammad Amzad Hussain, Farhana Rahman

Abstract

Objective: *The aim of this study was to determine the frequency of structural variations in nose & Paranasal sinuses in computed tomography in Bangladeshi people.*

Methods: *The retrospective study was done at the Sir Salimullah Medical College Mitford hospital and Apollo Hospitals, Dhaka. Fifty CT scan of Nose and Para nasal sinuses were collected from the patients presented with different sinonasal pathologies in OPD, IPD of both hospitals from July 2013 to June 2014.The scans were reviewed for the presence of different structural variations of nose and paranasal sinuses.*

Results*: The age range of the patients were 25 to 65 years.The most common anatomical variation in this study was Hypertrophied Inferior turbinate (82%) followed by Ethmoidal bulla (70%), Deviated nasal septum (64%)), Agar nasi cell (40%), Concha Bullosa(38%) and Haller cell (10%). In most of the patients we found more than one variation.*

Conclusion*: There are wide range of anatomical variations in nose and paranasal sinuses which might be regarded as the aetiological factors of different sinonasal pathologies . To maximize patients' benefit and to avoid unexpected situations during surgeries as well as dreadful complications, individualized pre-planning through tomographic study should be considered.*

Key Words*: Tomography, Nasal septum, Inferior turbinate, Chonca bullosa, Ethmoidal Bulla.*

(Sir Salimullah Med Coll J 2016; 24: 40-44)

Introduction

Nose and paranasal sinus Computed Tomography (CT) has become a widely accepted tool for providing detailed anatomy of PNS[1]. Growing attention is now being directed toward the anatomy of nose and paranasal sinus, as the use of endoscopy for preoperative evaluation and operative treatment of paranasal sinus disease is increasing[2]. This changing scenario of widening spectrums for endoscopic sinus surgeries urge detailed anatomical acquaintance of the patient before surgery.

High resolution CT scanning of PNS provides excellent bony detail and soft tissue mapping[3].For this reason Computed Tomography of the paranasal sinuses has nowadays become the investigation of choice for radiological evaluation.

Different school of thoughts identified anatomical variations in nose and paranasal sinuses i.e. osteo-meatal complexes as the aetiology of sinonasal disease[4]. Incidence of this variations may differ among different ethnic group. Surprisingly, no data is available on anatomical variations of nose and paranasal sinuses in our population.

The aim of this study was to determine the frequency of structural variations in nose & Paranasal sinuses in computed tomography in Bangladeshi people.

Materials & Methods

This retrospective study was done over a period of one year (July 2013 to June 2014) comprised of CT evaluation of 50 patients with clinical symptoms

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of sinonasal pathologies who attended at the Dept of Otolaryngology & Head-Neck Surgery of SSMC & Mitford Hospital Dhaka and also from Apollo Hospitals Dhaka.

Patients with Rhinosinusitis, symptoms of nasal obstruction, Headache due to nasal pathologies, anosmia/hyposmia due to structural pathologies etc. were included in this study. Each scan was reviewed for the presence of haller cell, onodi cell, paradoxical middle turbinate, deviated nasal septum (DNS), pneumatisation in the nasal septum, superior and middle turbinate, and uncinate process.

Patients with septal pathologies like haematoma, abscess, perforation ,fractured nasal bone, nasal trauma, sinonasal malignancies and patient with previous nasal surgery were excluded from the study. The scans were 1 to 3 mm cut, 0 degree, coronal, axial and sagittal cuts.

A total of 50 patients were included in this study. Out of them 34 were male, 15 were femal, and a boy of 8 years old. The most common age group was between 20 to 40 years.

Results

Nasal septum: Deviation was present in 64% cases. Septal Mucosal hypertrophy was 20%cases.

Inferior Turbinate: Hypertrophied in 82% cases. It may be unilateral or bilateral.

Middle turbinate: Bilateral Concha Bullosa found in 30%,Unilateral Concha Bullosa in 8% and Paradoxical middle turbinate in 4% cases.

Haller cell: Present in 10% cases**.**

Agar Nasi cell: Found in 40% cases**.**

Uncinate Process: No pneumatization is seen.

Maxillary sinus: Normal shaped cavities present in 98% cases, rest are oval shaped.

Ethmoidal sinus: Ethmoidal bulla was found in 70% cases.

Sphenoidal sinus: Normal in almost all cases. Position of septum was not counted as variation.

Olfactory fossa: Keros type 1: 90% (less than 4mm).
Keros type-2: 10% (4 to 7 mm)
Keros type -3; 0% (more than 7 mm).

Space in nasal cavity: narrow in 4% cases.

Main anatomical variants found:

Table-1
Main anatomical variants found in Nose and Paranasal sinus

Type of structural Variations	Number	Percentage
Hypertrophied Inferior Turbinate	41	82%
Deviated Nasal Septum	32	64%
Concha Bulosa	19	38%
Ethmoidal Bula	35	70%
Agar Nasi cell	20	40%
Septal Mucosal Hypertrophy	10	20%
Onodi Cell	2	4%

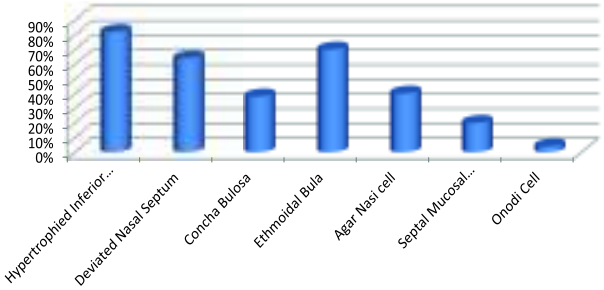

Fig.-1: *Frequency of different anatomical variation in nose and PNS (in percentage)*

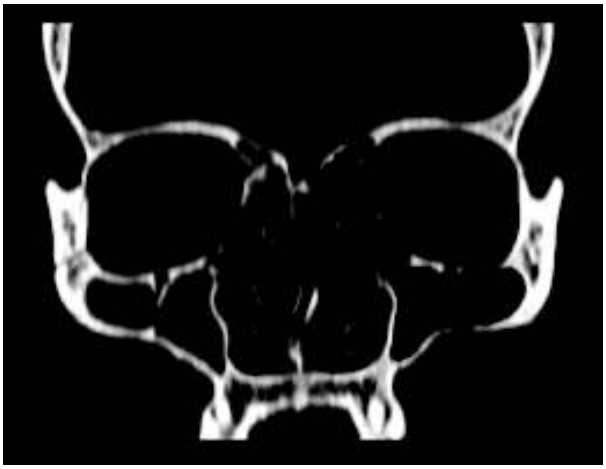

Figure 2. *Shows bilateral concha bullosa, DNS & septated right maxillary sinus.*

Discussion

Sinonasal diseases are common now-a- days. The under lying cause is anatomical variation in many cases. Only medical treatment cannot solve the problem, surgical intervention is must[4]. Endoscopic sinus surgery gives us that scope with minimal

intervention, short hospital stay and patient satisfaction. The complete knowledge of these variations is important before the surgical procedure to avoid dreadful complications.

1. **Hypertrophy of inferior turbinate**:The most common variation(82%) found in this study. Though it is not an anatomical variation but it is a structural variation. No such study was found about tomographic evaluation of hypertrophied inferior turbinate in sinonasal pathologies.

2. Deviated Nasal Septum

Nasal septal deviation has an important role in causing sinusitis. Asymmetric nasal septum can force nasal turbinates laterally and result in narrowing of the middle meatus and ultimately blocking drainage of the ipsilateral maxillary, anterior ethmoid and frontal sinuses[5]

In current study, DNS was the 2nd most common anatomical variation with prevalence rate of 64%. Prevalence of this particular anatomical variation ranging from 13% - 80% has been reported. Stammberger et al. (2005), considered notable DNS, only when it was more than 4 mm deviation and found its prevalence 15%to 20%[6]. On the other hand, Perez-Pinas et al. (2000) considered DNS, when any visually detectable nasal deviation from the midline was seen and observed prevalence of it to be 80%[5]. Different criteria applied to diagnose and consider septum to be deviated in different studies, accounted for variation in prevalence.

Majority of the studies showed DNS as the most common anatomical variations[7], Y. Talaiepour et al. (2005)[8] had prevalence rate of DNS as an anatomical variation 65%, 41.9%, 65% and 65.2% in their respective studies.

3. Concha Bullosa

The middle nasal concha is normally a flat bone. When it is pneumatised by extension of anterior ethmoid cells or less frequently, posterior ones, it is referred to as Concha Bullosa[10]. The true concha bullosa is produced following pneumatisation of both portions (vertical lamina and inferior bulb) of the middle nasal concha[11]. Lamellar pneumatisation and conchal Pneumatization, both were included in Concha Bullosa in our study and with this criteria, its prevalence rate was 38%.

As per Stammberger et al. the concha bullosa must be distinguished from an interlamellar cell, which arises from pneumatization of the vertical lamella of the middle turbinate from the superior meatus[6]. Perhaps due to this, prevalence of Concha Bullosa varied from 11.5% (A. K. Gupta et al., 2012 [14]to 53% (Bolger et al., 1991)[15].

Talaiepour et al. (2005)[8]had seen Concha Bullosa in 35% subjects, which nearly corresponds to our study-data.

4. Agger Nasi Cell

The most anterior cells of the anterior ethmoid group, the prevalence of Agger Nasi cell ranges widely in different studies which can be attributed to loose anatomic definitions or due to technical miss-match. Herein the Agger Nasi cells are defined as those lying anterior to the upper end of the nasolacrimal duct[7]. The frequency of Agger nasi cell (AN cell) in our study population was 40%.

Bolger et al. (1991)[8] reported very high prevalence (98.5%) of Agger Nasi cell. A. K. Gupta et al. (2012)[14] observed a prevalence rate of 68.8%; Tonai and Baba's (1996)[16]and Talaiepour et al.'s (2005)[8]found prevalence of 56.7%.

Study by Badia L. et al. (2005)[9] revealed presence of AN cell in 44% - 57% of British population. Lower prevalence of AN cell has been reported by PerezPinas et al. (2000)[5] 2.7%, S. Lerdlum et al. (2005)[11] 7.9% and Kasapoglu et al. (2009)[12] 4.7%. Reason may lie in not so fixed criteria for diagnosis.

5. Pneumatised Septum

Pneumatised septum, an important anatomical variation, can compress the osteomeatal complex and has a potential to induce sinonasal mucosal diseases. The prevalence rate of Pneumatised septum in our study was 0%. . K. Dua et al. (2005) reported prevalence of 2% for pneumatised septum (pneumatisation of Vomer) [10].

6. Paradoxical Middle Turbinate

It is an anatomical variation of middle turbinate, where in it's convexity is reversed to face laterally. However it is not associated with any change in the normal middle turbinate attachments. This

may lead to impingement of the middle meatus and thus sinusitis or other mucosal diseases of sinus, specially the large ones[7].

In present study, prevalence of Paradoxical Middle Turbinate was 4%, which is similar to the study of K. Dua et al. (2005)[10]. But, Bolger et al.'s (1991)[15], Tonai and Baba's (1996)[16] and Fikret K. et al.'s (2009)[16] noted greater prevalence rate of 26.1%, 25.3% and 16.3% respectively. S. Lerdlum et al. (2005)[11] reported lower prevalence rate (5.3% only).

7. Septated Maxillary Sinus

Maxillary sinus was septated in this study in 0% of patients. Relatively lower prevalence rate of 6% and 2.1% were observed by K. Dua et al.'s (2005)[10] and A. K. Gupta et al.'s (2012)[14] respectively.

8. Haller Cell

The potential pathophysiologic importance of a Haller's cell is clear, but not the anatomic definition. As described by Albert von Haller in 1765, these cells grow into the bony orbital floor that constitutes the roof of the maxillary sinus[6]. The definition of ethmoid cells given by Haller in eighteenth century is now controversial. Some authors (Kennedy and Zinreich, 1988) considered Haller cell as ethmoid cells which are the air cavities projecting below the ethmoid bulla within the orbital floor in the region of the opening of the maxillary sinus[6]. However, Bolger et al. (1991) broadened the term to include any cell located between the ethmoidal bulla, the orbital lamina of the ethmoid bone and the orbital floor[15].

Considering the criteria laid down by Haller, our study showed a prevalence of 10%. It is nearly similar to the prevalence reported by S. Lerdum et al. (2005)[11](9.4%) and Fikret K. et al. (2009)[12] (9.3%). High prevalence was noted by Bolger et al. (1991)[15] (45.1%), Tonai and Baba (1996)[16] (36%), Badia L. et al (2005)[9] (10% - 15%), K Dua et al. (2005)[10] (16%), H. Mamatha et al. (2010)[13] (17.5%) and Talaiepour et al. (2005)[8] (3.5%)

9. Pneumatised Uncinate Process

Pneumatised uncinate process as an anatomical variation was seen in 0% of patients, in the present study. This is quite comparable to the prevalence of 4.7% and 4.34%, reported by Fikret et al. (2009)[12] and A. K. Gupta et al. (2012)[14] respectively. Bolger et al. (1991)[15] found it in only 2.5% cases.

Conclusions

Thus there is definite structural variations in nose and paranasal sinuses in patients suffering from different sinonasal pathologies. Only tomographic study can identify them accurately.So before going any surgical intervention or after failure of medical treatment tomographic study of nose and paranasal sinuses is must.

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Anandaraja's Formula for Calculation of LDL Cholesterol: An Alternative to Friedewald's Formula?

Nadia Nasrin[1], Md Mozmmel Hoque[2], Shaikh Nazmus Saqueeb[3], Md Shiblee Zaman[4]

Abstract:

LDL cholesterol is the main lipid marker in cardiovascular risk estimation and principal therapeutic target for prevention of atherosclerosis. Friedewald's formula is used in most of the laboratories to estimate LDL cholesterol. This formula cannot give accurate result and it has several limitations also. Anandaraja et al. proposed a new formula for estimation of LDL cholesterol as a substitute of Friedewald's formula without the use of HDL cholesterol. In this study we assess the validity of Anandaraja's formula as an alternative to Friedewald's formula. Our study included 460 study subjects and categorized as normolipidemic and dyslipidemic group. Friedewald's formula and Anandaraja's formula were compared to a direct homogenous method for estimation of LDL cholesterol. Comparison was done by Pearson's correlation test, agreement was done by Bland-Altman agreement test between measured and calculated LDL cholesterol. The limits of agreement were lower for all, normolipidemic and dyslipidemic subjects by Friedewald's formula. Friedewald's formula showed better agreement with measured LDL cholesterol (Direct method) than Anandaraja's formula for approximate calculation of LDL cholesterol.

***Key words**: Friedewald's formula, Anandaraja's formula, LDL cholesterol*

(Sir Salimullah Med Coll J 2016; 24: 45-50)

Introduction

Raised plasma LDL cholesterol concentration is an established risk factor for coronary heart disease among all lipoproteins[1]. Atherosclerotic lesion is strongly correlates with increased level of serum LDL cholesterol. If the level of serum LDL cholesterol deceases the incidence of cardiovascular events will also decreases[2]. The reference method for the measurement of serum LDL cholesterol is the ß-Quantification that is costly, labor intensive and requires expensive ultracentrifuges, rotors, and tubes[3]. It is time consuming and only a few number of samples can be investigated a day[4]. That is why most of the clinical laboratories estimate LDL cholesterol by Friedewald's formula in serum[5].The Friedewald's formula is LDL-c (mg/dL) = TC (mg/dL) " HDL-c (mg/dL) " TG (mg/dL)/5. TG is mainly from chylomicrons and VLDL[6]. Friedewald's have formula several limitations and cannot apply in samples with TG> 400ml/dl, in hyperchylomicronemia and in case of patient with dysbetalipoproteinemia[7-8]. Recently direct homogenous assays have been developed for estimation of serum LDL cholesterol with a satisfactory degree of accuracy[9]. These methods are quite expensive for most of the laboratories, thus direct estimation of LDL cholesterol is uncommon in most of the laboratories in developing countries[10-11]. Anandaraja et al. proposed a new formula[12] for calculation of LDL cholesterol without the use of HDL cholesterol. This formula need only two analytes so chances of analytical error are decreased[13].This study was done to assess the applicability of Anadaraja's formula for calculation of LDL cholesterol in Bangladeshi population as an alternative to Friedewald's formula. If this formula is found to be more valid, it can be proposed to be used clinically for correct estimation of LDL cholesterol with minimum cost and time.

