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Review article

Histological grade and Breast Cancer – are we using it right?

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ABSTRACT:

Breast cancer is the most common malignancy among women all around the world. There is geographical variation in the relative rate from one country to the other, Breast cancer is the most common malignancy among women all around the world. There is geographical variation in the relative rate from one country to the other, nevertheless it remains at the top. Not only it is the top most cancer among females but it is also the leading killer and the oldest known disease among women.

Introduction

Breast cancer is the most common malignancy among women all around the world (1-3). However there is geographical variation in the relative rate from one country to the other, nevertheless it remains at the top. Not only it is the top most cancer among females but it is also the leading killer and the oldest known disease among women. The history of breast cancer is as old as of human being which can be evidenced by the oldest books on medicine, written in different ages like Nei Jing; written by Huang Di Chinese Emperor in 2698 BC [(4)]. The oldest description of the breast cancer was given by Edwin Smith, written on Papyrus leaves in Egypt in 1600 BC (5)who described it as the bulging lump, cool to touch, having no treatment. In the period of Greeks the Hippocrates linked breast cancer with cessation of menstrual cycle in today's terminology menopause(6) . Major milestone in the history of breast cancer is the discovery of the lymphatic system in 1650s by Jean Pecquet, Thomas Bartholin and OlofRudbeck and another is the association of hormonal factors with the development and progress of the cancer by George Beaston in 1896 when he demonstrated the remission of the cancer after oophorectomy (5). Subsequently the discovery of oestrogen receptors in 1962 by Jenson & Jacobson, and identification of genetic mutations have added in the understanding of the disease[(6)].

The realisation of the importance of the understanding of the breast cancer biology also dates back to the

centuries ago, when the medical scholars and treating physicians observed that patients of the same age group and similar clinical parameters of the tumours behave so differently that with even minimal treatment survive almost natural life span while others with maximum possible aggressive treatment die within a short period of time(7). This realisation made them study inside of the tumours. This understating improved step by step, first the scientists noticed that morphological differences in terms of the histological type and grade and further advanced to the more sophisticated molecular and genetic differences(8, 9). Recent advances in the understanding of the disease have greatly improved survival. This improved understating of the biology has resulted in the development of more targeted therapy such as use of hormonal therapy in oestrogen receptor (ER) positive patients and trastuzumab in patients having Human epidermal growth factor (HER2)-2 positive(10, 11). The understanding of the biology of the disease therefore remains the fundamental to the oncologists dealing with the disease.

Histologically breast cancer arises from the duct cells or the lobular cells, thus the basic histological types of breast cancer are named after these cells of origin(12). Further specialised types of cancers are also seen but usually perceived as variants of ductal carcinoma. These cancer types include no special type, mucinous, papillary carcinoma, tubular and tubular mixed, comedo carcinoma etc. Breast cancer has potential to disseminate away from the breast, resulting in the metastases spread to lymph nodes and systemic spread to bone, liver, lung and brain. In order to identify the cases at highest risk of distant metastases a number of histopathological, molecular and genetic factors have been studied resulting in basic prognostic index such as Nottingham Prognostic index or more sophisticated such as Oncotype Dx are in practice. Given the economic burden associated with high tec analysis it is important to identify some economic tool. Grade could be one of those.

Histological grade of breast cancer

Histological grade of the breast cancer is the degree of differentiation of the tumours cells define in the terms of mitotic count, nuclear pleomorphic and the tubular formation. It was given by the Scarff-Bloom-Richardson in 1957, which was then modified by the Eliston and Elis in 1991[1,2]. The Eliston Ellis modification of the histological grade of the breast cancer is also known as Nottingham histological grading system. The Eliston Ellis modification of the histological grade considers three components of the tumour cells including tubule formation, mitotic count and the nuclear pleomorphism in a specified field, cumulative score of these three components is then considered as the tumour grade[2]. Figure 1 summarises the grading system of the breast cancer. Grade I is the well differentiated tumour while grade III is the poorly differentiated tumour. Biologically they pose different characteristics, their growth rate is also different, where poorly differentiated tumours show high rate of growth, resulting in poor prognosis.

three components is then considered as the tumour grade[2]. Figure 1 summarises the grading system of the breast cancer. Grade I is the well differentiated tumour while grade III is the poorly differentiated tumour. Biologically they pose different characteristics, their growth rate is also different, where poorly differentiated tumours show high rate of growth, resulting in poor prognosis.

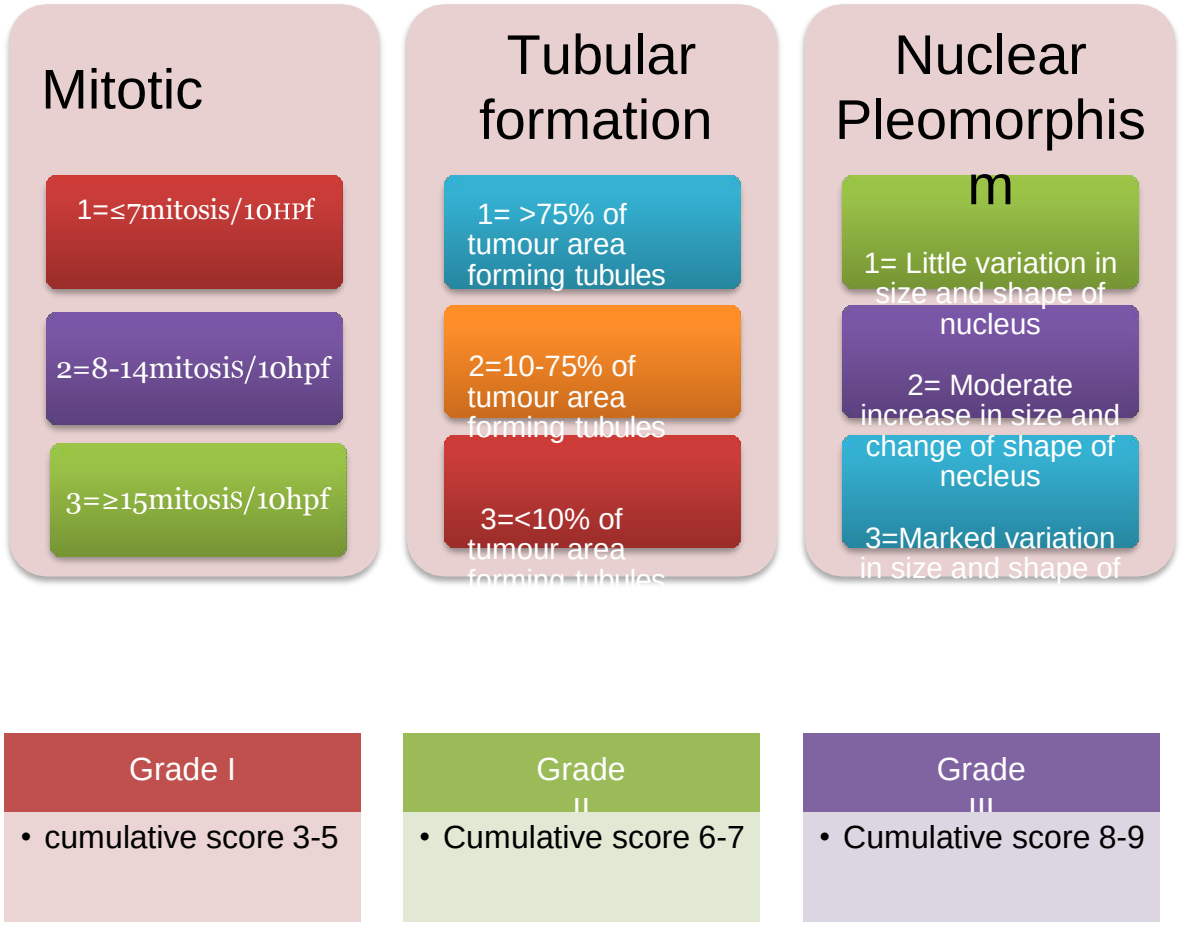

Figure.1. Histological grade of breast cancer

Correlation between the histological grade and the doubling time of tumour cells

The cell doubling time is the duration of the cell required to divide (ie mitosis). Figure 2 shows log of the doubling of cells in the cell division. The cell divides in log manner. One cell divides into two, two into four, four into eight, eight into sixteen and so on. The cells when divide at 20th time the theoretical number of cells would be around 1million and the clump of this number of cells make a mass of 1 mm[3]. When these cells divide 30 times they make a mass of 1 cm[3]. However in tumours all cells do not divide at one time. Some remain at resting stage for sometime before they enter into cell division again. Tumours with increased mitotic count and shorter interval between mitosis increase in size quickly.

