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EDITORIAL

Are we denying the Rights of Children?

Aisha Mehnaz

"Right" is the legal and moral entitlement to obtain or refrain from obtaining an action, in civil society. Rights are human constructs, created by society and enforced by the government. The constraints and obligations are placed on the individuals or groups.

It is not necessary that a right should be understood by the holder of that right; these rights may be recognized on behalf of another, such as children's rights. However, right must be understood by someone in order to have its legal and moral existence. Healthy and nurturing environment is the prerequisite for rearing a healthy child. Protection of children requires safeguarding children from all kinds of exploitation, abuse, maltreatment, neglect, abandonment, and discrimination. The responsibility lies with all individual within the society, Do children have rights? Who decides the basis and contents of the rights on legal or moral ground? This has to be done with unanimous consent at global level. After deliberating on the issue of children rights for 10 years, the United Nation (UN) reached a unanimous decision regarding Children Rights. UN adopted the resolution in Nov. 20, 1989 .In 1990 the world summit was held, all the member states present signed and ratified the CRC.[1]Pakistan was among the countries which has signed and ratified the CRC in 1990 for implementing it in accordance with Pakistan's Law and Islamic Ideology.

CRC provides a universal standard on the rights of the world's children. It contains 54 articles and deals with 4 major areas of children.(1)Survival, (2) Development, (3) Protection and (4) Participation. Survival, includes the right to live, registration at birth, preservation of identity. Development involves, right to education, health, standard of living, social security, leisure and recreation. In protection , articles included are concerned with protection of lost/missing children, children in labor, sexual abuse & exploitation, sale, trafficking, abduction, drug abuse, armed conflict, refugee children, neglect, torture and juvenile injustice.[1] Participation is the right to express opinion, freedom of thoughts, freedom of associations, protection of privacy and access to information.

It is the obligation of every state, civil society and parents to ensure the implementation of CRC in every country to give the children their right to survival, development, protection or participation. By ratifying the convention Pakistan is bound to the provisions in the CRC and is now answerable to the International Community.
According to CRC a child should have an access to adequate health care, medical assistance education and health promotion.

Are we able to provide protection to our children? Let us look at the situation of children in Pakistan. Children comprises of almost 50 percent of the population of Pakistan. More than 500,000 children under five years of age, die every year, i.e. 101 out of 1000 born do not reach their 5th birthday.[2] Nearly 40% of them do not complete even the first month of life, simply because their medical and nutritional needs are neglected. The common killers of these children are diseases which can be easily prevented like pneumonia, diarrhea, measles, malaria, typhoid etc. Malnutrition accounts for the major underlying cause of death in children. [2-3]

More than 40% of our children (Nearly 8 million) are suffering from undernutrition and anemia. [4-5] On the other hand we are increasingly seeing overweight obese children. This is a dangerous situation; these overweight children are as more at risk of developing diseases like diabetes, hypertension and other related disorders.
Lack of safe water, inadequate sanitation shelter and low education, directly effect rights of children. School enrolment ratio is 70% for 5-11 years, only 37% the age of 5 years .The literacy rate of youth is around 58%.Many children are deprived of schooling because they are working as laborers. Many organizations have estimated that there could be anywhere from 8 to 19 million child laborers in the country.[7] According

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to a survey by the Federal Bureau of Statistics, 40 million children are working as child laborers in the country.[8-9] In many cases, the parents of child laborers cannot afford an education for their children or they believe that education does not lead to marketable skills. More than twenty thousand (20,000) children are on the streets of Karachi alone. [8]

Children who are in conflict with law or are in prison, live in miserable conditions. Separate system for juvenile justice is not yet available to our children. [7]

Many other children who deserve serious attention include physically, emotionally & sexually abused children, refugees, kidnapped and the disabled .In Pakistan 15-25% children are sexually abused.[10] No official data exist on various types of Child Abuse but the figures are rising. There has been fourfold increase in the reported cases of maltreatment of Children during the past five years.

Nearly 25000 children were reported to be abused every year ; of these more than 4% were killed after committing heinous crime, unspecific number of cases go unreported, therefore the crime remains hidden.[10] These children are from all socio-economic and cultural strata. Karachi is the second largest city with the highest crime rate against children. Who are the abusers or perpetrators? In 80% of the cases the abuser is someone who is a close acquaintance, someone the child and the family know and trust.[11] Almost 50% of children who are abused are also murdered.