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Materials and methods

This cross sectional study was conducted during the period from January, 2014 to December, 2015, in the department of Biochemistry, BSMMU, Shahbagh, Dhaka. Blood samples were obtained from 460 study subjects who came in one point collection center of BSMMU by non probability sampling. Fasting venous blood were collected in blank tubes and allowed to clot at room temperature, then serum was obtained by centrifugation at 3000rpm for 10-15 minutes. All blood lipid analyses (Serum triglyceride, HDL cholesterol and total cholesterol) were performed within 1day. Serum was stored in ultra freezer at -20p c for the measurement of LDL cholesterol directly. All lipid parameter are (Serum triglyceride, HDL cholesterol, total cholesterol and LDL cholesterol) were measured by using the ARCHITECT auto analyzer System (Abbott Diagnostics, USA) in the department of Biochemistry, BSMMU. All kits, calibrators and quality control materials were obtained from Abbott Diagnostics, USA through local distributor. LDL cholesterol was also calculated by Friedewald's formula (LDL-C=TC-HDL-C-TG/5) and Anandaraja's formula (LDL-C=0.9TC-0.9TG/5-28). According to the third report of the National Cholesterol Education Program Adult Treatment Panel III[14], study subjects were categorized as-normolipidemic subjects and dyslipidemic patients. Patients having TG ≥ 400 mg/dL were excluded when LDL cholesterol was calculated by Friedewald's formula. Statistical analysis was performed by statistical package for social science (SPSS). Results was expressed as mean ± SD. Comparison was done by Pearson's correlation test between measured LDL cholesterol and calculated LDL cholesterol by Friedewald's and Anandaraja's formula. Agreement between measured LDL cholesterol and calculated LDL cholesterol by Friedewald's and Anandaraja's formula was done by Bland-Altman agreement test[15-16]. A p-value of <0.05 was considered as statistically significant.

Results

The mean age of the 460 study subjects were 45±12 years, out of them 71 were found normolipidemic and 389 were found dyslipidemic. 47(66.2%) were male, 24(33.8%) were female and 226(58.1%) were male and 163 (41.9%) were female out of 71 normolipidemic and 389 dyslipidemic subjects respectively. The mean concentration of TC, TG and HDL cholesterol were 129.11, 111.99, 47.87 mg/dL and 204.06, 198.21, 37.26 mg/dL in case of normolipidemic and dyslipidemic subjects respectively. The mean values of LDL cholesterol measured by direct method, Friedewald's formula and Anandaraja's formula were 132.99, 121.39 and 111.92±39.40 mg/dL respectively. Patients having TG ≥ 400 mg/dL were excluded while calculating Friedewald's formula. Correlation of measured LDL cholesterol (direct method) with calculated LDL cholesterol in all, normolipidemic, dyslipidemic subjects, showed significant positive correlation between measured and calculated methods (Table-I).

Bland- Altman agreement plot was done to see the agreement between measured LDL cholesterol (Direct method) and calculated LDL cholesterol by Friedewald's formula and Anandaraja's formula for all(Table-II & Figure-1), normolipidemic(Table-III & Figure-2), dyslipidemic subjects (Table-IV & Figure-3) within 95% limit. For all study subjects within 95% limit the mean difference was 7.19, 21.07, upper limits of agreement was 58.19, 95.78 and lower limit of agreement was -43.82, -53.64 for Friedewald's formula and Anandaraja's formula respectively (Table-II & Figure-1).

Table I

Correlation of measured LDL cholesterol (direct method) with calculated LDL cholesterol

Calculated method	Total subjects (n = 460)		Normolipidemic (n = 71)		Dyslipidemic (n = 389)	
	r value	p value	r value	p value	r value	p value
Friedewald's Formula	0.749#	<0.001	0.696	<0.001	0.635#	<0.001
Anandaraja's Formula	0.500	<0.001	0.351	0.003	0.320	<0.001

#Patients having TG ≥ 400 mg/dL were excluded.

Pearson's correlation was done to measure the level of significance.

Table II

Summary of Bland-Altman agreement plot between measured LDL-C and calculated LDL-C for all study subjects

	Mean	SD	Upper limit of agreement	Lower limit of agreement	Limit of agreement
Difference between LDL-C (Direct) and LDL-C (Friedewald's Formula) #	7.19	26.02	58.19	-43.82	102.01
Difference between LDL-C (Direct) and LDL-C (Anandaraja's Formula)	21.07	38.12	95.78	-53.64	149.42

#Patients having TG ≥ 400 mg/dL were excluded.

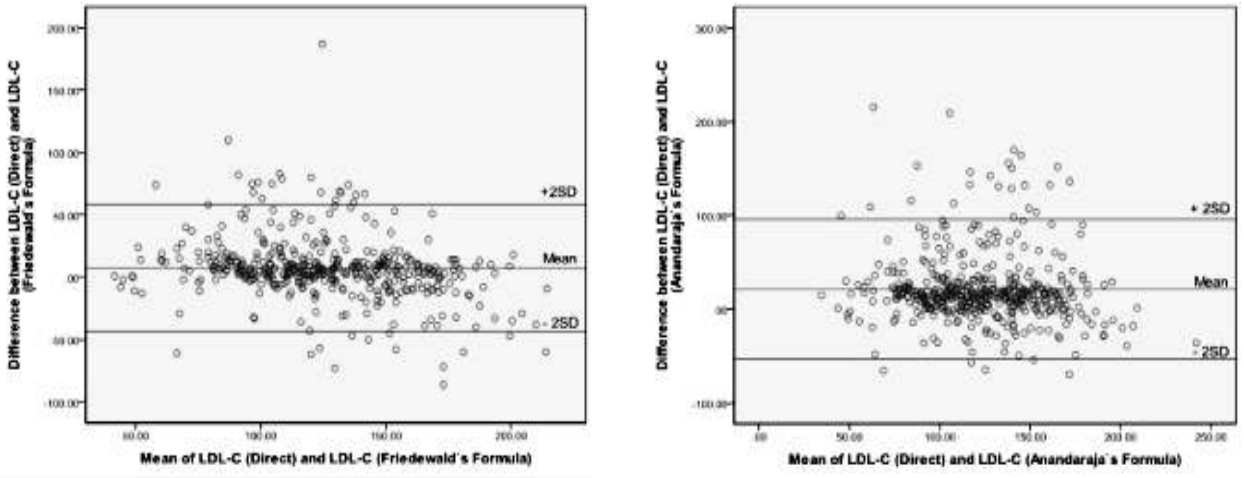

Fig.-1: *Bland-Altman agreement plot of the measured LDL cholesterol (direct method) and calculated LDL cholesterol for all study subjects (Patients having TGe" 400 mg/dL were excluded in case of Friedewald's formula).*

For normolipidemic subjects within 95% limit the mean difference was 11.04, 17.32, upper limits of agreement was 38.04, 57.67 lower limit of agreement was -15.96, -23.02 for Friedewald's formula and Anandaraja's formula respectively (Table-III & Figure-2).

Table III

Summary of Bland-Altman agreement plot between measured LDL-C and calculated LDL-C for normolipidemic subjects

	Mean	SD	Upper limit of agreement	Lower limit of agreement	Limit of agreement
Difference between LDL-C (Direct) and LDL-C (Friedewald's Formula)	11.04	13.77	38.04	-15.96	53.99
Difference between LDL-C (Direct) and LDL-C (Anandaraja's Formula)	17.32	20.59	57.67	-23.02	80.70

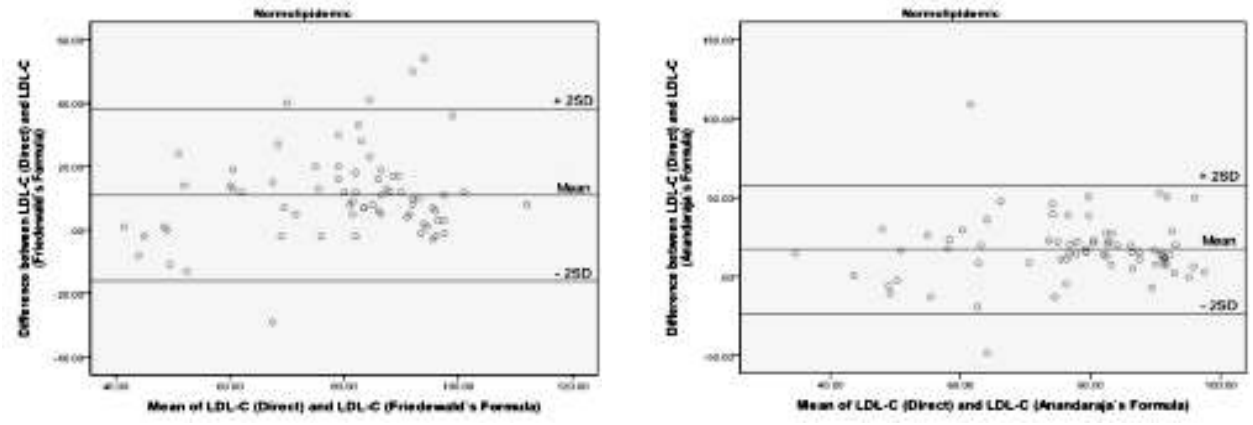

Fig.- 2: *Bland-Altman agreement plot of the measured LDL cholesterol (direct method) and calculated LDL-C for normolipidemic subjects*

Bland-Altman plot showed better agreement of the Friedewald's formula than Anandaraja's formula in dyslipidemic subjects as limits of agreement was lower in case of Friedewald's formula (60.81,- 47.95 vs 101.11, -57.61) within 95% limit (Table-IV & Figure-3).

Table IV

Summary of Bland-Altman agreement plot between measured LDL-C and calculated LDL-C for dyslipidemic subjects

	Mean	SD	Upper limit of agreement	Lower limit of agreement	Limit of agreement
Difference between LDL-C (Direct) and LDL-C (Friedewald's Formula) #	6.43	27.75	60.81	-47.95	108.76
Difference between LDL-C (Direct) and LDL-C (Anandaraja's Formula)	21.75	40.49	101.11	-57.61	158.71

#Patients having TG ≥ 400 mg/dL were excluded.

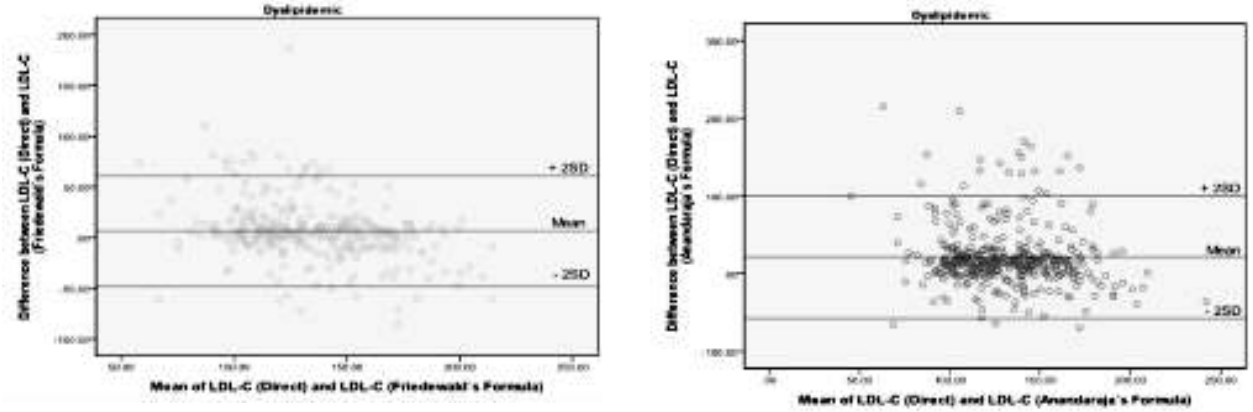

Fig.-3: *Bland-Altman agreement plot of the measured LDL-C (direct method) and calculated LDL-C for dyslipidemic subject (Patients having TG ≥ 400 mg/dL were excluded)*

Discussion

Accurate estimation of LDL cholesterol is crucial in the management of ishchemic heart disease patients[17]. However Friedewald's formula is used in most of the laboratories, it underestimates and sometimes overestimates LDL cholesterol compared to direct method[18]. In Parvin et al reported the mean ± SD of measured LDL cholesterol was138.3 ± 54.58 mg/dL, Friedewald's formula was 123.5 ± 65.75 mg/dL respectively. Compared to measured LDL cholesterol Friedewald's formula was 17.20 mg/dL lower[19]. In our study the mean concentration of total cholesterol, triglyceride and HDL cholesterol were 129.11mg/dl, 111.99mg/dl, 47.87mg/dl in case of normolipidemic and 204.06mg/dl, 198.21mg/dl, 37.26mg/dl in case of dyslipidemic subjects. The mean values of LDL cholesterol measured by direct method, Friedewald's formula and Anandaraja's formula were 132.99, 121.39 and 111.92 mg/dl respectively.

Sahu et al. found a good correlation between LDL cholesterol calculated by Friedewald's formula with measured LDL cholesterol correlation coefficient (r) was 0.88[20].In all, normolipidemic and dyslipidemic study subjects the correlation coefficient of LDL cholesterol calculated by Friedewald's formula with the measured LDL cholesterol was statistically highly significant and better than the correlation coefficient of LDL cholesterol calculated by Anandaraja's formula with the measured LDL cholesterol (0.749 vs 0.500,0.696 vs 0.351, 0.635 vs 0.320, $p< 0.001$) (Table-I). A similar study was done in Bangladesh by Kamal et al. they found a strong correlation between measured LDL-C and calculated LDL-C (Friedewald's formula and Anandaraja's formula). Correlation between measured LDL cholesterol with Friedewald's formula ($r =0.786$, $p < 0.001$). Correlation of measured LDL cholesterol with Anandaraja's formula ($r = 0.810$, $p < .001$)[1]. In a similar study in Brazil over 10,000 people, Gasko reported better performance of Anandaraja's formula compare to direct homogenous method than Friedewald's formula[21].

In this study Bland-Altman plot showed better agreement for the Friedewald's formula than Anandaraja's formula in all (58.19,-43.82 vs 95.78,-53.64,) (Table-II & Figure-1), normolipidemic (38.04,-15.96 vs 57.67, -23.02) (Table-III & Figure-2) and dyslipidemic (60.81, -47.95 vs 101.11, -57.61) (Table-IV& Figure-3) subjects as limits of agreement was lower in case of Friedwald's formula within 95% limit. Some study supports our study. Gupta et al. found Friedewald's formula was better than Anandaraja's formula for estimation of LDL cholesterol. Limits of agreement were lower in Bland-Altman graphs showed better agreement for the Friedewald's formula than Anandaraja's formula[8].

Conclusion

From this study it may be concluded that estimation of LDL cholesterol by Anandaraja's formula did not show better agreement with measured LDL cholesterol (Direct method). So Friedewald's formula was better than Ananaraja's formula for calculation of LDL cholesterol.