When tumours have adequate blood supply the cells can divide at almost constant rate and also the supply of the activators of the cell growth such as oestrogen and progesterone e.g rapidly growing tumours have high mitotic count, shorter mitotic interval and reached to palpable stage quickly. The grade I tumours having <10% mitotic count show low growth rate because only 10% of cells dividing thus the increase in the size of the tumour takes longer duration as compared to the tumours having >75% of cells dividing. The study by Mehara et al analysed computer simulated doubling time to assess tumour growth rate. They used doubling time of the cells[4]. They measured tumour at day 1 and 200[4]. They concluded that the doubling time is not the sole predictor of the growth rate. Here again comes the theory that the tumour mass has heterogeneous pattern of cells. Some divide rapidly some at slow rate and at the same time some cells may be lost. Thus the increase in the size of tumour takes into account all these factors. However if 75% of cells are dividing and a constant rate of 10% are lost even then 65% of cells will increase in number with each cell division making the tumour mass grow rapidly as compared to the tumour where only 10% of cells are dividing and 5% of which are lost during that period.

Practical example is of the ER positive tumours (where majority show low grade tumours) in older women where great majority present with low grade and show very slow growth rate. Sometimes it may take years to increase in size. In contrast triple negative breast cancer in younger population where majority show high grade of tumours grow rapidly and show a poor prognosis. The rate of mitotic count and average doubling time of the tumours have potential to accurately predict the growth rate of the tumours (Figure 3.).

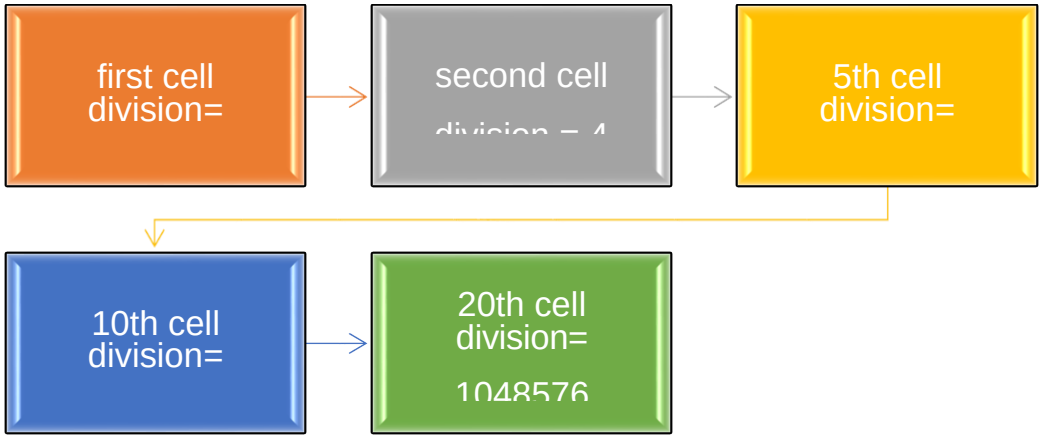

Figure 2. Log of the cell division pattern

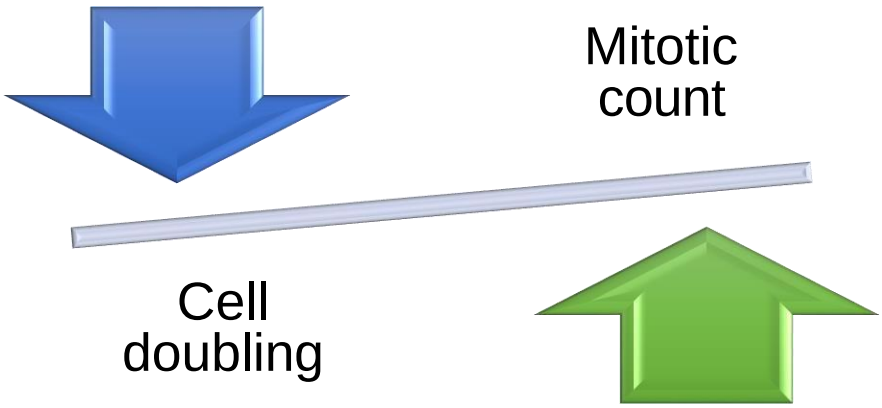

Figure 3. Relationship of the mitotic count with the cell doubling time in correlation with the tumour growth

Prognostic and predictive significance of grade in Breast cancer

Overall there is predominance of Grade II and the Grade I & III remain at the nearly similar pattern(13, 14). The studies have reported change in the pattern of grading distribution with age. In young patients there is predominance of the aggressive tumours with (ie. Grade III) while with advancing age there is change in the pattern and there is predominance of the less aggressive phenotype (ie grade I & II). As a result of change in the demographic characteristics of the breast cancer in different continents, African and Asian national have predominance of younger age group resulting in majority of high grade tumours. The SBR grading system was known for years but couldn't get acceptance as the prognostic factor in breast cancer due to lack of reproducibility and high level of subjectivity. However Eliston Ellis modification was tried on a large Nottingham/ Tenovus series (N=1831) with operable primary breast cancer in patients <70 years of age(15, 16). This series was aimed to analyse prognostic factors in breast cancer. The Nottingham tenovus series analysis showed that tumour grade is the strong prognostic factor which differentiates between the patients enjoying better survival and those who develop progression of disease early. As a result histological grade was also included in the Nottingham Prognostic Index. A number of studies then followed and tested prognostic significance of the histological grade(17-19). The histological grade has even maintained it prognostic significance in the modern era of the genetic testing. In other words it wont be wrong if we say that the morphological pattern in terms of grade in a way represents molecular and genetic pattern of the tumours. However with advancements in the management and targeted therapies the survival has improved though categorical distribution among the grade ranks remained the same. There is also indirect relation of the grade with the prognosis in terms of direct relationship with poor prognostic factors such as the S-phase fraction and Ki-67 and inverse relation with good prognostic factors such as hormone receptor status(14, 20).

Histological grade represents cell differentiation and the rate of growth. Thus theoretically it can predict response to the therapeutic agents which act on dividing cells such as cell cycle specific chemotherapy ie methotrexate(21). Technically it is not appropriate to assess the predictive response of a therapy when tumour is being removed. Once tumour is removed there are a number of confounding factors which may affect the predictive value of the histological grade. Chemotherapy on the other hand is the systemic therapy indicated as neo-adjuvant (before surgery) in locally advanced breast cancer, Adjuvant (after surgery) in operable primary breast cancer or as primary therapy in advanced cancer. There are different groups of cancer chemotherapy drugs classified on the basis their pharmacological groups or their mechanisam of action. A summary of the drugs used in breast cancer is given in figure 4. On the basis of their phamocological groups they are divided as cell cycle specific and non specific drugs. The cells have mixed pattern of cells having some cells dividing and others are at resting state. Thus a mixture of both group is given. Cell cycle specific chemotherapy affects the cells dividing actively. These drugs act on the cells in the log manner such that 50% of the dividing cells will be killed by one cycle. These drugs include fluorouracil, Methotraxate, Paclitaxil and docitaxel. Thus these drugs would ideally be useful for high grade tumours. Cell cycle non specific chemotherapy affects resting cells. These drugs include cyclophosphamide, Epirubicin and Doxorubicin. Low grade tumours can get maximum benefit from this group of drugs.