The violence against children has increased beyond imagination over the last few years, children are not only subjected to violence but are being used as a tool to perpetrate violence. Lately, there has been a marked rise in suicide bombing, large majority of the bombers are below 18 years of age. Their rights were violated from birth, they have no identity, most are subjected to brutality. We have failed to protect our children, this is a shameful situation for all of us, why have children rights never been implemented? Why we as a nation are unable to keep our promises to children? Considering the aftermath of violence and lawlessness, can we afford to continue denying the rights of our children?

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ORIGINAL ARTICLE

Knowledge of Anatomy: Where Do we Stand?

Muhammad Saeed[1] , Rameez Iqbal Hashmi[2], Ashraf Hussain[1], Syeda Arooj[1], Muhammad Aamir[3] and Attiya Khalid[4]

ABSTRACT

Objectives: Anatomy education has undergone enormous transformation in the last decade. Traditional topographic anatomy taught by didactic lectures and complete dissection of the body has been replaced by a wide range of special study modules, learning strategies and teaching tools. The present study was designed to provide a snapshot of the working knowledge of anatomy prevalent among Pakistani medical students and residents in this changing scenario.

Study Design: A cross-sectional study conducted through a mini-test questionnaire completed by 325 medical students & junior doctors randomly selected from four different medical institutions of Pakistan.

Results: Among 325 medical students and junior doctors, only 10% were able to identify all anatomical structures correctly, whereas 24% candidates identified =70% anatomical landmarks. Thirty nine percent of the junior doctors and medical students were able to identify =50% anatomical structures. The best anatomical knowledge was reflected by first year medical students whereas the worst performance was shown by residents. There was insignificant variation in the performance of candidates from different private medical schools; however the results of candidates from public sector institution were astounding.

Conclusion: The decay in anatomical knowledge over time portrays that residents would try to fill their anatomy deficiency during surgical residency rather learning surgical skills. The medical educationists should find-out the root cause of this deterioration. We propose a randomized trial using problem solving test items to identify the exact intensity of the problem prevalent among Pakistani medical students and residents.

Key words: Prosections, topographic anatomy, dissection, cadavers, anatomy teaching

INTRODUCTION

Current curricular reforms stress on integrated medical education and holistic patient care.[1-3] Traditional topographic anatomy taught by didactic lectures and complete dissection of the body has been replaced by a wide range of special study modules, problem based learning (PBL), case based learning (CBL), computer aided learning (CAL), prosections, plastinated specimens and many other teaching tools.[4-5] In many medical schools, dissected cadaver-based anatomy is no longer taught.[6-7] There is a great divergence in the teaching of anatomy, the

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educational impact of which has not been reflected in published scientific literatures.[8-13] However medical students at all levels of their professional learning think anatomy to be a very important subject for their clinical training.[14] The reduction in undergraduate teaching of anatomy has caused a great concern among undergraduate as well as postgraduate students, especially in surgery. This gave a safe passage to young men and women with poor knowledge of anatomy, to become surgeons.[15-16] Like us, anatomists across the globe are concerned with the deterioration in anatomical knowledge of their trainees.[17-18] The present study was designed to provide a snapshot of working knowledge of anatomy prevalent among Pakistani medical students and junior doctors. The carpal bones and important neurovascular structures in arm were chosen as a benchmark for assessment of anatomical knowledge, considering it clinically relevant to junior doctors in a number of disciplines and being feasible for objective assessment.

METHODS

A snapshot of anatomical knowledge was conducted through a mini-test survey questionnaire completed by randomly selected 325-medical students and residents. This cross-sectional study was completed by random sampling in four different medical schools located in different geographical locations of Pakistan over a period of two months during 2008. An informed verbal consent was taken from all the participants of the study. We grouped the participants of the study into three different categories, i.e, Residents, Clinical and Pre-clinical medical students. Two separate drawings of hand (Fig. 1) and arm (Fig. 2) with lines for labeling were used. Maximum fifteen minute time was allowed to label these diagrams in the presence of one of the authors, in addition to other invigilators. The study was conducted as a surprise mini-test questionnaire and the candidates were not allowed to communicate with each other by any means. The residents were chosen at random from general surgery and allied surgical specialties. No prior intimation was given and prospective candidates who knew of the test were excluded from the study, to give fair representation of current knowledge of anatomy as far as possible.