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Frequency of Hepatitis B and C Viral Infection Among the Medical Waste Handlers

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Abstract

Chronic viral hepatitis is a major health problem worldwide. Medical waste handlers (MWH) are particularly vulnerable to such hepatitis due to nature of their job. This cross-sectional study was done from January 2015 to June 2015 to know the frequency Hepatitis B and Hepatitis C among medical waste handlers in SSMC Mitford Hospital. 96 medical waste handlers wereenrolled. The mean age was 38.39 (SD ±10.057) years (range: 20 to 60 years). The leading age group was 31-40 years (32%). Most of them had little educational attainment and more than half of the respondents (53.1%) were working in the hospital for more than 10 years. Frequency of HBV and HCV were 6.3% and 1% respectively in MWH. More percentage of HBsAg was identified in female (8.1%), in age group between 30-39 years (17.9%), in MWH who were in the service for 7-10 years. 7.2% of the MWHs were found to have needle stick or sharp injuries while 7.7% had mucous membrane contamination. More than three-fourths of the MWHs wore thick disposable gloves, 30 (31.3%) protective gown and only 14 (14.6%) wore boots. Male MWHs were significantly more likely to wear Boots (OR: 1.505: P < 0.002) compared to Female. Due to needle stick puncture, infectious disease like Hepatitis B & C can be transmitted to the health care workers. Health education, prophylaxis by vaccination universal precautions and proper hospital waste management are crucial in the prevention of HBV and HCV infection.

Key words: *Medical waste handlers,Hepatitis B, Hepatitis C.*

(Sir Salimullah Med Coll J 2016; 24: 51-56)

Introduction

Viral hepatitis is inflammation of liver due to viral infections. It is caused by Hepatitis A, B, C, D and E Virus and also some other viruses.[1,2] Among them chronic viral hepatitis are caused by Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV). Chronic viral hepatitis subsequently causes cirrhosis of liver, liver failure, hepatocellular carcinoma and death. So chronic viral hepatitis is a major health problem worldwide. Viral hepatitis is the tenth leading cause of death and the leading cause of hepatocellular carcinoma worldwide.[3, 4] HBV and HCV can be ended with development of cirrhosis and hepatocellular carcinoma.[5] More than 500 million people worldwide are persistently infected with either of these two viruses thus presenting a major global health problem.[6] Because the two hepatotropic viruses share the same modes of transmission, co-infection with the two viruses is common, especially in areas with a high prevalence of HBV infection and among people at high risk for infection.[7, 8] There are several million carriers worldwide which provide a huge reservoir for HBV and HCV. It may progress to chronic liver disease (CLD) including hepatocellular carcinoma.[9,10] According to the estimate by World Health Organization (WHO), about two billion people worldwide have been infected with HBV and about 350 millionpeople become chronic carriers and over one million people die each year as a result of acute fulminate liver disease or HBV induced cirrhosis and liver cancer.[7,8]The burden of HBV infection is highest in the developing world particularly in Asia and

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sub-Saharan Africa.[9-11]WHO estimated that the prevalence of HBV infection in Africa is more than 10%.[12, 13]WHO estimated that approximately 170 million people are infected with HCV and about 130 million are carriers and three to four million persons are newly infected each year and more than 350,000 people estimated to die from hepatitis C-related liver diseases each year worldwide .[14-16]HCV infection in the world varies from 0.3 to 13% or more with the highest prevalence recorded in Central Africa and South-Eastern Asia.[17, 18]

Both HBV and HCV are an important occupational hazard for medical waste handlers and chronically infected HBV and HCV carriers are able to transmit through contact with their blood and body fluids, which includes occupational exposure to their blood and body secretions. The current treatment for hepatitis B virus infection is not curable after the infection progress to chronic stage and very expensive for individuals in developing countries like Bangladesh. Thus early screening of People who are at risk including medical waste handlers is mandatory.[19]

Generally, medical waste handlers (MWH) who are working in collection, transportation, cleaning and disposal of medical wastes in health institutions have been consistently shown to have higher prevalence of HBV and HCV infection than non-clinical waste handlers that directly or indirectly have no contact with medical wastes.[20, 21]

Objectives of the present study to know the frequency Hepatitis B and Hepatitis C among medical waste handlers in Bangladesh.

Study regarding prevalence of hepatitis B and hepatitis C among medical waste handlers is uncommon in Bangladesh. So this study may produce awareness among medical waste handlers working in different hospital and thus helping the management of such case in future.

Method

This was an observational, cross-sectional study. The study period was from January 2015 to June 2015 and was conducted in Sir Salimullah Medical College Mitford Hospital, Dhaka, Bangladesh. Total 96 medical waste handlers of Sir Salimullah Medical College Mitford Hospital, working for at least one year were enrolled in the study.Ethical approval from the ethical approval committee of Sir Salimullah Medical College Mitford Hospital was obtained prior to the commencement of the study. Informed written consent was taken from the participant after explaining all the facts. Primary data was collected by face to face interview of the medical waste handlers. Individual who have been previously positive for HBV and HCV ,who are vaccinated for HBV,having any history of unsafe blood transfusion,parenteral drug abusers, taking acupuncture treatment, having spouse of hepatitis B and/or hepatitis C patient. Medical waste were excluded from the study as defined by Waste generated by health care activities includes a broad range of materials, from used needles and syringes to soiled dressings, body parts, diagnostic samples, blood, chemicals, pharmaceuticals, medical devices and radioactive materials. The people who handle these waste products are medical waste handler. In this study we selected medical waste handlers as follows Sweeper working in ward, laboratory and Operation Theater, Ward boy, OT boy, Aya who works in wards. Hepatitis B virus infection was defined by HBsAg and/or anti-HBc positive detected by ELISA Method. In our study we defined Hepatitis C viral infection as Anti-HCV antibody positive detected by ELISA Method. Collected data were sorted and screened for any discrepancy. The scrutinized data was then entered on to the template of SPSS® 20. Data were presented in the form of table and graphs. Descriptive statistics was presented with frequency table. Association was done by test statistics. Chart was generated to illustrate descriptive statistics. Sampling technique and data collection process through enumerators were performed very carefully.

Result

Ninety six medical waste handlers working at Sir Salimullah Medical College Mitford Hospitalwere enrolled during the study period. Mean age ranged from 20 to 60 years. The mean age was 38.39 (SD ±10.057) years. The leading age group was 31-40 years with more than 32% presentation. More than 28% respondents' age was 30 years or less. Almost equal numbers (26) of respondents were from 41-50 years age group. Majority of respondents were male (59/96) and the remaining (37/96) were female. Most of the respondents (67/96) were working in the general ward.

About 19% respondents were the employee of operation theatre.Least numbers of respondents were working at emergency department, laboratory (4.2% each) or in the labourroom.Out of 96 patients 82 (85%) were married. The remaining 14 (15%) respondents were single. Majority of the respondents (52, 54%) had attained up to primary level education. Remaining forty six percent respondents at least completed secondary level education. More than half of the respondents (53.1%) were working in the hospital for more than 10 years. About 20% respondents had 4-6 years working experience.In 86.5% cases there were H/O needle prick & others sharp object injury; however, in most of the cases (86.7%) the injury occurred occasionally. In 81.3% cases splash of body fluid to the eye, nose & mucus membrane happened. In most of the case the accident occurred occasionally.

In case of needle prick & others sharp object injury 86.7% cases asked for expert consultation but most of them (96.2%) disinfected the material. Only 3 respondents did not take any action.

Out of 96 respondents only 6 (6.3%) patients were found to be HBsAg positive. However, most of the

Table-I

Use of Personal Protective Equipment (PPE) among male & female medical waste handlers.

Type of PPE	Male(N=59) n (%)	Female(N=37) n (%)	Total(N=96) n (%)	OR	95% CI	p-value
Thick disposable glove	42 (71.2)	31 (83.8)	73 (76.0)	1.091	0.739-3.917	0.159
Face mask	37 (62.7)	22 (59.5)	59 (61.5)	0.832	0.357-1.942	0.671
Boots	11 (18.6)	3 (8.1)	14 (14.6)	0.385	0.100-1.485	0.155
Protective gown	20 (33.9)	10 (27.0)	30 (31.3)	0.722	0.292-1.783	0.482

Table-II

Distribution of anti-HCV antibody in relation to Sociodemographic characteristics of medical waste handlers (MWH)

Socio-demographic characteristics	N (%)	Anti-HCV antibody in MWH (n=96)		
		n (%)	OR (95% CI)	p-value
Gender				
Male	59 (61.5)	3 (5.1)	0.565 (0.042-7.557)	0.666
Female	37 (38.5)	1 (2.7)		
Age (yrs)				
20-29	26 (27.1)	0 (0.0)	1	
30-39	28 (29.2)	2 (7.1)	5.549 (UD)	0.997
40-49	24 (25.0)	1 (4.2)	3.09 (UD)	0.998
≥ 50	18 (18.8)	1 (5.6)	1.126 (0.060-21.158)	0.937
Length of service (yrs)				
1-3	51 (53.1)	2 (3.9)	1	
4-6	13 (13.5)	1 (7.6)	UD	0.999
7-10	19 (19.8)	1 (5.3)	UD	0.997
>10	13 (13.5)	0 (0.0)	UD	0.998
Level of education				
Primary or less	44 (45.8)	1 (2.3)	0.953 (0.027-33.387)	0.979
Secondary or more	52 (54.2)	3 (5.7)		
Marital status				
Married	82 (85.4)	4 (4.9)	3.377 (UD)	1.000
Unmarried	14 (14.6)	0 (0.0)		

UD= Undefined; MWH= Medical waste handlers; OR= Odds Ratio; N = Total number of individuals in each category; n = Total number of positive individuals in each category

Table-III

Distribution of HBsAg, anti-HBc antibody and anti HCV antibody positivity among medical waste handlers with potential risk factors.

Risk factors	N (%)	n (%)	p-value*
For HBsAg			
Needle prick and other sharp object injury	83 (56.5)	6 (7.2)	1.00
Body fluid splash in MM	78 (81.3)	6 (7.7)	
For anti-HBcAg			
Needle prick and other sharp object injury	83 (56.5)	4 (4.8)	1.00
Body fluid splash in MM	78 (81.3)	4 (5.1)	
For anti HCV			
Needle prick and other sharp object injury	83 (56.5)	4 (4.8)	1.00
Body fluid splash in MM	78 (81.3)	4 (5.1)	

* Fisher's Exact test

respondents (90, 93.8%) were not infected with hepatitis B virus. Out of 96 respondents only 1 (1.0%) patient as found to be infected with HCV.

Bivariate analysis of HBV prevalence in MWHs indicated more percentage of HBsAg was identified in female (8.1%), in age group between 30-39 years (17.9%), in married person (7.3%), in MWH who were in the service for 7-10 years. However; none of them showed statistical significant association. Bivariate analysis in MWHs indicated more percentage of anti HBcAb was identified in female (5.4%), in age group between 30-39 years (10.7%), in married person (4.9%), in MWH whose length of service was more than 10 years. However; none of them showed statistical significant association ($p>0.5$).Bivariate analysis of HCV prevalence in MWHs showed more percentage of anti HCV antibody was identified in male (5.1%), in age group between 30-39 years (7.1%), in married person (4.9%), in MWH who were in the service for 4-6 years (7.6%). However; none of them showed statistical significant association ($p>0.05$).

Almost equal proportion of HBsAg was identified in those with a history of needle prick (including other sharp object) injury and body fluid splash in mucous membrane (7.2% and 7.7% respectively). This statement was also applicable for anti-HBcAb and For anti HCV antibody. However, none of the studied risk factors was associated with HBV or HCV markers.

Table III shows use of Personal Protective Equipment (PPE) among male & female medical waste handlers.More than three-fourths of the medical waste handlers wore thick disposable gloves, 59 (61.5%) face masks, 30 (31.3%) protective gown and only 14 (14.6%) wore boots. Male MWHs were significantly more likely to wear Boots (OR: 1.505: $P < 0.002$) compared to Female. However; use of PPE was not associated with gender ($p>0.5$).

Discussion

Medical waste poses the potential problems to health care workers, particularly to waste handlers.[23,24]Among the biohazards the occupational risks posed by hepatitis B virus (HBV), hepatitis C virus (HCV) and HIV are well documented.[36] HBV has been found to infect about 350 million people globally.[25] Hepatitis B and C are global problems mostly in the developing countries. Hepatitis B virus is one of the major public health problems globally and is the tenth leading cause of death. In a study HBsAg prevalence among the general population in Bangladesh was reported as 5.5%, which places our country in an intermediate HBV endemicity zone.[22]In Bangladesh representative population study regarding the prevalence of the HCV is lacking. Except for the work by a Japanese group, that reported 5% prevalence of HCV in Bangladesh,[26] others reported extremely low prevalence of HCV in Bangladesh.[27]

The present study shows prevalence of HBV and HCV were 6.3% and 1% respectively in MWHs,

which is comparable with other studies. Vipul MK [28] reported 2.4% prevalence of HBV while Attaullah et al reported 2.18% prevalence of the same [29]. Alam S et al. [30], Petrosillo N et al.[31]and Arankalle VA et al.[32] reported HCV prevalence as 1.4%, 2% and 4% respectively.

In married person (4.9%), in MWH who were in the service for 4-6 years (7.6%), none of them showed statistical significant association. These findings are well supported by that of the Vipul et al. study who reported almost similar results.[28]

More percentage of HBsAg was identified in female (8.1%), in age group between 30-39 years (17.9%), in married person (7.3%), in MWH who were in the service for 7-10 years. However, none of them showed statistical significant associationwhich is in correlation with the study of Vipul MK[28]. One study in Ethiopia reported high prevalence rate of HBsAg (6.0%) positivity among female as compared to male (1.0%).[31]Totally opposite result reported in Libyan's study where none of female medical waste handlers were positive compared to 2.9% HBsAg positivity in male [33].

Sharps injuries & splash exposures Blood Born Virus (BBV) infection may follow needle or sharps injury, contamination of pre-existing skin lesions or splash inoculation to the eyes, nose or mucous membranes.[34]The present study reported that, 7.2% of the MWHs were found to have needle stick or sharp injuries while handling medical waste. This finding was inconsistent with the studies revealed in Nigeria where needle stick injuries among healthcare workers were the commonest forms of exposure to HBV infections[35]. The current study reported 7.7% mucous membrane contamination. In Italy, needle sticks constituted the most common source of exposure (58.4%), followed by non-intact skin and mucous membrane contamination (22.7% and 11.2% respectively), and cuts (7.7%)[36].

More than three-fourths of the MWHs used thick disposable gloves, 59 (61.5%) face masks, 30 (31.3%) protective gown and only 14 (14.6%) used boots. This may be the result of lack of training and shortage of supply since this is a common scenario of our govt. Hospitals. This finding is consistent with other study where only 55% of MWH used personal protective equipment (PPE).[37] Male MWHs were significantly more likely to wear boots (OR: 1.505: $P < 0.002$) compared to Female. However; use of PPE was not associated with gender ($p>0.5$).

Conclusion

Medical waste chiefly infectious medical wastes are potential depot of numerous microbes, which implicates health care workers and also public health. Due to needle stick puncture infectious disease like Hepatitis B & C can be transmitted to the health care workers. In our country, among these infectious diseases hepatitis B has much more potentiality to be transmitted to the health care worker through puncture or any way contact of body fluid or blood. This study showed that 6.3%% and 1% of medical waste handlers (MWH) are infected with hepatitis B & C respectively which might be due to medical waste handling. One fourth of the MWH did not use any personal protective equipment. Health education, prophylaxis by vaccination, universal precautions and proper hospital waste management are crucial in the prevention of HBV and HCV infection.