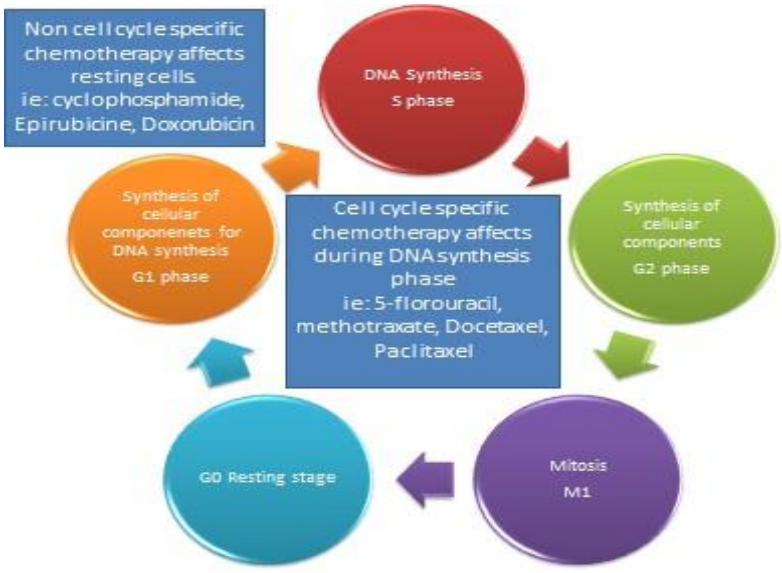

Figure. 4. Chemotherapeutic agent groups in relation to cell division

Conclusion

Histological grade of the breast cancer is not merely a morphological feature of the tumour but it portrays a whole spectrum of the molecular architecture of the tumour. Thus have potential to predict survival and role of different therapeutic agents. Further exploration of the factors governing histological grades need further exploration.

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Research article

Attention Deficit Hyperactivity Disorder (ADHD) in Children Consuming Junk Food

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Abstract:

Objective: The current study was aimed to determine relationship between junk food consumption and Attention Deficit Hyperactivity Disorder (ADHD) in school going children.

Methods: Standard questionnaires for the diagnosis of ADHD, junk food consumption among children and their mothers during pregnancy and lactation were distributed among children and parents of enrolled students.

Results: Parents of 84 students were contacted, of whom 47 were enrolled into the study. A total of 8 (17.02%) student were found to have ADHD, with a higher frequency in male students. Junk food consumption was found to have an association with ADHD.

Conclusion: ADHD is a common finding in school going children of district Kohat. The frequency is particularly high in male children and is strongly associated junk food consumption.

Key words: Attention Deficit Hyperactivity Disorder, Junk food, District Kohat, Artificial Food Colours

INTRODUCTION

Attention Deficit Hyperactivity Disorder (ADHD) is a neuro-developmental disorder, characterized by impairment in the executive functions of brain that include inattention, hyperactivity and impulsivity.[1] ADHD is diagnosed before the age of six years. In around 60% of the cases the individuals carry ADHD symptoms in their adulthood.[2] ADHD is a predisposing factor for other psychiatric conditions such as antisocial personality disorder, substance abuse, low educational level and tendency towards criminality.[3, 4] Several genetic and environmental factors have been implicated in the pathogenicity of ADHD.[5] It has recently been found that Artificial Food Colors (AFCs) and preservatives have a role in the development of ADHD among children in the general population.6

The strength of association between the two is, however, still a subject of debate.7 AFCs are frequently used in junk foods. The term junk food was first coined by Michael Jacobson (1972) for certain food categories with little or no nutritional value, or the ones which contain unhealthy ingredients (AFCs, preservatives etc.) as well.8

Junk foods also interfere with the neuronal activity of brain and have an addictive potential.9 The neuronal pathways affected are seriously interrupted by the in-utero exposure of fetus to junk food which increases the offspring's preference for junk food leading to its overuse of junk food in later life.10, 11. A study conducted on rats reported that the fetal exposure of maternal junk food significantly interrupts the neuronal pathways of the mesolimbic reward center by altering the receptor expression in these specialized areas of brain resulting in increased preference for junk food in the offsprings.[9] Studies supporting this hypothesis in the humans are lacking. This study elicits the inter-relationship of junk food preference in the children with that of mothers during pregnancy. The study also determines frequency of ADHD among children who prefer junk food.

METHODOLGY

This school based study was conducted from October 2011 to February 2012 on 150 children (4–7 years old) in three schools, namely the Educators, Beacon House School System, The Islamic Happy land Montessori School System, and the Pakistan Foundation Academy, all located in Kohat city, Khyber Pakhtunkhwa province of Pakistan. The approval was taken from the Institutional Review Board for Bioethics, KMU Institute of Medical Sciences. Letters were sent to the principals of the above mentioned schools explaining the objectives, methodology, and the duration of the study. After approval, a research team visited the schools and distributed the consent forms and self-reporting questionnaires among the class teachers of the study subjects. Another consent form and three different questionnaires were sent to the parents of students from the respective schools.

The questionnaires used in this study was translated bilingually from English to Urdu and then from Urdu to English by three language experts separately, to establish reliability. The questionnaire distributed among the school teachers was the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) diagnostic criteria for the diagnosis of ADHD. It consisted of 18 items, for the teacher rating of children hyperactivity level. Three different types of questionnaires were distributed among parents. The first questionnaire encompassed the demographic details and a detailed dietary history during their respective pregnancies, specifying the food taken during the first, second and third trimester. The second questionnaire delineated food preferences of the children towards healthy or junk food items. The third questionnaire was the DSM-IV diagnostic criteria for the parental rating of children hyperactivity. The questionnaires were collected after one month of distribution. The hyperactivity scores recorded at the school (by teachers) and at homes (by the parents), were compared for determining the ADHD status. All the children labeled as ADHD or hyperactive but below the threshold of ADHD were examined by the psychiatrist for the confirmation of the hyperactivity. Finally, the hyperactivity level was compared with the food preferences among the children.

RESULTS

Initially, parents of a total of 84 students were contacted of whom only 47 consented and were enrolled into the study. These included 21 female and 26 male students. The mean age of students enrolled was 5.7 years (age range: 4-7 years). It was found that 9 (19.15%) students were mild, 11 (23.40%) were moderate and 27 (57.45%) were excessive junk food users (Figure 1). A total of 27 students were found to be consuming junk foods; a majority of these were males (Table 1).

Among children with mild junk food preferences, only one (11.11%) had ADHD (Figure 1). In the moderate junk food consuming group of children, 2 (18.18%) had ADHD, a male and a female child. Similarly, among excessive junk food consuming students 5 (18.52%) had ADHD. It was found that among the study participants ADHD had a propensity for male gender, The maternal feedback depicting dietary histories in pregnancy and at the time of lactation was very poor; none of the mother could recall dietary history with certainty. This segment of research project was hence left unprocessed. The common coloring agents used in the junk food items found in this study included E122 (Carmoisine), E129 (Allura Red), E133 (Brilliant Blue) and E150.

Figure 1Activity status among junk food using children.