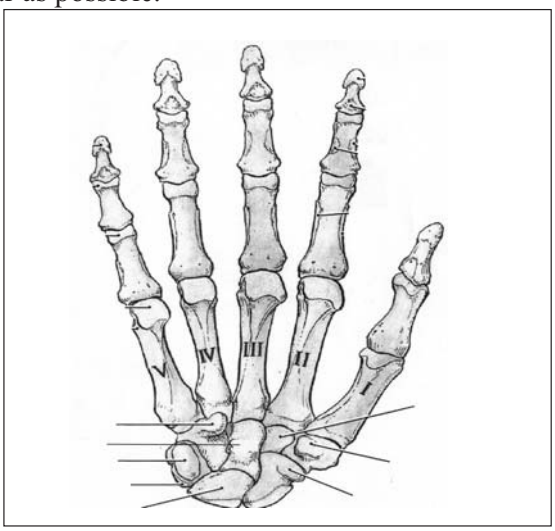

Figure – 1

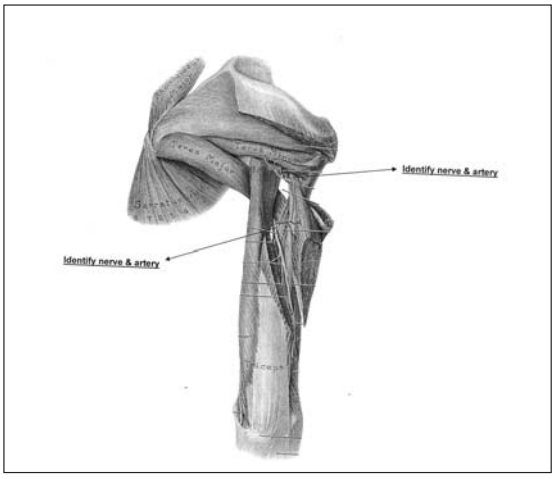

Figure – 2

RESULTS

Among 325 medical students and junior doctors, only ten percent (10%) were able to identify all anatomical structures correctly, whereas twenty four (24%) of the participants identified ≥70% anatomical landmarks. Thirty nine percent (39%) of the junior doctors and medical students were able to identify ≥50% anatomical structures. Most commonly identified structure was radial nerve (61%) followed by axillary nerve (60%), scaphoid (48%) and lunate (45%) bones. Surprisingly 61% of the participants were unable to identify even half of the structures asked in the mini-test questionnaire. The least identified structure was profunda brachial artery which was identified by 22% candidates only. The least identified bone among the carpals was Triquetrum (26%). Inability to identify the chief artery of the arm (profunda brachial artery), by 78% participants reflects the deterioration in anatomical knowledge of our future physicians / surgeons. Larger and more commonly injured bones were the most recognized by all groups of participants. Trapezium, trapezoid and triquetrum were frequently mixed up. Only one third of the participants were able to identify trapezium as the bone at the base of thumb.

The mean score of public sector medical school was 10.6 as compared to mean score of (5.6702, 5.1250 & 3.3929) for other three private medical schools. The results were analyzed for descriptive statistics and significance among different groups using one way ANOVA. The best anatomical knowledge was reflected by first year medical students whereas the worst performance was shown by the residents (Fig. 3). There was insignificant variation in the performance of participants from different private medical schools, however the result of factual anatomical knowledge of participants from public sector institution was astounding (Fig 4).

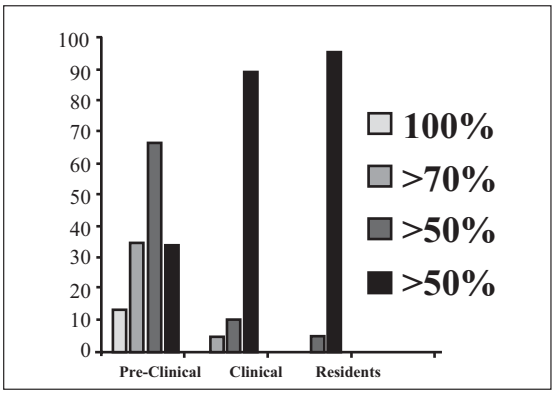

Figure – 3 : Comparison of Anatomical Knowledge of Residents, Clinical & Pre-clinical Students of Shifa College of Medicine

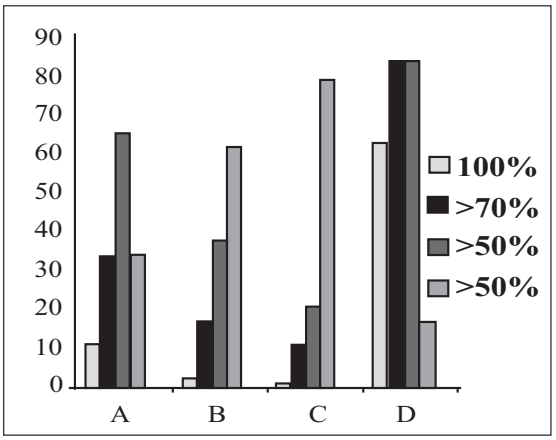

Figure – 4 : Comparison of anatomical knowledge of 1st year Students in different medical schools of Pakistan