Limitations of the study: Like all other research work the current study was also not flawless. The study included only a single centre with a relatively small sample size which limits generalizability. The observational study design was also weak to extract underlying information Multi-centre studies with larger sample and sound study design could bring more insight regarding this issue.

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Effect of Ethanol Extract of *Swertia Chirata* on Blood Glucose Level in Diabetic Rats

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Abstract

Background: *Diabetes is a multisystem disorder marked by elevated blood sugar level. The prevalence of diabetes is increasing day by day. To face the increasing needs of diabetes management, traditional herbs may be one of the approaches parallel to therapeutic drugs. Due to this fact, the blood glucose lowering effect of ethanol extract of swertia chirata (family-gentianaceae) in alloxan induced diabetic rats was assessed.*

Methodology: *The ethanol extract was orally administered for 4 weeks at a dose of 250 mg/kg body weigh t(b.w),500 mg/kg b.w &750 mg/kg b.w. Weekly estimates of fasting blood glucose level were recorded in normal non-diabetic rat as well as in alloxan induced diabetic rats.*

Result: *Ethanol extract of swertia chirata showed no blood glucose lowering activity in non-diabetic rats. But there was a significant reduction in blood glucose level ($p<0.001$), when compared with diabetic control. Similar results were found when compared with a standard antidiabetic drug, metformin at a dose of 100ìg/kg b.w. Ethanol extract at a dose of 750mg/kg b. W. Produce the maximum response in reducing blood sugar level of diabetic rats.*

Conclusion: *The ethanol extract of swertia chirata has shown to lower the blood glucose level in experimentally induced diabetic rats and the effect is almost near to that of metformin. It has no hypoglycaemic effect in normal rats. Effects of different doses of swertia chirata need to be investigated further.*

Keywords: *Ethanol extract of swertia chirata; alloxan induced diabetic rats; blood glucose level; metformin.*

(Sir Salimullah Med Coll J 2016; 24: 57-62)

Introduction

Diabetes mellitus is becoming a major public health problem. The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The prevalence of diabetes is more pronounce in third world countries like Bangladesh. In 2000, about 3.2 millions of people of Bangladesh were recognized to have diabetes and this ranked 10th position of global consideration. It is estimated that in 2030 about 11.1 millions of people of Bangladesh will be affected by diabetes which will rank the 7th position of global consideration. The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity.

World Health Organization (WHO) fact sheet (2011),reveals that 346 million people worldwide have diabetes. In 2004, an estimated 3.4 million people died from the consequences of high blood sugar[2].

More than 80% of diabetic deaths occur in the low and middle-income countries. WHO projects that diabetic deaths will double between 2005 and 2030. Type 2 diabetes comprises about 95% cases worldwide[3].

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According to WHO (1999)4 Diabetes is a chronic disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. This leads to an increased concentration of glucose in the blood (hyperglycaemia).

Lack of insulin affects the metabolism of carbohydrate, protein and fat that can cause a significant disturbance in water and electrolyte homeostasis. Death may result from acute metabolic disorder. WHO states, because of poverty and lack of access to modern medicine, about 65-80% of the world's population in developing countries depends essentially on plants for primary healthcare[5]. The conventional anti-diabetic drugs available in the market have several common adverse effects & they also pose a financial burden to many diabetic patients. On the contrary, the herbal alternatives may be a better option due to lack of such adverse effects & are a cheaper choice of treatment. The suitable weather and fertile soil has enabled Bangladesh a great source of medicinal plants. Many studies have confirmed the benefits of such plants with hypoglycaemic effects useful in the management of diabetes mellitus. New bioactive drugs isolated from hypoglycaemic plants showed antidiabetic activity with more efficacy than hypoglycaemic agents used in clinical therapy[6].

One of such plant that has been the subject of interest in research is *Swertia chirata*, has a long history as a multipurpose folk remedy in India, Nepal, Bhutan, Bangladesh and China. Useful chemical compounds (Swerchirin, Swertiamarin, Xanthones, Mangiferin) in the *Swertia chirata* plants are typically isolated[7].*Swertiachirata*is used to treat diabetes due to its ability to reduce blood glucose levels[8].

The present study has therefore been undertaken to investigate the glucose lowering effect of ethanol extract of *Swertia chirata* on normal and experimentally induced diabetic rat model. Clinical investigation of this natural product would reveal better potentiality of *Swertia chirata* in alleviating diabetes mellitus.

Materials and Methods

The study was carried out in the Department of Pharmacology and Therapeutics of Sir Salimullah Medical College in collaboration with the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh.

Preparation of extract: Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells) when administered to rodents and many other animal species. 1180 grams of grinded powder chirata was soaked in 5000 ml ethanol for 5days with occasional shaking, and then filtered. The filtrate was condensed by rotatory vacuum evaporator and stored in refrigerator.

Animal housing: 42 healthy male Swiss Albino rats, aged 10-12 weeks and weighing between 130-140 gm were purchased from the animal resource division of International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR,B), Mohakhali. They were housed in clean metallic cages individually in the animal house of the Institute of Nutrition and Food Science, in a well ventilated room within room temperature of about 26-28C. The animal house was maintained under a constant light and dark cycle alternating every 12 hours. The rats were allowed to feed upon standard food pellets and drink water ad libitum, except for the overnight fast, the day before blood glucose estimation. During fasting, they were allowed free access to water only9.

Collection of reagents, chemicals, drug and medicinal plant: Alloxan monohydrate was purchased from local chemical market Hattkhola, Dhaka; Metformin as Comet of Square pharmaceutical Pvt. Ltd, was used as positive control and *Swertia chirata*, was purchased from Mouluvi Bazar, Dhaka.

Animal Experimentation: The animal experiment comprised of two experiments_ Experiment I and Experiment II. Diabetes was not induced in the rats of experiment I. Alloxan (150mg/kg b.w.) was injected to induce diabetes in animals of experiment II.

Experiment – I: This part of experiment was carried out to observe the effect of *Swertia chirata*, on blood glucose level in normal fasting rats. 12 rats were divided into 2 groups A and B, each comprising 6 rats. All rats were fasted overnight before collection of blood to determine the fasting blood glucose level.

***Group-A* (Non-diabetic control):** Rats were given standard rat feed and water for 4weeks. Fasting blood glucose level was estimated on every week during the experiment.

***Group-B* (Non-diabetic *Swertia chirata* control group):** Rats were given ethanol extract *of Swertia chirata*250 mg/kg b. w. orally along with rat pellets and water for 4weeks. Fasting blood glucose level was estimated every week during the experiment.

Experiment – II: This part of study was carried out to see the effect of ethanol extract *of Swertia chirata* on blood glucose level in alloxan induced diabetic rat. The glucose lowering effect of the extract was compared with a standard oral antidiabetic drug, Metformin.A total of 30 rats were divided into five groups, each comprising 6 rats. All animals were made diabetic by intraperitoneal administration of alloxan at 150 mg/kg b.w.

***Group-C* (Diabetic control group_ negative control):** Rats received standard food and water. Fasting blood glucose level was estimated before alloxan injection, 72hrs after alloxan injection to confirm diabetes induction and thereafter every week throughout the experiment.

***Group-D* (Experimental group):** Diabetic rats were divided into three sub groups- D_1, D_2 & D_3, fed with ethanol extract *of Swertia chirata*(250mg/kg b.w,500mg/kg b.w,750mg/kg b.w) orally by means of micropipette along with standard food and water for 4weeks. Fasting blood glucose level was estimated on before alloxan, 72hrs after alloxan and thereafter every week throughout the experiment.

***Group-E* (Anti-diabetic drug group_ positive control):** Metformin (100ìg/kg b.w.) was given orally along with standard food and water for 4weeks after confirmation of induced diabetes. Fasting blood glucose level was estimated before alloxan, 72hrs after alloxan and thereafter every week throughout the experiment.

Collection of blood: Every week during the treatment, rat blood sample was collected from the tail vein by tail tipping for the estimation of fasting blood glucose level. All the animals were then sacrificed under light chloroform anesthesia after completion of treatment on the last day of the experiment. Blood was obtained in eppendorf tubes and centrifuged at 3000 rpm for 15 minutes for the separation of serum. This serum was then used for biochemical analysis.

Estimation blood glucose level: Determination of serum glucose concentration was done by oxidase and peroxidase (GOD-POD) method using glucose estimation kit (Human, Germany) and ELISA plate reader (Multiskan EX, Labsystems, Finland).

Statistical analysis: The results are given as mean and standard deviation(SD) for the independently performed experiments. Unpaired students' t test was used to see the level of significance. p value <0.001 was considered as statistically significant.

Result

Table -I shows the weekly blood analysis of the changes in blood glucose level in all groups during the entire study. Zero week started from the day since when administration of ethanol extract *of Swertia chirata* was initiated (after confirmation of induction of diabetes with alloxan). Each treatment with ethanol extract *of Swertia chirata* for 1 week ended with a fasting serum glucose level estimation. This method was repeated for 4 weeks.

In Experiment I, the effect of ethanol extract of *Swertia chirata* on fasting blood glucose level in the non- diabetic rat was observed (table II). It was found that there was no statistically significant ($p>0.05$) change of serumglucose level in group B compared to those in control group A.

Experiment II showed the effect of ethanol extract of 3 different dose of *Swertia chirata* on alloxan induced diabetic rat in group D_1, group D_2 and group D_3, (table III). Compared to glucose level in diabetic control (group C), a significant ($p<0.001$) decrease in serum glucose level was observed in the experimental D_1, D_2 and D_3groups.

In table IV, effect of ethanol extract of *Swertia chirata* in lowering the blood glucose level was compared with that of Metformin 100µg/kg b.w. It was noted that after 28 days of treatment, the blood glucose lowering effect of *Swertia chirata* extract at dose 750mg/kg body weight had no significant difference ($p>0.05$) with that of Metformin.

Table-I

Effect of ethanol extract of Swertia chirata and metformin on fasting blood glucose level in alloxan induced diabetic rats

Group (n=6)	Fasting blood glucose level (mmol/L) (mean ± SD)				
	0 week (after alloxan)	1st week	2nd week	3rd week	4th week
Group C	12.65 ± 0.41	12.85 ± 0.42	12.88 ± 0.39	12.95 ± 0.39	13.13 ± 0.34
Group D_1	12.82 ± 0.15	9.05 ± 0.19	8.55 ± 0.15	8.12 ± 0.12	7.83 ± 0.08
Group D_2	12.93 ± 0.22	8.45 ± 0.10	7.95 ± 0.10	7.60 ± 0.09	7.37 ± 0.08
Group D_3	12.90 ± 0.24	7.53 ± 0.22	7.25 ± 0.22	7.03 ± 0.14	6.85 ± 0.10
Group E	12.88 ± 0.25	6.97 ± 0.08	6.90 ± 0.09	6.82 ± 0.08	6.72 ± 0.15

n: number of rat used

Table – II

Effect of ethanol extract of Swertia chirata on fasting blood glucose level in non-diabetic rats

Group (n=6)	Treatment	Fasting blood glucose level (mmol/L) (4th week) (mean ± SD)	P value*
Group A	Standard lab diet and water *ad libitum*	5.38 ± 0.15	p>0.001
Group B	Ethanol extract *of Swertia chirata*(250mg/kg b.w.)	5.30 ± 0.14	

*level of significance determined by independent sample t test
ns: non-significant

Table - III

Effect of ethanol extract of Swertia chirata on fasting blood glucose level in alloxan induced diabetic rats

Group (n=6)	Treatment	Fasting blood glucose level (mmol/L) (4th week) (mean ± SD)	P value*
Group C	Standard lab diet and water *ad libitum*	13.13 ± 0.34	
Group D_1	Ethanol extract *of Swertia chirata* (250mg/kg b.w.)	7.83 ± 0.08	p<0.001 *s* C vsD_1
C vsD_2	Ethanol extract *of Swertia chirata* (500mg/kg b.w.)	7.37 ± 0.08	p<0.001 Group D_2
Group D_3	Ethanol extract *of Swertia chirata* (750mg/kg b.w.)	6.85 ± 0.10	p<0.001 C vsD_3

*level of significance determined by independent sample t test
s: significant ; ns: non-significant

Table - IV

Effect of ethanol extract of Swertia chirata and Metformin on fasting blood glucose level of alloxan induced diabetic rats

Group (n=6)	Treatment	Fasting blood glucose level (mmol/L) (4^{th} week) (mean ± SD)	P value*
Group E	Metformin (100ìg/kg b.w.)	6.72 ± 0.15	
Group D_1	Ethanol extract *of Swertia chirata* (250mg/kg b.w.)	7.83 ± 0.08	p<0.001 ^{s} EvsD$_1$
Group D_2	Ethanol extract *of Swertia chirata* (500mg/kg b.w.)	7.37 ± 0.08	p<0.001 EvsD$_2$
Group D_3	Ethanol extract *of Swertia chirata* (750mg/kg b.w.)	6.85 ± 0.10	p>0.001 E vs D$_3$

*level of significance determined by independent sample t test
s: significant ; ns: non-significant

Discussion

The aim of this study was to investigate the blood glucose lowering effect of *Swertia chirata* in experimentally induced diabetic rat. Diabetes was induced after 48 hours of intraperitoneal administration of a single dose of 150 mg/kg body weight alloxan monohydrate. This dose of alloxan had significantly (p<0.001) increased the blood glucose level as it is claimed.[10]

The results of the present study shows that ethanol extract of *Swertia chirata* has blood glucose lowering effect in alloxan induced diabetic rat, but poses no effect on blood glucose level of non-diabetic rat. Similar results was also previously reported. Arya Renu had shown his study that ethanolic extract of *Swertia chirata significantly* (p<0.001) *r*educes blood sugar level in diabetic rats.[11]

Treatment with 750mg/kg body weight was observed to be significantly (p<0.05) more effective in lowering of blood glucose level than that of 500mg/kg & 250mg/kg body weight.

However, it is revealed that *Swertia chirata* extract significantly reduces blood glucose levels in diabetic rat. The possible mechanism of glucose lowering action of *Swertia chirata* extract may be due to its ingredient Swerchirin & Mangiferin.Swerchirin acts by stimulating insulin release from islets of Langerhans.Mangiferin directly stimulate ² cells to release insulin, enhances glycolytic enzymes which stimulates glycogenesis in the liver, and also inhibits dipeptidyl peptidase IV mediated degradation of glucagon like peptide-1(GLP-1)[12] Mangiferin showed ±-glucosidase inhibitory activity indicating anti-hyperglycemia potential.[13]

Conclusion

The results of this study provide information that the ethanol extract of *Swertia chirata* has glucose lowering effect and dose 750mg/kg body weight is almost near effective to that of Metformin. It has no hypoglycaemic effect in normal rats. Moreover, the molecular components of the active ingredients of *Swertia chirata* should be investigated in future for the development of new drug for the treatment of diabetes mellitus.