ADHD, Attention Deficit Hyperactivity Disorder

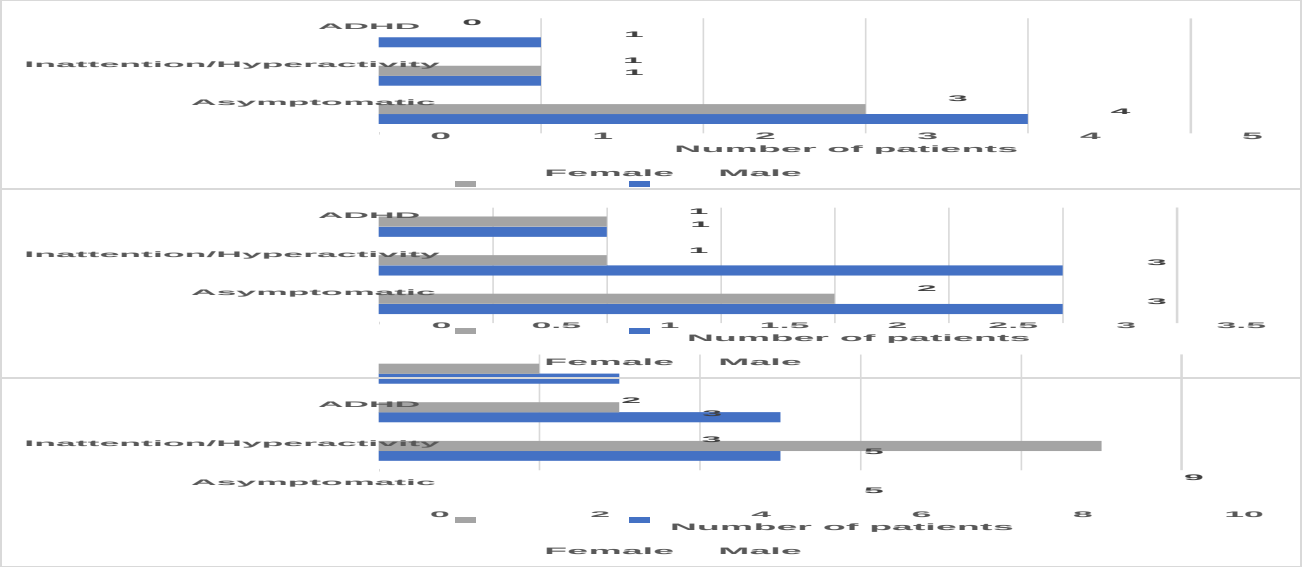

Table 1. Gender wise distribution of study participants in various junk food usage categories

	Junk Food Usage		
	Mild n (%)	**Moderate n (%)**	**Excessive n (%)**
Male	6 (12.76)	7 (14.89)	15 (31.94)
Female	4 (10.64)	4 (8.51)	12 (25.54)
Total	9 (19.14)	11 (23.40)	27 (57.44)

n, number of patients

DISCUSSION

In the current study, among 47 students, 8 (17.04%) were found to have ADHD. In a study conducted in the US, the ADHD prevalence rate among school going children was found to be 6.9% [12]. In another study from Germany, a prevalence rate of 4.8% was found.[13] This significant disparity may be attributed to the geographical, cultural, and social differences.

An increased propensity of ADHD for male gender was identified in the current study. Previously studies have also reported similar findings of increased frequency in male children.14, 15 It has been found that the decreased frequency among female children is mainly due to the relatively vague expression of the disorder.16 Another contributing factor is the frequent occurrence of co-morbid psychiatric conditions in female children which may have a masking effect on ADHD.[17]

The study was delimited by small sample size. Initially parents of 84 students were contacted, however, most of them (n = 37) denied to participate in the study. This higher frequency of denial may relate to the native cultural dilemma of strict traditional practices which prevents females from disclosing whereabouts of their pregnancies and lactation period. Among those who consented, recall bias compromised the data generated. The segment of study encompassing dietary details among mothers at the time of pregnancy and lactation was hence omitted. It is, however, strongly suggested that a prospective study addressing these limitations be carried out in larger set of students. It is also suggested that comprehensive questionnaires, encompassing the dietary histories in detail, discerning the quality, quantity and timing of meals be prepared while carrying out such studies. In addition, we could not correlate the ingredients in junk foods with the psychiatric clinical outcome. We suggest studies elucidating the relationship of these agents with level of hyperactivity in students.

CONCLUSION

ADHD is a common psychiatric disorder among school going children of district Kohat. Males are affected more frequently by the disease as compared to females. ADHD is strongly correlated with junk food consumption. Abstinence of children from such food items is therefore warranted.

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Research article

Does Ice Slush Placed Inside Pericardial Well During Mitral Valve Replacement Cause Injury to Phrenic Nerve?

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ABSTRACT

This study was conducted to determine frequency of phrenic nerve injury during mitral valve replacement, with application of Ice Slush, and compare it with international data. This study was performed at Punjab Institute of Cardiology Pakistan from January 2010 to December 2015. A total of 931 patients with only primary rheumatic mitral valve disease were prospectively observed. A pre-designed proforma was used, that recorded phrenic nerve injury. Out of 931 patients who underwent mitral valve repair or replacement, 466 (50.1%) were males. Time spent on CPB ranged from 40 to 120 min; in 58.1% patients the time remained less than 80 minutes. Post-operative chest X-ray remained normal in 97.5% patients whereas it showed atelectasis in 2.3% and elevated left hemidiaphragm in 0.2% of the patients. The total in-hospital mortality was 3.2% during study period. No patient was found to have phrenic nerve injury, unilateral or bilateral, during the study period. Topical cooling of heart, during mitral valve replacement, with Ice slush per se is not responsible for Phrenic nerve injury.

Key Words: Cardiopulmonary Bypass, Mitral Valve, Phrenic Nerve, Ice Slush

INTRODUCTION

Phrenic nerve is the principal innervation of the diaphragm. It is formed in the neck, from cervical nerve roots C3, C4 and C5. It runs vertically downwards on the anterior surface of scalenus anterior, behind the prevertebral fascia. It leaves the medial side of the muscle near its lower end and enters the thorax

between the main arterial and venous structures lateral to the vagus nerve. The nerve is related to the mediastinal pleura and passes in front of the hilum as it descends across the pericardium.

This nerve on each side supply ipsilateral sensory fibres to the fibrous pericardium, the parietal serous pericardium and the mediastinal pleura, before terminating in diaphragm.[1] During Mitral valve repair or replacement procedures, the Cardiopulmonary Bypass (CPB) with hypothermia is used.[2,3] After cross clamping and cardioplegia, the surface of the heart is further cooled down to reduce the local Basal Metabolic Rate (BMR), especially over the left ventricle, in order to reduce myocardial damage.[2,3] This makes left phrenic nerve at risk of paresis or paralysis; this may be short term or long term.[1,2,3] Once phrenic nerve injury occurs it can result into abnormal diaphragmatic movements & transitory diaphragmatic paralysis. This then results into higher rates of prolonged mechanical ventilation, failure of extubation, nosocomial pneumonia, pleural effusions & thoracentesis, prolonged ICU stay, increased treatment cost and may be mortality.[3,4] Thus, this study was conducted to sort out issue of phrenic nerve injury, whether unilateral or bilateral, with application of Ice Slush, and compare it with international data.

METHODOLOGY

This study was performed at Department of Cardiothoracic Surgery, Punjab Institute of Cardiology, Lahore, Pakistan, from 1st January 2010 to 31st December 2015. A total of 1105 patients with only primary rheumatic Mitral valve disease, both stenosis and regurgitation, were prospectively observed, without coming into knowledge of primary surgeons who were either doing repair or replacement. Both men and women aged 20-50 years, undergoing elective Mitral valve repair or replacement were included in the study. However, patients for emergency Mitral valve replacement, calcified mitral valve disease, Redo Mitral valve Surgery, & combined valve surgery, those patients with associated additional cardiac pathologies (Coronary artery disease, aortic aneurysm and congenital heart disease, detected preoperatively on echocardiogram and angiography), those patients with chronic obstructive pulmonary disease, chronic cough, diabetes mellitus, obesity (BMI (body mass index, ≥30) and neurological disorders, were excluded from the study. This left us with only 931 patients, suitable for study.

All cases were operated at the same Institute. Patients were preoperatively assessed as per routine. As the left phrenic nerve injury is more common, we performed central venous catheter placement through the internal jugular vein of right side, for all cardiac operations. All operations were performed with sternotomy under cardiopulmonary bypass with double venous cannulation, and after cooling to 28-32°C, with cardiac diastolic arrest by antegrade blood cardioplegia with potassium. Ice slush was applied all the time during repair or replacement of the mitral valve, either directly into the pericardial well or Ice slush wrapped inside gauze and then applied directly over the left anterolateral surface of heart. Once the mitral procedure was over, the Left atriotomy was closed with deairing. Cross clamp was made off from the ascending aorta, CPB was disconnected along with its connections.