DISCUSSION

A sound knowledge of anatomy remains central to the understanding of disease processes and the principles of surgical management.[18] Defining the anatomical site of the lesion is crucial if the physician and surgeons have to resolve the problem effectively and compassionately.[19] Therefore, contextual learning of anatomical knowledge is essential from the very beginning of undergraduate medical education. This can only be achieved by exposing and examining the tissues and structures in situ, best revealed and studied

by dissection.[20-21] Dissection-based anatomical analysis facilitates the classification of body components, the development of vocabulary and 3-dimensional mapping of viscera along with their surface projections, which would be later applied in clinical practice.[22-23] Biomedical informatics and new modalities of imaging have further magnified our knowledge of structural organization. Educating medical students in the principles of anatomy introduces them to the reality of death, concept of biological variations, uniqueness of each patient and instructs them the ways & means of accessing information.

Performance in gross anatomy is a better predictor of performance in the United States Medical Licensing Examination (USMLE) part-1; in comparison to A-level, Medical Colleges Admission Test (MCAT) and GPAs.[24] Although the assessment method applied in this study is a crude measure of the factual anatomical knowledge, however, it provides an insight into the level of anatomical knowledge at different stages of training in medical profession and the level of decay in this knowledge over time. Moreover, such studies may be helpful in the identification of "struggling" students whose performance could be improved by timely counseling.

The results of radial & axillary nerve identification were good among all groups and reflected the logical retention of these anatomical structures because of their potential implication in various clinico-pathological conditions. The best anatomical knowledge was reflected by first year medical students whereas the worst performance was shown by the residents. This finding was surprising to the assumptions of the authors. Although, medical students in their final year are preparing for their examinations in clinical disciplines, however, their knowledge of clinical anatomy is also formally tested at that stage. It is therefore, disappointing that none of them identified important anatomical structures in the upper limb. The residents' score demonstrated the worst performance among all levels of learners. Anatomy is central to general surgical training as well as allied surgical specialties. Therefore, it is also formally examined in the membership and fellowship examinations. The poor results of residents suggest that deficiency in their anatomical knowledge would be filled during their surgical training. The time available during surgical residency should not be used to learn the relevant anatomy; instead it should be utilized for the training of surgical skills. There was insignificant variation in the performance of candidates from different private medical schools (Fig 4), however the results of candidates from public sector institution were astounding (Fig 4). The variance among public and private institutions may be multi-factorial; linked to available anatomical learning resources, learning strategies in practice, faculty training and learning culture at the medical school; however the different entry stuff (input) for private and public institutions may be a single most important factor responsible for this remarkable difference. Usually, the private medical schools in Pakistan recruit the left-over candidates of the public sector institutions. The decay in anatomical knowledge with advancing professional life is reflective of non-coherent, disintegrated anatomy instruction which promotes rote learning, and fails to apply basic sciences knowledge in clinical context during later years of training

The results of present study are comparable to the similar exercise conducted in British medical schools.[17] Surgical colleges had already expressed their grave concern at the decline of undergraduate anatomical knowledge.[15-17,19] In prevailing scenario it is the responsibility of the medical educationists in general and anatomists in specific to find out the root cause of this deterioration in anatomical knowledge and propose remedial measures for this invasive and erosive problem. We propose a randomized cross-sectional study using problem solving test items to augment the findings of this snapshot of anatomical knowledge prevalent among Pakistani medical students and residents. As the learning strategies employed to learn anatomy

correlate positively with the quality of learning, retrieval and retention of anatomical knowledge; therefore an integrated undergraduate medical curriculum, where anatomical knowledge is structured in a clinical context and fosters critical thinking and problem solving, may be a remedy to our problem.

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ORIGINAL ARTICLE

Perinatal Outcome in High Risk Pregnant Women According to Antenatal Attendance at a Tertiary Hospital

Saima Aziz Siddiqui and Nargis Soomro

ABSTRACT

Objective: To analyze the perinatal outcome in two categories of patients on the basis of number of antenatal visits to three or less against more than three visits.

Study Design: Prospective analytical study.

Setting: Obstetrics & Gynaecology Unit II, Civil Hospital Karachi.