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The Prevalence of the Carabelli cusp in selected Bangladeshi Population

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Abstract

***Aims**: The aim of the study was to find out the prevalence and degree of expression of the carabelli trait structure in the permanent first and second maxillary molars in selected Bangladeshi population residing in City Dental College of Dhaka city.*

***Study Design:** A descriptive type of cross sectional study.*

***Place and Duration of Study:** Oral examination was done to identity the Prevalence of Carabelli Trait for 250 Bangladeshi patients attending in different public dental clinic and City dental college between 20-55 years of age. They were also interviewed through a structured questionnaire for collecting other relevant information.*

***Result:** Among all the respondents, the prevalence of the Carabelli structure was 56% at first molar and only 14.8% at second molar, while the absence of the Carabelli structure was found to be 44% at the first molar and 85% at second molar. Bilateral occurrence with a tendency toward concordance of expression between sides was also observed. Patients with severely carious, restored or missing upper first or second maxillary molars on any side were excluded.*

***Conclusion:** It was concluded that more than half of the Bangladeshi population have a degree of expression of the Carabelli structure.*

Clinical relevance: *Prominent pits and groove can be foci for plaque retention and caries development, a large tubercle can pose some problems with orthodontic bands. Patients should know about the problems of teeth, oral activity. And must visit dentist regularly and also be properly instructed about maintaining oral hygiene.*

***Keywords:** Carabelli, Dental Anthropology, Maxillary Molars.*

(Sir Salimullah Med Coll J 2016; 24: 63-67)

Introduction

The Carabelli structure is a tubercle or a small cusp, often seen on the palatal surface of the mesiopalatal cusp at maxillary permanent molars and maxillary second deciduous molars[1]. It includes a variety of expressions that range from present to complete absence of the tubercle, or to be as a cusplet[2]. It represents the end product of the interaction of a complex system of ontogenetic and environmental factors[3].

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Table 1

Prevalence of patients having the Carabelli trait

Structure	Patient frequency	Prevalence (%)
Smooth surface	105	42%
Carabelli trait	145	58%
Total	250	100

Alvesalo et al[3] (1975) examined 233 patients in a Finnish rural population for the presence of the carabelli cusp. 79% of them had the cusp in first upper molars. The occurrence of the structure was bilateral with varying degrees of asymmetry. Their results suggested low heritability of the character. They classified the Carabelli structure as follows:

1. Smooth surface.
2. Single pit or furrow.
3. Double furrow or Y- shaped furrow.
4. Slight protuberance or small cusp.
5. Large cusp.

In a study made by Rusmah[4] 320 maxillary cusp of Malaysian children were examined for the occurrence of the Carabelli trait on the maxillary first permanent molars. The total trait frequency was observed to be 51.6%. The cusp was observed to be more bilateral[5]. 17.43% occurrence of the Carabelli cusp in maxillary molars was reported by Falomo[5] after examining 2,604 Nigerians; there was 70.71% bilateralism in the upper first molars, with 1.98% simultaneously bilateralism in the upper first and second molars. Unilateralism in the upper first molars accounted for 25.99%[7].

The purpose of this study was to determine the prevalence, degree of expression of the Carabelli structure in the permanent first and second maxillary molars in a selected Bangladeshi population.

Material and methods

250 Bangladeshi patients attending to the different public dental clinic and City Dental College, Dhaka city were randomly selected for examination. Their age ranged from 20 – 55 years old. Any patients with severely carious restored or missing upper first or second maxillary molars on any side was excluded. Both the first and the second upper molars were carefully examined for the occurrence of tubercle structure, from the occlusal view using a mouth mirror and dental explorer. Under efficient light condition, tubercle presence was examined by pulling the explorer tip over the groove created by the cusp looking for stickiness, and avoiding apically directed force[8].

The structure and its degree of expression were classified into three categories:

1. Smooth surface or absence of the structure.
2. Depression or groove.
3. Cusp prominence.

Three examiners collected the data. For inter-examiner calibration, and before starting data collection, a training session with a senior examiner was carried out to standardize examination. At the beginning, 20 patients were examined by all examiners, in which the first examiner performed examination and recorded data followed by the second and third examiners in an orderly manner.

All statistical analysis were calculated using the SPSS program version 10 and then descriptive data analysis using frequency distribution was used to compare prevalence.

Results

A high degree of inter-examiner variation was observed with the first twenty patients examined by the three examiners. The mean Kappa values were 0.89 for the detection of the Carabelli structure. One hundred and forty five individuals ($N = 250$) had some sort of expression of Carabelli trait (58%), while 105 patients did not have any expression of the structure (42%) i.e. smooth surface (Table 1). Table 2 shows that 39.6% of all patients had cusp expression, while 32.7% had groove expression in at least one tooth.

Table-II

Frequency and percentage of patients having the Carabelli trait expression in relation to number of teeth.

Structure present	Groove or depression		Prominent cusp	
	Patient frequency	Percentage (%)	Patient frequency	Percentage (%)
One out of the four molars	6	2.4	9	3.6
Two out of the four molars	68	27.2	72	28.8
Three out of the four molars	3	1.2	3	1.2
Four out of the four molars	4	1.6	15	6
Total	81	32.4	99	39.6

Table-III

Degree of expression of the Carabelli trait in the upper first molars

Criteria	Upper first molar	Total %	Frequency Percentage (%)
(*N* = 500)			
Absence of structure	220	44	44
Groove or depression	120	24	56
Prominent cusp	160	32	
Total	500	100	100

Table-IV

Degree of expression of the Carabelli trait in the upper second molars.

Criteria	Upper second molar	Total %	Frequency Percentage (%)
(N = 500)			
Absence of structure	425	85	85
Groove or depression	41	8	14.8
Prominent cusp	34	6.8	
Total	500	100	100

Degree of expression of the Carabelli trait in the first and in the second maxillary molar teeth is shown in Table 3 and 4 , respectively. It was found that 56% of all first molars have the Carabelli trait, in which a higher frequency was recorded for a prominent cusp 32 % compared to a groove expression 24 (Table-III). It was also found that the frequency of the trait in the second molar was lower (14.8) compared to the first molar, but with almost equal frequency regarding groove or cusp expressions (Table 4).

Table -V

Patients' Carabelli structure bilateralism in relation to the upper first molars.

Criteria Carabelli structure	Percentage (%)	Frequency (*N* = 150)
Groove or depression unilaterally	3	2
Groove or depression bilaterally	55	36.6
Groove on one side – cusp on other side	5	3.3
Prominent cusp unilaterally	5	3.3
Prominent cusp bilaterally	82	54.6

Table-VI

Patients' Carabelli structure bilateralism in relation to the upper second molars.

Criteria Carabelli structure	Percentage (%)	Frequency (*N* = 52)
Groove or depression unilaterally	4	7.6
Groove or depression bilaterally	22	42.3
Groove on one side – cusp on other side	2	3.8
Prominent cusp unilaterally	4	7.6
Prominent cusp bilaterally	20	38.4

The frequency of patients' Carabelli structure bilateralism for the maxillary first and second molars is shown in Tables 5 and 6 respectively. In the first maxillary molar, the total bilateralism, regardless of the type of expression, was found to be 91.2%, of which 36.6% was groove bilateralism, and 54.6% was cusp bilateralism. And in the second maxillary molar, Bilateralism was 80.7% , of which 42.4% was groove bilateralism, and 38.4% was cusp bilateralism.

Discussion

Since three examiners collected the data, it was important to unify and standardize their method of examination and data collection. The mean Kappa values obtained were high, which suggest that the three examiners reached an excellent inter-examiner reliability.

The present study reveals that more than one half of the Bangladeshi population has a degree of expression of the Carabelli structure (Table-I). The results were very close to those reported by Rusmah (1992) (51.6%). Prominent pits and grooves can be foci of plaque retention and caries development, and a large tubercle can pose some problems with orthodontic bands.

The frequency percentage of Carabelli trait for the first molar was 56%, which is statistically significantly higher compared to the second molar (14.8%), at P = 0.00 (Tables 3 and 4). Regarding the Carabelli trait expression in first molar, it was 24% as a groove and 32% as a cusp, which is statistically significantly different at P = 0.00 (Table 3). It was 8% as a groove expression and 6.8% as a cusp expression for the second molar, but this difference was not statistically significant P = 0.00 (Table 4).

Bilateralism of the trait, in this study, was very high. This supports the findings of other researchers (Alvesalo et al., 1975; Falomo, 2002; Joshi, 1975; Rusmah, 1992; Thomas et al., 1986); which they agree that bilateralism of the trait is more frequent. Although some investigators, such as Biggerstaff (1973, 1972), suggested that bilateralism or mirroring is not a factor in the Carabelli trait, even among twins. Simultaneous Bilateralism in the upper first and second molars was found to be low, which agree with Falomo's findings (2002).

An interesting result was found, which is if the second upper molars had prominent cusps bilaterally, the upper first molars would have prominent cusps bilaterally as well. But it is not true for the grooves. This could be attributed to genetics, as some investigators reported that it may have a big influence on the Carabelli trait (Biggerstaff, 1973; Dahlberg, 1963; Goodman, 1965; Jordan et al., 1991; Lasker, 1950).

Many studies (Alvesalo et al., 1975; Rusmah, 1992; Saunders and Mayhall, 1982; Thomas et al., 1986) failed to show any sex dimorphism in the occurrence of the trait, although some investigators (Kaul and Prakash, 1981; Kieser and Preton, 1981) observed a sex-linked pattern. Therefore, the relationship of sex to the Carabelli trait was not investigated in this study.

In the first molars, it was found that the cusp expression showed 32% Carabelli when groove expression 24%, this is true for the second molar as well. This finding can be explained by the fact the cusp expression has a tubercle in addition to the groove; this tubercle may act as a physical barrier against the cleansing mechanisms of a tooth brush and physiological cleansing by the tongue movement and the salivary flow. In cases where the Carabelli structure was absent, caries was not detected which supports the idea that the Carabellistructure presents a foci for plague accumulation and caries development.

Further studies should be made to assess the relationship of the Carabelli trait to genetics and its heritability, the Carabelli trait should also be studied in relation to sex.

Conclusion

Our findings lead us to propose that, in individuals with the genotype for Carabelli trait expression, larger molar crowns are more likely to display Carabelli cusps, whereas molars with smaller crowns are more likely to display reduced forms of expression of the trait, we suggest that the pattern of folding of the internal enamel epithelium in developing molar crowns, articularly in the protocone region, can be modified by a developing Carabelli cusp.

Within the limitation of the present study, the following conclusions can be drawn:

1. The prevalence of Carabelli trait in a selected Bangladeshi population was 58%.
2. The Carabelli trait is bilaterally present.
3. Dentists are advised to perform a careful examination to the lingual surface of the maxillary first and second molars to rule out the presence of the Carabelli structure and caries.

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Help-seeking Practices to Cope Mental Trauma due to Domestic Violence among Rural Women of Reproductive Age

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Abstract

Bangladesh is a highly populated country having a male dominating society. A cross-sectional, descriptive study was carried out in different households in rural areas of KeraniganjUpazilla of Dhaka district between March 2016 and June 2016. The study population comprised of married women of reproductive age group (15-49 years) with a sample size of 201. Purposive sampling technique was applied. An interviewer administered structured questionnaire was developed and used after pre-testing to collect primary data. Data was collected by face to face interview of the respondents by the researchers during data collection period. After collection, data were checked and verified. Omission and errors were corrected properly. Data were analyzed by using Social Package for the Social Sciences (SPSS version 22). Majority 174(86.6%) suffered from mental trauma due to domestic violence. Most of the women were traumatized due to use of abuse language 70%, followed by 46.6% stopped talking, 21% stop taking care of the women. It was observed that, the women in reproductive age 28% (n=184) suffered some sort of domestic violence and about 72% of sufferer did not seek any support or help from others. Just about 43% of wage earning women sought help during or immediately after the violence whereas the 26% among them were non-earning women. Women of 25 - 34 age groups (31%) shows the tendency of seeking help and those of other groups sought very little help. Majority of the women (31%) try to discuss with husbands on the burning issue to stop violence whereas only 1% of them try to get help from neighbor or from spiritual leaders, rest had other means of getting help. Most of the respondents (71%) tried to find some ways out so that the situations never happen in future. Almost 66% percent tried to end the violence by finding out a way by themselves and 45% wanted to strengthened their capacity or become stronger economically (11%) so that they can cope the violence. A good number of the respondents (35%) left the situation to the Merciful to have enough courage to cope the situations. A very little number of the respondents (10%) felt like killing the perpetrators. Unless domestic violence causing mental trauma appear to be life threatening, victims tend not to seek formal or legal support.

Key Words: *Help-seeking Practices, Cope, Mental trauma, Domestic violence.*

(Sir Salimullah Med Coll J 2016; 24: 68-73)

Introduction

"Domestic violence is a burden on numerous sectors of the social system and quietly, yet dramatically, affects the development of a nation… batterers cost nations fortunes in terms of law enforcement, health care, lost labor and general progress in development. These costs do not only affect the present generation; what begins as an assault by one person on another reverberates through the family and the community into the future".[1]

Domestic violence is not only widely dispersed geographically, but its incidence is also extensive, making it a typical and accepted behavior inside the households. It is wide spread, deeply ingrained and has serious impacts on women's health and

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well-being, its cost to individuals, to health systems and to society is enormous and Yet no other major problem of public health has been so widely ignored and so little understood.[2]

Mental or psychological violence against married women is extremely common and persistently practiced by their husbands in Bangladesh, as over 80% have ever experienced it in their life time with 72% in the past 12 months (VAW survey 2011).[8]Anxiety and mood disorders both are more common in women suffering from domestic violence compared to those who have not experienced IPV.[3] Eating disorders. And diagnosis of an eating disorder of women suggests that they have experienced some sort of lifetime violence.[4]Suicidality is a common outcome of violence.[25] and attempts to commit suicide.[5] Women who reported partner violence at least once in their lifetime are nearly three times as likely to have suicidal thoughts and nearly four times as likely to attempt suicide, compared to women who have not been abused by a partner.[6] Eighty percent of women who experienced mental or physical violence by an intimate partner reported significant effects including posttraumatic stress disorder (PTSD) for a short or a long time.[7]

Although several legal and protective policy measures have been adopted to safeguard women's rights, effectively outlawing gender-based violence and discrimination—implementation through enforcement remains difficult, greatly impeding the advancement of women's rights in the society.[8]

United Nations Secretary General in a video conference on violence against women stated that "violence has no boundaries of geography, culture or wealth and as long as it continues, we cannot claim to make real progress towards equality development and peace in the world".[8] Thus it is highly appreciable to adopt effective strategies for the prevention of violence which involve public awareness campaigns and community based network to combat violence and support victims. However, Bangladesh Government has recently passed the Domestic violence (prevention and protection) act/policy/mandate, (year). Despite having many legal protections of women in the society many forms of violence is still continuing where domestic violence constitutes a prime number. In recent years, domestic violence is increasing day by day as evidenced by the recent magnitude of eve teasing and acid abuse, rape and murder even to the minors.[10]In this context the aim of this article is to examinethe socio economic factors those effect the domestic violence occurring in the communities of Bangladesh and to look for national and international legal protection for the victims, and finally, some interventions are recommended to be taker in compliance with national and international legal instrument.[8]

Methods

A cross-sectional, descriptive study was carried out in different households in rural areas of Keraniganj Upazilla of Dhaka district between March 2016 and June 2016. The study population comprised of married women of reproductive age group (15-49 years). The sample size was 201,the number of the sample was so because ofthe situations, ability and the provision of time. Only married women of reproductive age group were taken to make the sample more specific. Women beyond reproductive age, widowed and unmarried women were excluded from the study. Purposive sampling technique was applied. Aninterviewer administered structured questionnaire was developed and used after pre-testing to collect primary data.Data was collected by face to face interview of the respondents by the researchers during data collection period. After collection, data were checked and verified. Omission and errors were corrected properly. Data were analyzed by using Social Package for the Social Sciences (SPSS version 22), an IBM software and was represented in tables.

Results

Table I

Types of domestic violence(n=184)

Types of domestic Violences	Frequency	Percentage
Mental	174	86.6
Physical	81	40.3
Sexual	4	2.0
Economical	3	1.5

*Multiple responses

The table depicted that most of the respondents suffered from mental trauma due to domestic violence. The second large category suffered from physical violence. The number of respondents suffering from sexual and economical violence was negligible.