Patient was normothermized (37oC). Hemostasis maintained and drains along with pericardial pacing wires placed. After the procedure Stainless-steel no.5 wire were used in all cases for sternum closure. Following sternal closure pre-sternal fascia, subcutaneous tissue and the skin closure was performed as per standard. A pre-designed proforma was used, that recorded demographic data of patients, type of valvular pathology, type of procedure performed, the size of the valve used, whether pleura was opened, cardiopulmonary bypass time, cross clamp time, degree of hypothermia, total time of hypothermia, total operation time, time on mechanical ventilation, delayed extubation, re- intubation, total ICU stay, pleural effusion, nosocomial pneumonia, reciprocal or paradoxical diaphragmatic / abdominal movement, total hospital stay, Chest x-ray findings (elevation of diagram, pleural effusion, no movement of diaphragm in expiration and inspiration) and ultrasonological detection of diaphragmatic movements, before operation during hospital stay and after 6 weeks of operation, need for chest intubation, Echocardiographic Findings (LVESD, LVEDD, EF, LA diameter), whether pericardial patch was taken, whether pericardium was closed before sternal closure, time to diagnosis of phrenic nerve injury, and mortality. Statistical Package for Social Sciences (SPSS) for Windows version 21 was used for the analysis of data. All qualitative variables, like diaphragmatic paralysis and gender, were presented in the form of frequency tables, percentages, graphs and pie charts. All quantitative variables were presented in the form of mean ± standard deviation and bar charts. Frequency of sternal wound dehiscence was compared in both groups by using Pearson chi-square test, Fischer Exact Test. A p-value of ≤0.05 was taken as significant.

RESULTS

Out of 931 patients who underwent Mitral valve repair or replacement, 466 (50.1%) were males. In 243 cases the primary pathology was stenosis, whereas regurgitation was found in 388 cases and mixed valvular disease in 300 patients. The preoperative echocardiography showed that the End systolic dimensions of left ventricle were less than 45 mm in 89 % patients (829) while End diastolic dimensions were between 56-60mm in 67.7% patients (630). The ejection fraction was more than 51% in 77.8% (725). The left atrial diameter was more than 56mm in 47.6% patients (419). Mitral valve repair was performed in 10 patients, whereas the major treatment remained the valve replacement (921 patients, 98.9%). Valve size of St Jude mechanical prosthesis varied between 27-33, where valve size 29 was most used (502 patients) followed by size 27 (326 patients). The time on CPB varied from as little as 40 min to as high as 120 min. but in 58.1% patients the time remained less than 80 minutes.

As far as the Ascending aortic cross clamp time was concerned 83.1% patients had less than 40 minutes of cross clamp time. Pericardial closure was performed in 7.7% patients. Pleura remain intact in 27.1% patients. The right pleura was most common to open, either willingly or inadvertently, in 36.8% patients, left pleura opened in 17.6% patients, while both pleura were found opened in 18.5 % patients. Total ICU stay was 2 days in 85.7% patients while the total hospital stay was 6-7 days in 75.5% patients. 93% patients remained on mechanical ventilation for 1 day or less. Post-operative chest X ray remained normal in 97.5% patients, showed atelectasis in 2.3% and elevated left hemidiaphragm in 0.2% patients.

The total in-hospital mortality was 3.2% (30 patients) during study period. No patient was found to have phrenic nerve, unilateral or bilateral, injury during the study period.

Table 1. Various study variables in comparison with immediate postoperative x-ray chest findings

	Normal Chest X-Ray	Atelectasis (n=21)	Elevated left hemidiaphragm (n=2)
Age			
• **Mean**	33.10	37.33	45
• **Minimum**	20	21	45
• **Maximum**	50	49	45
• **SD**	7.719	8.575	0.000
Gender			
• **Male**	457	8	1
• **Female**	451	13	1
Valve Pathology			
• **Stenosis**	238	4	1
• **Regurgitation**	379	8	1
• **Mixed disease**	291	9	0
CPB Time			
• **40-60 min**	251	5	-
• **61-80 min**	366	10	2
• **81-100 min**	194	4	-
• **100-120 min**	97	2	-
Cross Clamp Time			
• **20-30 min**	559	14	1
• **31-40 min**	194	4	1
• **41-50 min**	155	3	-
Hypothermia			
• **28ºC**	274	4	2
• **30 ºC**	282	9	-
• **32 ºC**	312	7	-
• **34 ºC**	40	1	-
Hypothermia Time			
• **20-30 min**	559	14	1
• **31-40 min**	194	4	1
• **41-50 min**	155	3	-
Pleura			
Intact	252	0	-
Right pleura opened	343	6	-
Left pleura opened	158	0	-
Both pleurae opened	155	15	2
Pericardial Closure	70	2	0
Total ICU Stay			
• **1 Day**	519	12	1
• **2 Days**	259	6	1
• **3 Days**	95	3	-
• **4 Days**	35	0	-
Mechanical Ventilation			
<1 Day	740	17	2
1 Day	103	4	-
2 Days	43	-	-
3 Days	21	-	-
> 3Days	1	-	-
Nosocomial Pneumonia	3	0	0
Mortality	30	0	0

DISCUSSION

Topical hypothermia over Heart into pericardial well is clinically applicable for myocardial preservation during heart operations, because during temporary individual coronary artery occlusion it protects regional myocardium detected as early return of function and decreased necrosis. This is the reason, an ice slush is used as topical cooling agent for conventional heart tissue preservation during open heart surgery.1-4

Diaphragmatic dysfunction results from damage to phrenic nerve, a well-recognized complication observed in cardiac surgery. Myriad of mechanism for phrenic nerve injury are proposed e.g. decreased body temperature, mechanical trauma and probably decreased blood flow. Hypothermia, especially Topical cooling with ice slush decreases cell membrane integrity during ischemia.2-5 Canbaz et al found 5 cases from a total of 78 patients, who developed left phrenic nerve dysfunction, all in the hypothermic cardiopulmonary bypass groups and were of the opinion that hypothermic CPB and topical ice-slush application may be related to phrenic nerve dysfunction.6 Similar results were shown by four studies by Efthimiou, & Dimopoulou, & Mazzoni, & Mills GH et al. 7-10 However, Canbaz S et al also suggested that as both left and right phrenic nerves have been equally exposed to CPB and systemic hypothermia, hypothermic cardiopulmonary bypass is not itself the cause of the damage.6 They explained phrenic nerve injury caused by topical cardiac cooling.

Sarnowski W et al gave their impression that ice/saline slush used addition to cold cardioplegia for heart arrest during cardiac surgery can cause hypothermic injury of phrenic nerve. Paralysis, partial or complete, of Phrenic nerve results in raised ipsilateral diaphragm and delayed recovery of the patients.2 Alassar A et al in their randomized study of patients undergoing elective cardiac surgery found significant phrenic nerve injury ($P = 0.009$) and failure of extubation ($P = 0.034$) with the use of with iced slush.11 Efthimiou J7 found Twenty (36%) patients developed unilateral diaphragm paralysis when topical hypothermia was used in open heart surgeries. Similarly, Cassese et al found that phrenic nerve injury and failure of extubation occurred more frequently with the use of iced slush ($P = 0.009$ and $P = 0.034$, respectively).12 Additionally, Maccherini et al in their study titled "Warm heart surgery eliminates diaphragmatic paralysis", found that topical hypothermia causes transitory diaphragmatic paralysis, pleural effusions, and thoracentesis.13 The opponents of Topical cooling with ice- slush suggested that such maneuver does not provide additional cardioprotective effects, and is an unnecessary adjunct to myocardial protection in patients undergoing cardiac surgery.2,14,15

Fortunately, we have found no Phrenic nerve injury during the study period. This study does not compass the whole cardiac surgical procedures and was performed only on those patients who underwent mitral valve surgical procedure, whether repair or replacement. In this group of patients iced slush was used whether covered in gauge and then applied over the anterolateral surface of heart or applied directly. The slush that turned water was immediately suctioned back. Secondly, due to shorter cross clamp time, the ice slush remains for a short time in pericardial well. It is also our routine practice to lift the left and right leaves of pericardiotomy and hitch them with the skin or with the self-retaining sternal spreader.

This does not allow the spilling over of the ice or water slush into the pleural space, once found open, thus reducing direct contact of hypothermic solution with ipsilateral phrenic nerve. It is also our routine practice just to drift a little hypothermia (mostly to 32-34oC. All these factors may have contributed to No phrenic nerve injury noted in any of the cases in the study group during specified period. The main limitation of this study was the inability to conduct electrophysiological studies to detect phrenic nerve injury perioperatively.