Table II

Taken any help-seeking measure (n=184)

Taken any help-seeking measure	Frequency	Percentage
Yes	52	28.3
No	132	71.7

Significantly it was observed that, among the study population, most of the women in reproductive age 28% (n=184) suffered some sort of domestic violence and among themabout 72 percent of sufferer did not seek any support or help from others.

Table III

Types of mental trauma due to domestic violence (n=174)

Types of mental trauma	Frequency	Percentage
Using abuse language	122	70.1
Stop talking	81	46.6
Stop taking your care	37	21.3
Stop giving household expenses	30	17.2
Stop giving pocket money	19	10.9
Restricting going out for job	20	11.5
Restricting freedom to go out for a visit to family and friends	30	17.2
Restricting from doing things which you like	20	11.5
Restricting decision making in the family	22	12.6
Creating pressure to do things which the respondent does not like to do	9	5.2
Forceful sexual intercourse	5	2.9

*Multiple Responses

The table showed that most of the women were traumatized due to use of abuse language 122 (70%),followed by 81 (46.6%) stopped talking, 37 (21%) stop taking care of the women. A moderate number of women suffered from stop giving household expenses and restricting freedom to go out for a visit to family and friends are in the same categories 30 (17%). About 22(12.6%) agonized from restricting decision making in the family 22(12.6%). Stop giving pocket money, restricting going out for job, restricting from doing things which she like were in almost same percentage (20%). A negligible percent of women suffered from creating pressure to do things which the respondent does not like to do and forceful sexual intercourse.

Table IV

Association of occupation with help-seeking measure taken by victim (n=184)

Taken help-seeking measure	Occupation		p value
	Not working	Working	
Yes	40 (25.6)	12 (42.9)	0.062
No	116 (74.4)	16 (57.1)	
Total	156 (100.0)	28 (100.0)	

The study found that, there is an adverse relation between help seeking measures and working (earning) status among the women victim of domestic violence. Data shows that, after any incidence, about 43 percent of wage earning women sought help during or immediately after the violence whereas the percentage is 26 among the non-earning women.

Table V

Association of age with help-seeking measure taken by victim (n=184)

Age (years)	Taken help-seeking measure		p value
	Yes	No	
15 - 24	10 (19.2)	39 (29.5)	0.201
25 - 34	31 (59.6)	56 (42.4)	
35 - 44	10 (19.2)	32 (24.2)	
>=45	1 (1.9)	5 (3.8)	
Total	52 (100.0)	132 (100.0)	

Table demonstrated that, most of the ever married women (56%) of age between 25 and 34 take no measure after being victimized by domestic violence. On the other hand, the same age group shows the tendency of seeking help(31%).Married women aged 15-24 and 35-44 sought support in case of domestic violence representing 10 percent of the study population.

Table VI

Types of help-seeking measure taken (n=52)

Types of preventive measure taken	Frequency	Percentage
Try to discuss the problem with husband to stop violence	31.0	59.6
Try to get help from elder-in-law	14.0	26.9
Try to get help from the members of paternal side of the respondents	16.0	30.8
Try to get help from neighbour	1.0	1.9
Try to get help from village leaders	2.0	3.8
Try to get help from spiritual/ religious leaders	1.0	1.9
Tolerate all by herself without protest	4.0	7.7
Respondent protested with all her energy	2.0	3.8
Try to get help from friends	3.0	5.8

*Multiple Responses

Table VII

Way to cope the violence (n=184)

Way to cope the violence	Frequency	Percentage
Try to find out a way to end the violence	66	35.9
Try to find a way so that violence never happens	71	38.6
Try to find out a way that will make you stronger	45	24.5
Try to find out a way that will make you stronger economically	11	6.0
Pray to the Merciful so that you have enough courage to cope the situations	35	19.0
Feel like killing the perpetrators	10	5.4

*Multiple Responses

Regarding help seeking measures, the study found that, majority of the women (31%) try to discuss with husbands on the burning issue to stop violence whereas only 1% of them try to get help from neighbor or from spiritual leaders. About 2% sought help from village leaders and 3% from friends.Respondents (4%)tolerated all by herself without protest and (2%) protested with all her energy. As another strategy, 16% of the women try to get support or help from parents. As the elder members in in-laws house plays an important role in domestic violence, 14% of the respondent try to get help from elder members from in-laws family. A negligible (1%) tried to get help from spiritual or religious leaders.

Women who ever experienced domestic violence; take different measures to cope with the violence. The study found that, most of the respondents (71%) tried to find some ways out so that the situation never happen in future. Approximately 66% percent of the respondents try to end the violence by finding out a way by themselves. 45% of the respondents try to strengthened their capacity or become stronger economically (11%) so that they can cope the violence. A good number of the respondents (35%) left the situation to the Merciful to have enough courage to cope the situations.A very little number of the respondents (10%) felt like killing the perpetrators.

Discussion

The physical and mental health consequences of domestic violence are often obscure, indirect and emerge over the long term. For example, women

who were subject to violent attacks during childhood are bothered by menstrual problems and irritable bowel syndrome in later life.[8]

This cross-Sectional type of descriptive study was carried out in rural area of KeraniganjUpazilla under district of Dhaka with the purpose to assess out magnitude of domestic violence causing mental trauma among married women of reproductive age in that rural area with an objective to measure the level of awareness of getting help during and after sustaining mental trauma due to domestic violence. Limitations of the study are addressing the sensitive issues of life and respondents were shy to express their opinions openly and willingly, thoughts of damaging self-images and that of families, chance of recall bias is very high in any study based on the self-reporting, illiteracy of the respondents.[9] Small study period forced thesample size to be 201 only.

Many studies show that that the majority of the women "most often" can anticipate potential violence and the usual strategic measure adopted by women is abstaining from the household, especially using the no-talk strategy with their intimate partner or any other perpetrators. In some cases, women leave the scene or try to divert attention of the husbands. The other strategies of abstinence include not talking to other family members, not talking to children, abstaining from food preparation, and abstaining from food intake, for a few hours to a few days. But women were found less likely to seek help from others. Women who sought help from others mostly turned to family members and neighbours and no one sought formal social or legal support.[10]

In 23.9% of the cases family members directly took a mediating role by requesting the husband not abuse his wife verbally or physically.[11] Women try to cope with the violent situation for the sake of their children (32.1%), followed by having no place to go (12.7%) and social stigma (12.2%). Women keep silent about their abuse due to fear of further assault, uncertainty for children's future and of their own, stigma and belief of men's right to be violent as well as other reasons. About 66% of women never told anyone of their abuse having been physically abused by their husbands.[12] The prevalence of physical violence and percentage of silence are directly proportional. Only 2% of women ever sought institutional help, doing so when the violence was really life-threatening. Abused women rarely seek recourse in the event of domestic violence. The majority of the women confirmed they had a very few options for preventing or stopping domestic violence. Interestingly, when they sought a way out of the domestic violence they had very little success.[13] Having no alternative women have to accept violence done by their husbands as an inevitable part of their marital life. Poverty, gender inequality, patriarchal attitude discourage abused women from seeking recourse. Women even avoided 'salish- a community-level dispute resolution mechanism traditionally dominated by men and elites, because women often do not like to make family matters public and the decisions made in 'Salish' are often biased towards men.[14] Women's Attitudes of women significantly varied by women's age, women's working status, experience of violence, receipt of micro-credit, women's decision-making authority, husband's age, husband's education, family economic status and family type. Around 72.4% women were in favour of seeking advice from social services regarding ways to improve their behavior toward husbands, 68.8% were in favour of asking social services to persuade the husband to seek therapy and 71.1% were in favour of going to a battered women's shelter.[15] A greater percentage of women wanted to change their own behavior in order to change their husbands' violent behaviour. Working women were more likely to seek help from both formal and informal agents compared to that of non-working women.[16]

Conclusions

Unless domestic violence causing mental trauma appear to be life threatening, victims tend not to seek formal or legal support. This includes all the women illiterate, qualified, working or not-working. Tolerance and finding out a way to end the violence is the staple mechanism of coping.

Recommendations

Rights of women to seek help after any type of domestic violence should be established by including women's rights in regular academic

education. Schemes should be taken within mainstream organizational routines, promote collaborative multi-agency striving steps need to be taken. Qualified staffs to ensure a reliable, continuous approach to help women cope mental trauma due to domestic violence.

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Review Article

Dengue Fever: An Update

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Abstract

Dengue is a mosquito-borne viral infection. The infection causes flu-like illness, and occasionally develops into a potentially lethal complication called severe dengue. The global incidence of dengue has grown dramatically in recent decades. About half of the world's population is now at risk. Dengue is found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas. Severe dengue is a leading cause of serious illness and death among children in some Asian and Latin American countries. There is no specific treatment for dengue/ severe dengue, but early detection and access to proper medical care lowers fatality rates below 1%. Dengue prevention and control depends on effective vector control measures. A dengue vaccine has been licensed by several National Regulatory Authorities for use in people 9-45 years of age living in endemic settings.

***Key words**: Dengue; Viral infection; Severe; Flu-like; Fatal*

(Sir Salimullah Med Coll J 2016; 24: 74-84)

Introduction

Dengue fever is a mosquito-borne viral disease that has rapidly spread in all regions of the world in recent years. It is caused by the dengue virus. Mild dengue fever causes high fever, rash, and muscle and joint pain. A severe form of dengue fever, also called dengue hemorrhagic fever, can cause severe bleeding, a sudden shock and death.[1] Symptoms typically begin 3 to 14 days after infection.[2] This may include a high fever, headache, vomiting, muscle and joint pains, and a characteristic skin rash.[1,2] Recovery generally takes less than 2 to 7 days[1]. In a small proportion of cases, the disease develops into the life-threatening dengue hemorrhagic fever, resulting in bleeding, low levels of blood platelets and blood plasma leakage, or into dengue shock syndrome, where dangerously low blood pressure occurs.[2] As we have come to understand this illness, fluid therapy has become the most important aspect in the management of dengue. In 2009, WHO new guidelines for management of dengue were published.[3] In 2012 the revised comprehensive guidelines were published by WHO.[4]

History

The first record of a case of probable dengue fever is in a Chinese medical encyclopedia from the Jin Dynasty (265-420 AD) which referred to a "water poison" associated with flying insects.[5] The primary vector, Aedes aegypti, spread out of Africa in the 15th to 19th centuries due in part to increased globalization secondary to the slave trade.[6] There have been descriptions of epidemics in the 17th century, but the most plausible early reports of dengue epidemics are from 1779 and 1780, when an epidemic swept across Asia, Africa and North America.[7] From that time until 1940, epidemics were infrequent.[7] In 1906, transmission by the Aedes mosquitoes was confirmed, and in 1907 dengue was the second disease (after yellow fever) that was shown to be caused by a virus. Further investigations by John Burton Cleland and Joseph Franklin Siler completed the basic understanding of dengue transmission.[8]

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The marked spread of dengue during and after the Second World War has been attributed to ecologic disruption. The same trends also led to the spread of different serotypes of the disease to new areas, and to the emergence of dengue hemorrhagic fever. This severe form of the disease was first reported in the Philippines in 1953; by the 1970s, it had become a major cause of child mortality and had emerged in the Pacific and the Americas.[7] Dengue hemorrhagic fever and dengue shock syndrome were first noted in Central and South America in 1981, as DENV-2 (one of the five strains) was contracted by people who had previously been infected with DENV-1(one of the five strains) several years earlier.[9] The origins of the Spanish word "dengue" are not certain, but it is possibly derived from "dinga" in the Swahili phrase "Ka-dingea pepo", which describes the disease as being caused by an evil spirit.[5] Slaves in the West Indies having contracted dengue were said to have the posture and gait of a dandy, and the disease was known as "dandy fever".[10,11] The term "break-bone fever" was applied by physician and United States Founding Father Benjamin Rush, in a 1789 report of the 1780 epidemic in Philadelphia. In the report title he uses the more formal term "bilious remitting fever".[12] The term dengue fever came into general use only after 1828. Other historical terms include "breakheart fever" and "la dengue". Terms for severe disease include "infectious thrombocytopenic purpura" and "Philippine", "Thai", or "Singapore hemorrhagic fever".[11]

Epidemiology

Dengue is common in more than 110 countries. The high risk regions for catching dengue fever are Central America, South America, the Caribbean and tropical Asia; more specifically - northern Argentina, northern Australia, the entirety of Bangladesh, Barbados, Bolivia, Brazil, Cambodia, Costa Rica, Dominican Republic, Guatemala, Guyana, Honduras, India, Indonesia, Jamaica, Laos, Malaysia, Mexico, Micronesia, Pakistan, Panama, Paraguay, Philippines, Puerto Rico, Samoa, Singapore, Sri Lanka, Suriname, Taiwan, Thailand, Trinidad, Venezuela and Vietnam, and increasingly in southern China.[3]

In many parts of the tropics and subtropics, dengue is endemic, usually during a season when Aedes mosquito populations are high, often when rainfall is optimal for breeding. These areas are, however, additionally at periodic risk for epidemic dengue, when large numbers of people become infected during a short period. Dengue epidemics require a coincidence of large numbers of vector mosquitoes, large numbers of people with no immunity to one of the four virus types (DENV 1, DENV 2, DENV 3, DENV 4), and the opportunity for contact between the two. It infects 50 to 528 million people worldwide a year, leading to half a million hospitalizations,[13] and approximately 20,000 deaths.[14] In 2015, 2.35 million cases of dengue were reported in the Americas alone, of which 10 200 cases were diagnosed as severe dengue causing 1181 deaths. The number of cases reported increased from 2.2 million in 2010 to 3.2 million in 2015.[1] The year 2015 was characterized by large dengue outbreaks worldwide, with the Philippines reporting more than 169 000 cases and Malaysia exceeding 111 000 suspected cases of dengue, representing a 59.5% and 16% increase in case numbers to the previous year, respectively. Brazil alone reported over 1.5 million cases in 2015, approximately 3 times higher than in 2014. Also in 2015, Delhi, India, recorded its worst outbreak since 2006 with over 15 000 cases.[16, 17, 18]

Infections are most commonly acquired in the urban environment.[19] In recent decades, the expansion of villages, towns and cities in the areas in which it is common, and the increased mobility of people have increased the number of epidemics and circulating viruses.[20] Rates of dengue increased 30 fold between 1960 and 2010.[21] This increase is believed to be due to a combination of urbanization, population growth, increased international travel, and global warming.[13] An infection with dengue is second only to malaria as a diagnosed cause of fever among travelers returning from the developing world.[22] It is the most common viral disease transmitted by arthropods[23], and has a disease burden estimated at 1,600 disability-adjusted life year per million population.[24] The World Health Organization counts dengue as one of seventeen neglected tropical diseases.[25]

Like most arboviruses, dengue virus is maintained in nature in cycles that involve preferred blood-sucking vectors and vertebrate hosts[15]. The viruses are maintained in the forests of Southeast

Asia and Africa by transmission from female Aedes mosquitoes-of species other than A. aegypti-to their offspring and to lower primates.[19] In towns and cities, the virus is primarily transmitted by the highly domesticated A. aegypti. In rural settings the virus is transmitted to humans by A. aegypti and other species of Aedes such as A.albopictus.[19] Both these species had expanding ranges in the second half of the 20th century.[26] In all settings the infected lower primates or humans greatly increase the number of circulating dengue viruses, in a process called amplification.[19]

Etiology

There are four dengue viruses (DEN-V) that cause dengue fever, all of which are spread by a species of mosquito known as the Aedes aegypti mosquito, and more rarely by the Aedes albopictus mosquito. Infection with one serotype does not protect against the others, and sequential infections put people at greater risk for dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Dengue fever virus (DENV) is an RNA virus of the family Flaviviridae; genus Flavivirus. Other members of the same genus include yellow fever virus, West Nile virus, St. Louis encephalitis virus, Japanese encephalitis virus, tick-borne encephalitis virus, Kyasanur forest disease virus, and Omsk hemorrhagic fever virus. Most are transmitted by arthropods (mosquitoes or ticks), and are therefore also referred to as arboviruses (arthropod-borne viruses).[27]

The dengue virus genome contains about 11,000 nucleotide bases, which code for the three different types of protein molecules (C, prM and E) that form the virus particle and seven other types of protein molecules (NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5) that are found in infected host cells only and are required for replication of the virus.[23,28] There are five[29] strains of the virus, called serotypes, of which the first four are referred to as DENV-1, DENV-2, DENV-3 and DENV-4.[30] The fifth type was announced in 2013.[29] The distinctions between the serotypes are based on their antigenicity.[27] The genetic variation in dengue viruses is region specific; suggestive that establishment into new territories is relatively infrequent, despite dengue emerging in new regions in recent decades.[26]

Transmission

Dengue is transmitted between people by the several species of mosquito of the Aedes type, principally Aedes aegyptii and Aedes albopictus, which are found throughout the world. In rare cases, dengue can be transmitted in organ transplants or blood transfusions from infected donors, and there is evidence of transmission from an infected pregnant mother to her fetus.[31] Other person-to-person modes of transmission have also been reported, but are very unusual.[22] But in the vast majority of infections, a mosquito bite is responsible[1].These mosquitoes usually live between the latitudes of 35° North and 35° South below an elevation of 1,000 meters (3,300 ft).[30] They typically bite during the early morning and in the evening,[32] but they may bite and thus spread infection at any time of day.[33]

Humans are the primary host of the virus,[27] but it also circulates in nonhuman primates.[34] An infection can be acquired via a single bite.[35] A female mosquito that takes a blood meal from a person infected with dengue fever, during the initial 2 -to 10-day febrile period, becomes itself infected with the virus in the cells lining its gut.[36] About 8-10 days later, the virus spreads to other tissues including the mosquito's salivary glands and is subsequently released into its saliva. The virus seems to have no detrimental effect on the mosquito, which remains infected for life. Aedes aegypti is particularly involved, as it prefers to lay its eggs in artificial water containers, to live in close proximity to humans, and to feed on people rather than other vertebrates.[19]

Symptoms of infection usually begin 4 - 7 days after the mosquito bite and typically last 3 - 10 days. In order for transmission to occur the mosquito must feed on a person during a 5- day period when large amounts of virus are in the blood; this period usually begins a little before the person become symptomatic. Some people never have significant symptoms but can still infect mosquitoes. After entering the mosquito in the blood meal, the virus will require an additional 8-12 days incubation before it can then be transmitted to another human. The mosquito remains infected for the remainder of its life, which might be days or a few weeks. Dengue is spread by the virus has five different types[37]; infection with one type usually gives

lifelong immunity to that type, but only short-term immunity to the others. Subsequent infection with a different type increases the risk of severe complications.[1] A number of tests are available to confirm the diagnosis including detecting antibodies to the virus or its RNA.[2]

Fig.-1: *The mosquito Aedes aegypti feeding on a human host*

Pathogenesis

When a mosquito carrying dengue virus bites a person, the virus enters the skin together with the mosquito's saliva. It binds to and enters white blood cells, and reproduces inside the cells while they move throughout the body. The white blood cells respond by producing a number of signaling proteins, such as cytokines and interferons, which are responsible for many of the symptoms, such as the fever, the flu-like symptoms, and the severe pains. In severe infection, the virus production inside the body is greatly increased, and many more organs (such as the liver and the bone marrow) can be affected. Fluid from the bloodstream leaks through the wall of small blood vessels into body cavities due to capillary permeability. As a result, less blood circulates in the blood vessels, and the blood pressure becomes so low that it cannot supply sufficient blood to vital organs. Furthermore, dysfunction of the bone marrow due to infection of the stromal cells leads to reduced numbers of platelets, which are necessary for effective blood clotting; this increases the risk of bleeding, the other major complication of dengue fever.[38]

Once inside the skin, dengue virus binds to Langerhans cells.[38] The virus enters the cells through binding between viral proteins and membrane protein on the Langerhans cell, specifically the C-type lectins.[23,24] The dendritic cell moves to the nearest lymph node. Meanwhile, the virus genome is translated in membrane-bound vesicles on the cell's endoplasmic reticulum, where the cell's protein synthesis apparatus produces new viral proteins that replicate the viral RNA and begin to form viral particles. Immature virus particles are transported to the golgi apparatus. The now mature new viruses are released by exocytosis. They are then able to enter other white blood cells, such as monocytes and macrophages.[23]

The initial reaction of infected cells is to produce interferon that raises a number of defenses against viral infection through the innate immune system by augmenting the production of a large group of proteins. Some serotypes of dengue virus appear to have mechanisms to slow down this process. Interferon also activates the adaptive immune system, which leads to the generation of antibodies against the virus as well as T cells that directly attack any cell infected with the virus. Various antibodies are generated; some bind closely to the viral proteins and target them for phagocytosis but some bind the virus less well and appear instead to deliver the virus into a part of the phagocytes where it is not destroyed but is able to replicate further.[23]

Predisposition/Risk factors

Severe disease is more common in babies and young children, and in contrast to many other infections, it is more common in children who are relatively well nourished.[39] Other risk factors for severe disease include female sex, high body mass index, and viral load.[40] While each serotype can cause the full spectrum of disease,[23] virus strain is a risk factor.[40] Infection with one serotype is thought to produce lifelong immunity to that type, but only short-term protection against the other three.[30] The risk of severe disease from secondary infection increases if someone previously exposed to serotype DENV-1 contracts serotype DENV-2 or DENV-3, or if someone previously exposed to DENV-3 acquires DENV-2.Dengue can be life-threatening in people with chronic diseases such as diabetes and asthma.[28]

Polymorphisms in particular genes have been linked with an increased risk of severe dengue complications.[13]

Clinical Features

As there are different severities of dengue fever, the symptoms can vary. Typically; people infected with dengue virus are asymptomatic (80%) or have only mild symptoms such as an uncomplicated fever.[13, 30] Others have more severe illness (5%), and in a small proportion it is life-threatening.[20] The incubation period ranges from 3 to 14 days, but most often it is 4 to 7 days.[19] Therefore, travelers returning from endemic areas are unlikely to have dengue if fever or other symptoms start more than 14 days after arriving home.[39] Children often experience symptoms similar to those of the common cold and gastroenteritis and have a greater risk of severe complications[39,40], though initial symptoms are generally mild but include high fever.[40] The characteristic symptoms of dengue are sudden-onset fever, headache (typically located behind the eyes), muscle and joint pains, and a rash. The alternative name for dengue, "break bone fever", comes from the associated muscle and joint pains.[13,22] The course of infection is divided into three phases: febrile, critical, and recovery. The febrile phase involves high fever, potentially over 40°C (104°F), and is associated with generalized pain and a headache; this usually lasts two to seven days.[22] Nausea and vomiting may also occur.[40] A rash occurs in 50-80% of those with symptoms[22,41]in the first or second day, 4 of symptoms as flushed skin, or later in the course of illness (days 4-7), as a measles-like rash[41]. A rash described as "islands of white in a sea of red" has also been observed.[27] Some petechiae that do not disappear when the skin is pressed can appear at this point, as may some mild bleeding from the mucous membranes of the mouth and nose.[22,39] The fever itself is classically biphasic or saddleback in nature, breaking and then returning for one or two days.[9,27]

In some people, the disease proceeds to a critical phase as fever resolves.[6] During this period, there is leakage of plasma from the blood vessels, typically lasting one to two days.[42] This may result in fluid accumulation in the chest and abdominal cavity as well as depletion of fluid from the circulation and organs.There may also be organ dysfunction and severe bleeding, typically from the gastrointestinal tract.[39,42] Shock (dengue shock syndrome) and hemorrhage (dengue hemorrhagic fever) occur in less than 5% of all cases of dengue, however those who have previously been infected with other serotypes of dengue virus ("secondary infection") are at an increased risk.[23,39] This critical phase, while rare, occurs relatively more commonly in children and young adults.[6]

The recovery phase occurs next, with resorption of the leaked fluid into the bloodstream.[42] This usually lasts two to three days.[39] The improvement

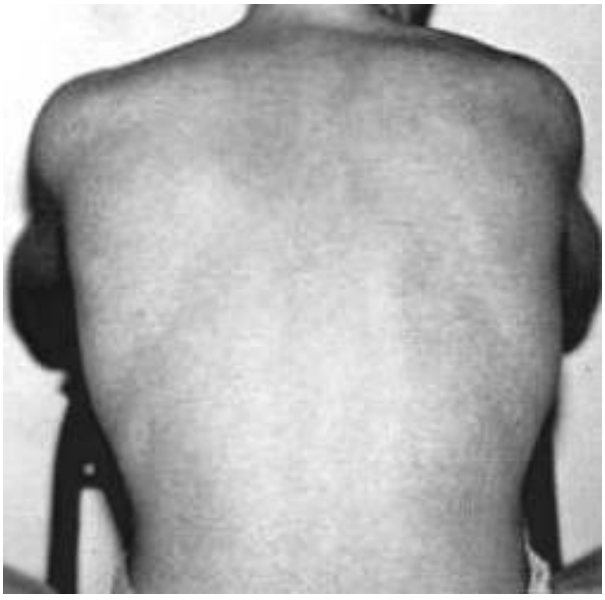

Fig.-2: *The typical rash seen in dengue fever*

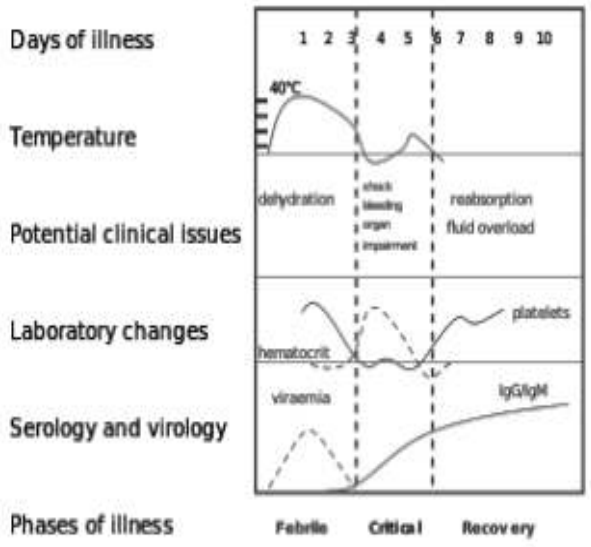

Fig-3: *Clinical course of dengue fever*

is often striking, and can be accompanied with severe itching and a slow heart rate.[39,42] Another rash may occur with either a maculpapular or a vasculitic appearance, which is followed by peeling of the skin.[6] During this stage, a fluid overload state may occur; if it affects the brain, it may cause a reduced leval of consciousness or seizures.[39] A feeling of fatigue may last for weeks in adults.[6]

Severe disease

It is not entirely clear why secondary infection with a different strain of dengue virus places people at risk of dengue hemorrhagic fever and dengue shock syndrome. The most widely accepted hypothesis is that of antibody-dependant enhancement(ADE). The exact mechanism behind ADE is unclear. It may be caused by poor binding of non-neutralizing antibodies and delivery into the wrong compartment of white blood cells that have ingested the virus for destruction[23, 28]. There is a suspicion that ADE is not the only mechanism underlying severe dengue-related complications,[43] and various lines of research have implied a role for T cells and soluble factors such as cytokines and the complement system.[38]

Severe disease is marked by the problems of capillary permeability and disordered blood clotting[6].These changes appear associated with a disordered state of the endothelial glycocalyx, which acts as a molecular filter of blood components. Leaky capillaries (and the critical phase) are thought to be caused by an immune system response.[6] Other processes of interest include infected cells that become necrotic - which affect both coagulation and fibrinolysis and low platelets in the blood, also a factor in normal clotting.[38]

Complications

Dengue can occasionally affect several other body systems,[42] either in isolation or along with the classic dengue symptoms. A decreased level of consciousness occurs in 0.5-6% of severe cases, which is attributable either to inflammation of the brain by the virus or indirectly as a result of impairment of vital organs, for example, the liver.[27]

Other neurological disorders have been reported in the context of dengue, such as transverse myelitis and Guillain-Barre syndrome[44]. Infection of the heart and acute liver failure are among the rarer complications.[39,42]

A pregnant woman who develops dengue may be at a higher risk of miscarriage as well as low birth weight and premature birth.[45]

Prognosis

Most people with dengue recover without any ongoing problems. The fatality rate is 1-5%,[39] and less than 1% with adequate treatment[46]; however those who develop significantly low blood pressure may have a fatality rate of up to 26%.[39]

Diagnosis

The diagnosis of dengue is typically made clinically, on the basis of reported symptoms and physical examination; this applies especially in endemic areas.[43] However, early disease can be difficult to differentiate from other viral infections.[39] A probable diagnosis is based on the findings of fever plus two of the following: nausea and vomiting, rash, generalized pains, low white blood cell count, positive tourniquet test , or any warning sign in someone who lives in an endemic area [46]. Warning signs typically occur before the onset of severe dengue.[42] The tourniquet test involves the application of a blood pressure cuff at between the dialostic and systolic pressure for five minutes, followed by the counting of any petechial hemorrhages; a higher number makes a diagnosis of dengue more likely with the cut off being more than 10 to 20 per 1 inch^2 (6.25 cm^2).[42]

The diagnosis should be considered in anyone who develops a fever within two weeks of being in the tropics or subtropics.[26] It can be difficult to distinguish dengue fever and chikungunya, a similar viral infection that shares many symptoms and occurs in similar parts of the world to dengue.[22] Often, investigations are performed to exclude other conditions that cause similar symptoms, such as malaria, leptospirosis, viral hemorrhagic fever, typhoid fever,meningococcal disease , measles, and influenza.[39,47] Zika fever also has similar symptoms as dengue.[48]

The earliest change detectable on laboratory investigations is a low white blood cell count, which may then be followed by low platelets and metabolic acidosis. [39] A moderately elevated level of aminotransferase (AST and ALT) from the liver

is commonly associated with low platelets and white blood cells.[26] In severe disease, plasma leakage results in hemoconcentration (as indicated by a rising hematocrit) and hypoalbuminemia. Pleural effusions or ascites can be detected by physical examination when large, but the demonstration of fluid on ultrasound may assist in the early identification of dengue shock syndrome.[39] Dengue shock syndrome is present if pulse pressure drops to d" 20 mm Hg along with peripheral vascular collapse.[26] Peripheral vascular collapse is determined in children via delayed capillary refill, rapid heart rate, or cold extremities.[42] While warning signs are an important aspect for early detection of potential serious disease, the evidence for any specific clinical or laboratory marker is weak.[49]

Laboratory tests

The diagnosis of dengue fever may be confirmed by microbiological laboratory testing[46].This can be done by virus isolation in cell cultures,nucleic acid detection by PCR, viral antigen detection(such as for NS1)or specific antibodies (serology).[24,47] Virus isolation and nucleic acid detection are more accurate than antigen detection, but these tests are not widely available due to their greater cost.[47] Detection of NS1 during the febrile phase of a primary infection may be greater than 90% sensitive however is only 60-80% in subsequent infections.[6] All tests may be negative in the early stages of the disease.[28, 39] PCR and viral antigen detection are more accurate in the first seven days[6]. In 2012 a PCR test was introduced that can run on equipment used to diagnose influenza; this is likely to improve access to PCR-based diagnosis.[50]

These laboratory tests are only of diagnostic value during the acute phase of the illness with the exception of serology. Tests for dengue virus-specific antibodies, types IgG and IgM, can be useful in confirming a diagnosis in the later stages of the infection. Both IgG and IgM are produced after 5–7 days. The highest levels (titers) of IgM are detected following a primary infection, but IgM is also produced in reinfection. IgM becomes undetectable 30–90 days after a primary infection, but earlier following re-infections. IgG, by contrast, remains detectable for over 60 years and, in the absence of symptoms, is a useful indicator of past infection. After a primary infection, IgG reaches peak levels in the blood after 14–21 days. In subsequent re-infections, levels peak earlier and the titers are usually higher. Both IgG and IgM provide protective immunity to the infecting serotype of the virus.[19, 22, 24] In testing for IgG and IgM antibodies there may be cross-reactivity with other flaviviruses which may result in a false positive after recent infections or vaccinations with yellow fever virus or Japanese encephalitis.[6] The detection of IgG alone is not considered diagnostic unless blood samples are collected 14 days apart and a greater than fourfold increase in levels of specific IgG is detected. In a person with symptoms, the detection of IgM is considered diagnostic.[19]

Management

There are no specific antiviral drugs for dengue; however maintaining proper fluid balance is important.[6] Addressing the plasma leakage and complications of it has become the mainstay of treatment of dengue.Treatment depends on the symptoms.[51] WHO in their 2012 handbook on management of dengue, have described stepwise approach to the management of dengue, where only isotonic solutions have been advised, followed by serial monitoring of clinical status, fluid balance and hematocrit. Judicious fluid resuscitation was advised to maintain effective circulation during the leak period. Those who are able to drink, are passing urine, have no "warning signs" and are otherwise healthy can be managed at home with daily follow up and oral rehudration therapy.[51] Those who have other health problems have "warning signs", or who cannot manage regular follow-up should be cared for in hospital.[39,51] In those with severe dengue care should be provided in an area where there is access to anintensive care unit.[51].