CONCLUSION

Once ice slush stays in pericardial well, for a shorter period of time, in cases of mitral valve surgeries, this remarkably reduces the chances of Phrenic Nerve injury. Thus topical cooling of heart with Ice slush per see is not responsible for Phrenic nerve injury.

Conflict of Interest: No conflict of Interest to be declared regarding publication of this paper.

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Research article

Emergence of Multi Drug Resistant Salmonella Typhi as Epidemic Among Lower Sindh Regions Patients of Pakistan

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Abstract

This study was aimed to determine the frequency of multidrug-resistant *Salmonella* Typhi (*S. Typhi*) among patients from lower region Sindh. This cross-sectional study was conducted at diagnostic and research laboratory, Hyderabad by evaluating cultures and sensitivity reports collected from different health care centers during one year period (November 2016 to November 2017). Total 92 patients were included in this study where most of patients were children with mean age of 6.2yrs and male population was predominant (63%). A detailed history and examination was done and blood culture reports were followed for sensitivity and resistance. Blood culture and sensitivity results showed resistance to ampicillin, cefixime, ceftriaxone, ciprofloxacin, and sulphamethoxazole/trimethoprim in 80.4%, 66.3%, 63%, 63% and 82.6%, in respectively. The highest sensitivity (89.1%) was recorded against meropenem. It was concluded that MDR salmonella infection is prevalent in Sindh, particularly in Hyderabad.

Key Words: Salmonella, Multidrug Resistance, MDR, Sindh, Enteric Fever

INRODUCTION

Typhoid fever remains as one of the major healthcare problems for this geographical location. It is one of those diseases which are diagnosed in areas where there is water contamination, poor hygiene and transmission of infection through feco-oral contamination from the affected person. It affects a large population and approx. 12-13 million cases are reported annually with high death toll of 1,90,000 people in the year 2010.[1] About 1% of the cases are fatal. Its occurrence is rare in developed countries, however it is endemic in developing countries especially in Africa and Asia.[2]

Southeast Asian countries seem to be the worst affected with high population density living in poor sanitary conditions and unavailability of clean water. Lack of education and hygiene further complicates the problem causing sudden outbreaks in such communities.[1, 3] These outbreaks can be attributed to contaminated food due to poor cooking practice in restaurants or food stalls failing to follow standard hygienic practice in kitchens, drinking or using water for washing and cooking especially contaminated with human or animal waste, infected poultry and eggs, from waste material of sick and or clinically unhealthy people especially involved in cooking.

Typhoid fever or enteric fever is caused by *Salmonella enterica typhi* (most commonly referred to as *Salmonella typhi*) Para typhi A, Para typhi B and Para typhi C.[4] Salmonella typhi is a motile, facultative gram-negative enteric bacillus, belonging to Enterobacteriaceae family. It has six subspecies and includes about 2500 serotypes.[5] Humans are the only known reservoir. It is a food borne pathogen. It is found in the intestinal tract of its host. Numerous strains of this particular organism have been isolated; characterized by variable metabolic characteristics, different levels of virulence, and possessing multi-drug resistance genes.[5] These genes then are responsible for complicating the treatment or resulting in failure causing death. A complete blood count test (CBC) may show decreased count of white blood cells (leukopenia), decreased neutrophil count (neutropenia), with decreased eosinophil count and a relative increase in lymphocyte count. Diagnostic criteria other than complete blood count includes stool D/R or a bone marrow cultures.[4] Bone marrow culture is more superior since it has more bacterial concentration and therefore considered gold standard for the diagnosis of S. Typhi. The growth on blood culture is attained on MacConkey and Eosin methylene blue (EMB) agars. This bacterium is non- lactose fermenting therefore no gas is produced when it is grown in Triple Sugar Iron (TSI) media, thus differentiating it from other *Enterobacteriaceae*. Symptoms of the disease include high grade fever spikes which may persists for several days with bradycardia, malaise, abdominal pain and occasionally cough. Abdominal pain can also be associated with diarrhea.[5] Hepatosplenomegaly may also be present. Dehydration can be found due to which patient may become delirious (typhoid state). About one third of the patients' develop rose spots on lower chest and abdomen. Complications include intestinal perforation which is a surgical emergency; septicemia, diffuse peritonitis, Encephalitis, pneumonia and acute bronchitis, cholecystitis, endocarditis, and osteitis are the notable. Timely empirical treatment and proper management can be curative for majority of the patients. However morbidity and mortality is significantly increased due to the emergence of multi drug resistant strains in endemic regions such as Asia.[6] Prevention is the key in high risk areas. Emergence of newer outbreaks of typhoid fever in large numbers formed the basis of this study.

METHODS

This cross-sectional study was conducted from November 2016 to November 2017. The consent of the patients were obtained both written and informed, before the commencement of study. The patients were selected from different areas of Sindh province **(Figure 1)**. The study included patients who presented to the outpatient department of healthcare units with features of typhoid fever.

Those with positive test for malaria or with signs of other infections on examination, having positive urine analysis for infection or any other systemic finding raising suspicion for any other infection were excluded from the study. History and systemic examination findings were recorded in predesigned data sheet. Patient's blood samples were collected for complete blood count (CBC) and peripheral smear examination. Blood culture and sensitivity using was carried out on MacConkey's agar following standard procedures. Currently recommended and commonly prescribed antibiotics were tested for sensitivity. The empirical treatment for enteric fever was continued in patients until the culture and sensitivity results were available.

The regular follow up of patients was kept in routine for treatment response and improvement or deterioration of clinical symptoms. The data was clinically analyzed. Statistical analysis was performed on SPSS version 16.0 employing descriptive statistics.

RESULTS

A total of 92 patients were included in this study, with a male to female ratio of 1.7:1. Mean age of the patients was 6.24 years. The study patients were selected from different cities and districts of Sindh Province. Majority of patients belonged from Hyderabad City Majority of the affected cases (51.1%) belonged to middle income group. The most affected ethnic group in this study were Urdu speaking community (47.8%) (**Figure 2**). Among the study patients, 52.2% presented with high grade fever (**Figure 3**). It was found that 41.3% of the patients relied on metropolitan board supplied water, which was neither filtered nor boiled; 92.4% of the patients relied on homemade food for their daily needs. Hygiene status was satisfactory in 55.4% of the patients. There was no history of pet animals in 88% of the patients. All the patient samples were 100% positive for polyvalent antisera for O antigen. Blood culture and sensitivity results showed resistance to ampicillin, cefixime, ceftriaxone, ciprofloxacin, and sulphamethoxazole/trimethoprim in 83%, 66%, 66%, 90% and 83%, in respective order (Table 1). Meropenem and Azithromycin were found to be the most effective ones, with no resistance in any case.

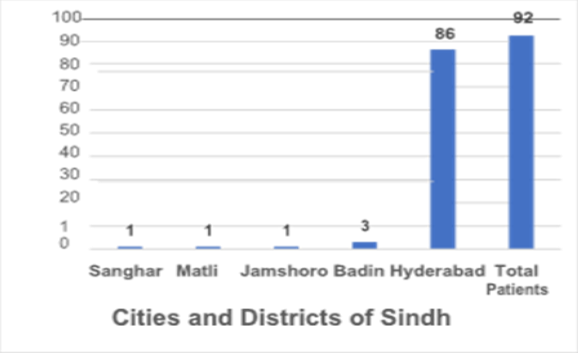

Figure 1. Frequency of Study Patients from different cities and districts of Sindh Province

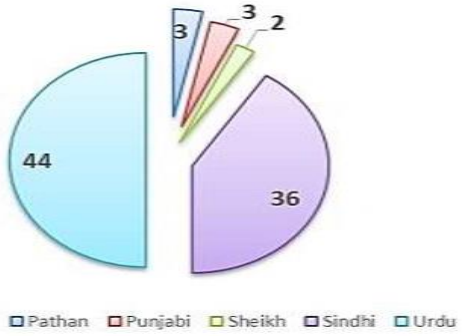

Figure 2. Representation of different ethnicities : Salmonella Typhi Infection

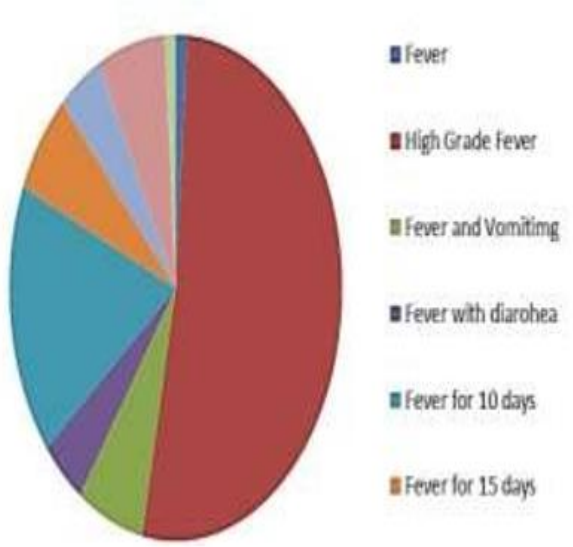

Figure 3. Distribution of fever found in patients with typhoid infection.

Table 1: Antimicrobial resistance of *S. typhi* isolated from blood cultures of cases included in the study

Antimicrobial agent	Resistance rate %
Ampicillin	83%
Trimet/Sulpha	83%
Chloramphenicol	83%
Ciprofloxacin	90%
Ceftriaxone	66%
Cefixime	66%
Meropenem	0
Azithromycin	0

Trimet/Sulpha, Trimethoprim/Sulphamethaoxazole

DISCUSSION

Multi drug resistance (MDR) salmonella typhi infection poses a worldwide threat. This disease is especially attributed to the developing countries including Asian and African countries; however, outbreaks have been reported in the developed world as well. Researchers worldwide have identified the MDR salmonella strains lineage, H58, being involved in Asian and African outbreaks and further studies are going on.[7, 8] This study reports MDR salmonella based on blood culture and sensitivity study and the resistant drug pattern is similar to an earlier study in Tajikistan India United states of America and Kenya.[7,10-13] Diagnosis of salmonella infection (typhoid fever) is important because of its complications and treatment challenges. In this particular study the number of patients was small, and most of the patients were children, there was an outbreak of a similar salmonella infection previously in Pakistan also.[14] Adults need to be included in further studies so that the sensitivity of drugs can also be studied. Patients from rural areas could not be included because the patient access was limited to tertiary care hospitals where all facilities are available for the diagnosis and treatment. Another limitation of this study is that those cases where blood cultures were negative for any growth of salmonella were excluded; however bone marrow cultures were not performed. We believe that a bigger study including patients enrolled from all over the country should be initiated. This would provide the complete spectrum of the disease and the status of MDR salmonella in Pakistan. We believe that a study should also be conducted for the identification of carriers of the disease and especially among workers involved

in the food industry. Health departments of provincial and federal governments should lead prevention efforts and implementation of laws related to clean drinking water and healthy food practices. Rational use of antibiotics, Proper education, creating awareness through print, broadcast and social media should be made, observing good sanitary hygiene and vaccination of the susceptible should be undertaken. The future research should be conducted to identify the MDR strains prevalent in this location and their genetic identification.

CONCLUSION

It was concluded that MDR salmonella infection is prevalent in Sindh. It is warranted that practice guidelines be developed to tackle this obvious threat.

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Research article

Hemostatic defects in Dengue infection at a tertiary care hospital in Karachi

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ABSTRACT

This study was aimed to investigate haemostatic defects in dengue infection. A cross sectional study was conducted from 2013-2014 at National Institute of Blood Diseases & Bone Marrow Transplantation, Karachi. Total 127 dengue patients of either sex were included. After clinical examination, serology was performed to confirm dengue. The complete blood picture (CBC), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, D-Dimer, liver function tests were performed. Out of 127 cases about 95.8% patients presented with myalgia and 88 had headaches. No splenomegaly or hepatomegaly was observed. Serologic antibodies were found in all patients. Average platelet count and white blood cell count were $47.2x10^3$ /ul and $5.3x10^3$/ul respectively. Eighty-three patients had prolonged PT while 92 patients had prolonged APTT value. Raised total bilirubin, alkaline phosphatase and SGPT were found in 7, 91 and 87 patients respectively. Highly elevated D-Dimer values were recorded in 96% cases while only 12% patients had higher fibrinogen levels. Marked hematological abnormalities were observed among all the patients diagnosed with DF regardless of age, sex and clinical presentation.

Key words: Dengue fever; fibrinolysis; D- Dimer; PT; APTT; hemostatic defects

INTRODUCTION

Dengue infection is a mosquito borne tropical illness caused by any of the four different viral serovers i.e. DEN I-IV. The common vectors for transmission in human are *Aedes aegypti* and *Aedes albopictus*. Clinically apparent disease due to dengue virus varies in severity from mild undifferentiated dengue fever (DF)

through to more severe forms such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The DF and DHF are more commonly occurring pathological events due to dengue virus world-wide; accounting to more than 100 million annual cases.[1,2] Acute febrile phase is manifested by headache, body aches, weakness, joint pains, GI discomfort and last upto 3-5 days. Capillary leakage due to increase vascular permeability is a key differentiating feature observed in DHF otherwise absent in DF. Excessive decrease of intravascular volume in DHF may lead to DSS. Common symptoms for DHF include skin petechiae or bruising, minor bleeding episodes but major hemorrhage is unusual.[3] The exact cause of bleeding in dengue infections is still obscured. Most evident physiological changes in DF include thrombocytopenia (platelet counts less than 150,000/l), raised hemoglobin, and hematocrit, and decreased total leucocyte counts. Viral attack on hepatocytes cause deranged liver function tests.[4]

Abnormalities in platelets function are common in dengue infections such as ADP induced impaired platelets aggregation response and increased secretory activities of platelets.[5] Furthermore, hyperfibrinogenemia, decreased fibrin monomers (FM) and slightly prolonged prothrombin (PT) and partial thromboplastin times (APTT) have also been reported in dengue infected patients.[6,7] According to some studies small fraction of DHF patients may exhibit alteration in levels of coagulation factors, such as factor II, V, VII, VIII, IX, X, antithrombin, and α-2–antiplasmin.[8] This study was aimed to investigate haemostatic defects in patients with dengue infection admitted to NIBD.

METHODS

A total of 127 dengue patients of either sex were enrolled to conduct a cross sectional study over a period of two years from 2013-2014 at National Institute of Blood Diseases & Bone Marrow Transplantation, Karachi. The study was conducted after approval by ethical review committee of NIBD. A clinical examination with detailed history was carried out. Serology was performed to confirm dengue using solid-phase immunochromatographic assays (MP Diagnostics MULTISURE dengue Ab/Ag Rapid Test kit). The primary and secondary infections were confirmed by presence or absence of NS-1 antigen and IgA, IgM, IgG. Primary dengue cases were NS-1, IgA or IgM positive while secondary dengue cases also showed positivity for IgG. Complete blood count (CBC) using EDTA blood sample analyzed by hematology analyzer XE- 2100. First line coagulation screening tests were performed to rule out the findings of hemostatic defects. Citrated plasma was used for Prothrombin time (PTT), activated prothrombin time (APTT), fibrinogen and D-Dimer levels using STA Compact R, Diagnostic Stago, France. Serum was used for Liver function test (LFT) using biochemical analyzer Mindray 200, China. The data obtained was subjected to simple descriptive analysis using SPSS version 23.0.

RESULTS

The dengue serological tests confirmed dengue infection in 127 patients. Out of these, 90 were males and 37 were females; mean age of the patients was 24.8(3-41 yrs). Overall, 79 patients had dengue secondary infection based on their serological findings i.e presence of IgG, IgM+IgG or IgA alone (*Figure-1*). Beside fever, vomiting and fatigue; other hematological manifestations observed were epistaxis, petechia, gum bleeding and hematemesis (*Table-1*). The diagnostic findings such as mean hematocrit, platelet count, PT, APTT, and liver function tests (LFTs) for DF patients are shown in *Table-1*.