Intravenous hydration, if required, is typically only needed for one or two days.[51] In children with shock due to dengue, a rapid dose of 20mL/kg is reasonable.[52] The rate of fluid administration is than titrated to a urinary output of 0.5–1 mL/kg/h, stable vital signs and normalization of hematocrit.[39] The smallest amount of fluid required to achieve this is recommended.[51]

Invasive medical procedures such as nasogastric intubation, intramuscular injections and arterial punctures are avoided, in view of the bleeding

risk.[39] Paracetamol (acetaminophen) is used for fever and discomfort while NSAIDs such as ibuprofen and aspirin are avoided as they might aggravate the risk of bleeding.[51] Blood transfusion is initiated early in people presenting with unstable vital signs in the face of a decreasing hematocrit, rather than waiting for the hemoglobin concentration to decrease to some predetermined "transfusion trigger" level. Packed red cells cells or whole blood are recommended, while platelets and fresh frozen plasma are usually not[53]. There is not enough evidence to determine if steroids have a positive or negative effect in dengue fever.[54]

During the recovery phase intravenous fluids are discontinued to prevent a state of fluid overload.[39] If fluid overload occurs and vital signs are stable, stopping further fluid may be all that is needed. If a person is outside of the critical phase, a loop diuretic such as furosemide may be used to eliminate excess fluid from the circulation.[53]

Prevention

The best method of prevention is to avoid being bitten by mosquitoes. Prevention depends on control of and protection from the bites of the mosquito that transmits it.[32,55] The World Health Organization recommends an Integrated Vector Control program consisting of five elements[32]:

- Advocacy, social mobilization and legislation to ensure that public health bodies and communities are strengthened;
- Collaboration between the health and other sectors (public and private);
- An integrated approach to disease control to maximize use of resources;
- Evidence-based decision making to ensure any interventions are targeted appropriately; and
- Capacity-building to ensure an adequate response to the local situation.

The primary method of controlling A. aegypti is by eliminating its habitats. This is done by getting rid of open sources of water, or if this is not possible, by adding insecticides or biological control agents to these areas.[32] Generalized spraying with organophosphate or pyrethroid insecticides, while sometimes done, is not thought to be effective.[20] Reducing open collections of water through environmental modification is the preferred method of control, given the concerns of negative health effects from insecticides and greater logistical difficulties with control agents.[32] People can prevent mosquito bites by wearing clothing that fully covers the skin, using mosquito netting while resting, and/or the application of insect repellent (DEET being the most effective).[35] However, these methods appear not to be sufficiently effective, as the frequency of outbreaks appears to be increasing in some areas, probably due to urbanization increasing the habitat of A. aegypti. The range of the disease appears to be expanding possibly due to climate change.[37]

Vaccine

There are ongoing programs working on a dengue vaccine to cover all four serotypes.[55] Now that there is a fifth serotype this will need to be factored in[29]. One of the concerns is that a vaccine could increase the risk of severe disease through antibody- dependent enhancement (ADE). The ideal vaccine is safe, effective after one or two injections, covers all serotypes, does not contribute to ADE, is easily transported and stored, and is both affordable and cost-effective.[56]

Six dengue fever vaccines are in development, but not yet available. The vaccine that's furthest in development is a three-dose vaccine for children. The results of a phase III trial were published in July 2014. This study showed that the vaccine appears to be safe, and it prevented dengue infections slightly more than half the time.

As of December 2015, there was no commercially available vaccine for dengue fever.[49] One that is partially effective is now available in Mexico, the Philippines, and Brazil from 2016.[57, 58] It received approval in December 2015.[58] The vaccine is produced by Sanofi and goes by the brand name Dengvaxia.[59] It is based on a weakened combination of the yellow fever virus and each of the four dengue serotypes.[60,61] Two studies of a vaccine found it was 60% effective and prevented more than 80 to 90% of severe cases.[62,63]

Research

Research efforts to prevent and treat dengue include various means of vector control,[64] vaccine development, and antiviral drugs.[55]

With regards to vector control, a number of novel methods have been used to reduce mosquito numbers with some success including the placement of the or copepods in standing water to eat the mosquito larvae.[64] There are also trials with genetically modified male A. aegypti that after release into the wild mate with females, and render their offspring unable to fly.[64]

Attempts are ongoing to infect the mosquito population with bacteria of the Wol bachia genus, which makes the mosquitoes partially resistant to dengue virus.[6] While artificially induced infections with Wolbachia are effective, it is unclear if naturally acquired infections are protective. Working is still ongoing as of 2015 to determine the best type of Wolbachia to use.[66]

Apart from attempts to control the spread of the Aedes mosquito there are ongoing efforts to develop antiviral drugs that would be used to treat attacks of dengue fever and prevent severe complications. Discovery of the structure of the viral proteins may aid the development of effective drugs.[67] There are several plausible targets. The first approach is inhibition of the viral RNA-dependent RNA polymerase (coded by NS5), which copies the viral genetic material, with nucleoside analogs. Secondly, it may be possible to develop specific inhibitors of the viral protease (coded by NS3), which splices viral proteins.[68] Finally, it may be possible to develop entry inhibitors, which stop the virus entering cells, or inhibitors of the 52 capping process, which is required for viral replication.[69]

Campaign: Anti-dengue day

International Anti-Dengue Day is observed every year on June 15.[70] The idea was first agreed upon in 2010 with the first event held in Jakarta, Indonesia in 2011.Further events were held in 2012 in Yangon, Myanmar and in 2013 inVietnam.[70] Goals are to increase public awareness about dengue, mobilize resources for its prevention and control and, to demonstrate the Asian region's commitment in tackling the disease.[71]

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Case Report

Pelvic Actinomycosis Infection - A Rare Case in IUD User

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Abstract

Pelvic inflammatory disease is one of the most commonly encountered serious infectious disease entities in gynaecology. Salpingoophritis is a part of the spectrum of PID which is more among the IUD users. Serious pelvic infection due to actinomycosis species can result in abscess formation, bowel complaints and other serious complications.

A 42years old lady admitted in gynae ward with complaints of continuous lower abdominal pain for 9 years but aggravated for last 3 years. Two and half years after the 1st delivery she used cu-T for contraception. One year after its use she developed low grade continuous fever and lower abdominal pain. Suddenly she suffered from acute abdominal pain following episode of severe diarrhoea.USG showed moderate thick collection in pelvic peritoneum that means pelvic abscess .Her Pelvic abscess was drained by colpotomy and Cu-T was removed. Repeated follow up USG showed Rt ovarian mass. Surgically she was treated by ovariotomy. But she did not get rid of pelvic pain .Gradually the pain was increased and was intolerable for last 3 years. She had dysmenorrhoea and dyspareunea. Abdomino pelvic CT scan showed left adenexal mass measuring about 6x5 cm. All ovarian tumor markers were within normal limit.TAH with left sided salpingoophrectomy and Rt sided salpingectomy was done. Histopathology of specimen showed presence of colonies of actinomycosis species.

(Sir Salimullah Med Coll J 2016; 24: 85-87)

Case report

A 42 years old woman p-1 , MR-2 admitted in gynae department of DMCH with complaints of lower abdominal pain for last 9 years but aggravated for last three years and low grade fever. Two and half years after her 1st confinement, she used CU-T for long term contraception. One year after the use of it she developed continuous and low grade fever. Then suddenly she suffered from one episode of severe diarrhea followed by acute abdominal pain. She got admission in hospital. An urgent USG whole abdomen was done which showed thick moderate collection in pelvic peritonium that means pelvic abscess and fever. Colpotomy was done to drain abscess and CU-T was removed.She left hospital. Follow up USG was done for constant lower abdominal pain after conservative surgery. There was a Rt ovarian mass. Again surgically she was treated by ovariotomy. The patient could not show any hospital document. But still she did not get rid of pain and had low grade fever.Hse was treated conservativey by antibiotics and analgesics. Gradually the pain was increased and become intolerable for last 3 years which affected her daily activities. She complaints about dysparunea and dysmenorrhoea with normal menstrual cycle and flow. So for further better management she admitted in DMCH.

On examination she was clinically stable with moderate anemia and low grade fever (100 C).Abdomen was soft , mildly tender with no palpable mass. On pelvic examination the cervix appeared normal, uterus was bulky, fixed R/V. Cystic tender mass was felt through left fornix about 5x5cm.Other fornices were shallow and

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slightly tender without significant vaginal discharge. On digital rectal examination finding was similar finding to vaginal finding.

Investigation showed low Hb% (7.5 gm/dl), WBC 13.5k/μL, ESR-97 mm in 1st hour. Others are normal. Pus cells were present in urine. Liver and renal functions were normal. USG showed left ovarian mass. Tumor markers for ovarian malignancy CA-125, CA 19-9, á-feto protein andCEA were normal.

CT scan of whole abdomen showed a normal finding except a mixed density lesion measuring about 6x5cm having enhancing solid component noted involving the left adenexa. Right adenexa appeared normal.

On admission she was treated with tab ciprofloxacin b.d for 7 days. Her anaemia was corrected by 3 unit fresh blood transfusion pre-operatively. Plan of treatment was laparotomy. On laparotomy after opening the peritoneum a left adenexal mass about 6x7 cm involving left ovary and F. tube was found. Right F. tube was thicken and oedematous. There was gross adhesion to surrounding structures. On digital adhesiolysis greenish pus like material came out from the mass.

TAH with left sided salpingoophorectomy and right sided salpingectomy was done with adequate plane of cleavage without any surrounding structure damage.

Post operatively she was treated with i/v Ceftriaxone, i/v Metronidazol and i/v Gentamycin for 7 days. Histopathology of specimen showed presence of colonies of actinomyces species. Then cap Doxycyclin 100 mg b. d for 7 days also added. Her post operative period was uneventful. One unite fresh blood was transfused. Her pain resolved. Fever was subsiden. She was discharged on7th post-operative day without any complication. After 7 days follow up the patient was quite painless except scar tenderness. After one year follow up the patient is symptomless and healthy.

Discussion

Actinomycosis is achronic infection characterized by presence of dense fibrous tissue and purulent fluid . Approximately 20% of the actinomycosis infection occurs in abdomen and pelvis. This species are normal inhabitants of human GIT. These are gm positive, nonacid fast anaerobic bacteria that exhibit branching and filamentous growth. The presence of actinomycosis in vagina has been subject of controversy .It is rather associated with the presence of a foreign body, most often IUCD. It is thought that IUD causes the initial tissue injury which permit subsequent colonization by these organisms. The relative risk of PID among IUD users has been reported to be increased and the risk may be slightly less with copper IUD users.[1,2,3]

In 1976 Gupta reported finding organisms resembling actinomycosis in the cervical smears of woman using IUD and he identified a majority of these organism as A. Israelli.[4,5,6] Overall rate of A. Israelli in cervical smear of IUD users at about 10%.Dr Kelly and Aaron when presented the case of pelvic actinomycosis, illustrated some new and important information for physician and patients concerned about the health of woman using IUD.IUD appears to predispose to colonization and tissue invation with actinomycosis species.The clinical spectrum of sickness ranges from low grade endometritis to tuboovarian abscess. Advanced pelvic actinomycosis with formation of solid masses can be difficult to differentiate from pelvic neoplasia i.e. ovarian malignancy, which can compress nearby structures and invade surrounding tissues.[7,8,9,10]

It extends to the abdominal wall and deep pelvic structures.The disease has several clinical presentations and non specific CT findings.[11,12]

Diagnosis of pelvic actinomycosis can be difficult preoperatively because of incidious onset and lack of sign of obvious pelvic inflammation .Pelvic exam. is often concerning for ovarian malignancy.[13] CT scan shows dense inhomogenous contrast enhancement in the solid component in 2/3rd of patients.[14] In our patient CT scan of whole abdomen showed normal finding except a mixed density lesion about 6x5cm having enhancing solid component noted involving left adnexa. Right adnexa appeared normal.MRI was not done .USG showed only left ovarian mass . Diagnostic imaging may be non- specific and misleading unless the possibility of actinomycosis is considered. Florino reviewed 92 cases of pelvic actinomycosis. The patients were usually in their reproductive years with a history of having an IUD. Multiple

symptoms can appear in varied frequencies, such as abdominal pain (85%), fever (60%).On lab tests,70% 0f them were anaemic,76% had leucocytosis, and 95% had raised ESR. Here my patient had chronic abdominal pain, low grade fever and moderately anaemic (7.5gm/dl). On laboratory tests she had leucocytosis moderately anaemic and raised ESR. All these findings are consistent to Florino reviewed findings .Diagnosis before operation can be done by CT or USG biopsy. But it was not done. My patient presented with pelvic pain and low grade fever .Gradually it became intolerable before hospital admission. Her presentation was raised the suspicion of PID which not completely responded to antibiotics. So after proper investigations exploratory laparotomy was preferred. Diagnosis was confirmed after histopathology.

I/V Penicillin is the preferred antimicrobial agent. Clindamycin, Tetracycline,Erythromycin, Doxycyclin , Amikacin, Clavulanic acid are also used for treatment of actinomycosis.[15]

Conclusion

Currently,IUD are one of the most common methods of reversible contraception worldwide Although IUD are highly effective for preventing unintended pregnancy. Users and medical persons should remember the risk of complications, including pelvic inflammatory disease. Whenever examining IUD users with GIT symptoms, pelvic actinomycosis should be included in the differential diagnosis .Antimicrobial agents can be tried for several attempt. If the symptoms remains unresponsive, surgery should be the preferable method for definitive diagnosis and cure the patient.

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