The hematological parameter were elevated with abnormally high count of reactive lymphocytes observed in all cases. Mean platelets count was 80.76±84 x 10^9/L (p<0.001) with significantly raised APTT (p=0.048) and D-Dimer (<0.001) values. Raised APTT was observed in 104 (81.88%) patients most of these patients were also presented with bleeding and other coagulopathy symptoms as expected. Deranged biochemical tests including abnormal LFTs were also observed (*Table-1*).

***Table 1*: Laboratory and clinical findings of Dengue infected patients (n=127)**

Variables	Mean ±S.D (range)	p-value
Clinical findings		
Duration of illness (days)	5±2.1 (1-14)	-
Temperature (°F)	101.97±0.87 (100-105)	-
Headache (n%)	34	-
Fatigue(n%)	48.4	-
Epistaxis (n%)	19.82	-
Petechia (n%)	81.62	-
Gastrointestinal discomfort (n%)	23.48	-
Gum bleeding (n%)	11.9	-
Hematemesis (n%)	5.0	-
Hematological parameters		
Hematocrit (%)	38.05±6.22 (18-49)	<0.001
TLC (x 10^9/L)	5.32±3.09 (1.09-16.05)	0.001
Reactive lymphocytes (%)	73 ±9.03 (40-82.03)	0.001
Hemostatic parameters		
Platelets (x 10^9/L)	80.76±84.8 (2-421)	<0.001
PT (seconds)	14.04±2.36 (10-18)	0.99
APTT (seconds)	37.39±7.29 (20-50)	0.048
D-Dimer (mg/L)	2.58±2.72 (0.009-13.08)	<0.001
Fibrinogen (g/L)	2.56±0.78 (1.01-4.39)	0.99
Biochemical parameters		
AST (U/L)	145.12±250.61 (18-1996)	<0.001
SGPT (U/L)	92.10±119.55 (18-940)	<0.001
Gamma GT (U/L)	290.46±157.44 (128-840)	<0.001
Alkaline phosphatase (U/L)	83.49±114.44 (11-664)	<0.001
Direct Bilirubin (mmol/L)	0.63±0.71(0.1-5.8)	0.94
Albumin (g/dL)	4.64±3.63 (3.1-6.4)	<0.001

n%, percentage of patient out of 127; SD, Standard deviation; PT, prothrombin time; APPT, activated partial thromboplastin time; AST, aspartate aminotransferase; SGPT, serum glutamic-pyruvic transaminase;Gamma GT, gammaglutamyl transpepsidase.

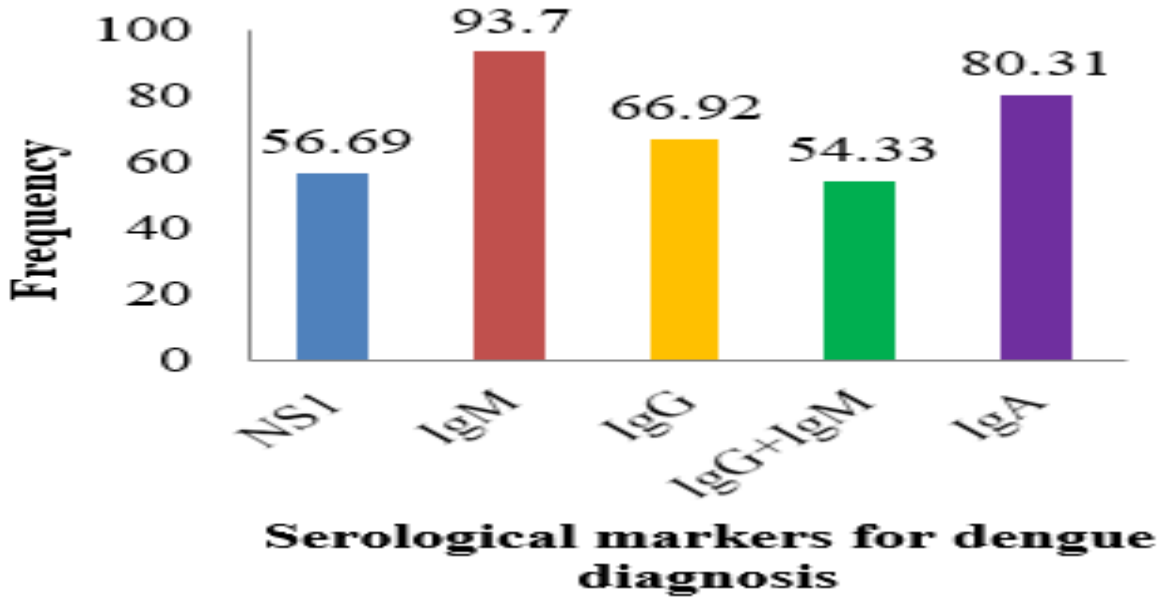

Figure 1: Frequency of serological markers in patients confirmed primary and secondary dengue infection

DISCUSSION

Over the past few decades DF has become one of the major viral infections in Pakistan. Post-monsoon seasonal outbreaks were a common observation throughout the country.[9,10] Untreated or misdiagnosed cases result in death due to hemostatic defects causing increased vascular permeability associated with severe form of DF i.e. DHF/ DSS with serious bleeding manifestations. Occurrence of DHF or DSS is more common in secondary dengue infections or reinfection due to different serover of dengue virus.[5] Past immuno-pathological mechanistic studies of DF suggest direct involvement of both humoral and cell-mediated immune responses.[11,12] Halstead proposed; that beside complement system; the antibodies from previous DF cause increased replication of virus infected macrophages/ mononuclear cells via an antibody dependent mechanism thereby contributing to pathogenesis of DHF.[13] Therefore, the present study was aimed to evaluate the possible association of defects in the extrinsic and intrinsic coagulation pathways with severity of DF. About 62.20% patients in our study population were presented with secondary dengue infections with 5% patients having episodes of hematemesis. The frequency of common symptoms such as elevated body temperature, fatigue and headache is comparable to other local and international studies.[9,7,14] Elevated hematocrit and activated lymphocytes were observed in most DF patients (*Table-1*). To study the cause of bleeding complications in DF; Kurane and Ennis hypothesized that activated lymphocytes such as T-cells along with monocytes release cytokines (IL-2, IFN-γ, TNFα, IL-6, etc.) along with chemical mediators (histamine and complement components etc.) which work synergistically to induce tissue injury due to dysfunctional vascular endothelial cells.[15] The shock due to plasma leakage causes defects in coagulation cascade resulting in hemorrhagic manifestations. Transient coagulopathy which last for few days during the course of disease have been reported in previous studies.[8,7]

The abnormal PT and APTT correlate with defects in the extrinsic and intrinsic coagulation pathways significantly therefore, raised APTT (p= 0.048) and D-Dimer (p=<0.001) were observed in 81.88% cases confirm abnormal events in intrinsic pathway in our study population. Another important factor is the level of fibrinogen, an acute phase protein, which is degraded to thrombin in case of vascular injury. Most of our patients had moderately low fibrinogen concentration which is in-line with previous studies.[7,14] The mechanism of fibrinogen consumption in Dengue is poorly understood. Wills et al., has proposed that the presence of dengue virus in DF directly activates fibrinolysis via molecular mimicry without the need of a usual thrombotic signal.[14] The fibrinogen degradation in turn initiates secondary activation of pro-coagulant homeostatic cascade. Therefore, hemorrhagic manifestations or bleeding episodes in DF does not follow the classical disseminated intravascular coagulation pattern rather it may be a combined consequence of low platelets count, altered platelets function, and augmented lysis of fibrinogen.[16] Increased D-Dimer values also confirmed increased fibrinolysis in our study. Like most viral infections abnormal LFT values were also observed as reported by Giri et al., in a past study.[4]

Conclusion: Significant association of transient coagulopathy was observed in most of the DF patients with suggested altered events in intrinsic coagulation and fibrinolytic pathways i.e. D-Dimer. Understanding the pathogenesis of DF is important and may lead directly to more effective treatment of patients with DF, particularly those with complex hemostatic disorders. Further research with large sample size and more parameters like determination of levels of clotting factors and inhibitors of coagulation pathways is suggested to confirm the clinical relevance of the current findings.

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