Methods: One hundred and twenty patients who attended high risk pregnancy clinic for one or more times during pregnancy, were prospectively followed till delivery for comparison of perinatal outcome in patients attending for three or less visits against more than three antenatal visits Outcome measures were analyzed by 'z' test and Fischer's exact test with a level of significance <0.05

Results: Perinatal mortality rate (PNMR) was **61.22/1000** in group A (three or less visits) versus **14.08/1000** in group B with more than three visits($p>0.05$). There was no significant difference in other pregnancy outcomes between the two groups but neonatal admission rate was significantly high in group A ($p<0.05$). Overall PNMR of the study population and that of group B was significantly lower than the annual PNMR of the department ($p<0.05$).

Conclusion: Minimal antenatal attendance adversely affects perinatal outcome in high risk pregnant women as compared to regular antenatal attendance with more than three visits.

Key Words: Antenatal care, high risk pregnancy, perinatal mortality.

INTRODUCTION

Antenatal care is a basic component of maternal care which is of major importance for the life of mothers & babies. World Health Organization (W.H.O) has defined antenatal care as a dichotomous variable, having one or more visits with a trained person during pregnancy.[1] Several Studies have identified link between lack of antenatal care and maternal mortality, poor maternal & neonatal health.[2-4]

According to WHO estimates 529,000 women died from obstetric causes in the year 2000 and 67% of these deaths occurred in South East Asia and Subsaharan Africa.[2,5]In contrast, the western and Northern Europe maternal mortality rate is around 10/100,000[6] live births. Maternal mortality ratio in

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Pakistan is estimated to be 276/100,000[7] births while according to hospital based studies in public sector hospital perinatal mortality rate(PNMR) is 97.7/1000 to 100.7/1000 total births.[8] Nordic countries have the lowest perinatal and maternal mortality i.e 4-7/1000 births.[9] Still birth rates are 3-5/1000 in developed countries in contrast to 100/1000 in developing countries.[10]Antenatal care is designated as one of the four pillars of safe motherhood initiative and the knowledge about danger signs in pregnancy is increased in women who utilize antenatal care.[11] The process of antenatal care and management of pregnancy[12] depends on identification of risk factors at first antenatal attendance and then pregnancy being classified as low or high risk pregnancy A high risk pregnancy is the one in which chance of adverse outcome to mother or baby is greater than general pregnant population.[13]

Teenage and maternal age 35 years or more, previous adverse outcomes in pregnancies previous still births & neonatal deaths have higher chances of unfavourable pregnancy outcomes.[14] A local study by Majeed R, et al identified antepartum and intrapartum factors such as maternal hypertension, antepartum hemorrhage, meconium stained liquor, multiple births, prolonged rupture of membranes and anemia are important in causing neonatal encephalopathy.[15] Post term pregnancy is also a recognized risk factor.[16] According to local studies bad obstetrical history, anemia, grandmultiparity & its association with advanced age, preterm births, malpresentations, multiple pregnancy, prelabour rupture of membranes and postpartum hemorrhage are important risk factors for adverse pregnancy outcome in our population.[17-18] One of the important tasks of antenatal care is to reduce perinatal mortality.[19] The recommended number of antenatal visits in different countries range from 8-13.[20-21] Studies have identified link between lack of antenatal care and poor pregnancy outcome including neonatal mortality.[22-23]According to Pakistan demographic health survey 2006-7, 61% mothers consult health professional, doctor, nurse or a lady health visitor, at least once for antenatal care while only twenty eight percent (28%) women make four or more prenatal visits throughout pregnancy.[7] There is lack of individualization of care for low and high risk pregnant women in public sector hospitals. Tertiary care hospitals face the problems in management of patients because of illiteracy, decreased awareness among women of high risk conditions during pregnancy, late antenatal attendance, missed visits, non compliance, unorganized health network,delay in timely referral to the consultant and increased workload. All these factors make antenatal management more difficult. WHO recommends minimum four antenatal visits during pregnancy. [1] There is no study, regarding comparison of perinatal outcome according to number of antenatal visits in high risk pregnancy clinics of public sector hospitals catering to the lower socioeconomic class. Aim of our study was to analyze perinatal outcome in high risk pregnant patients despite practical problems of minimal number of antenatal visits and late attendance.

SUBJECTS AND METHOD

Study Population & Setting: Study was conducted over a period of six months from 1st july 2008 to 31st December 2008 at Obstetrics and Gynaecology Unit II, Civil Hospital Karachi(CHK). It was a prospective analytical study.

One hundred and twenty patients who registered and delivered at obstetrics Unit II, CHK, were selected for study by non probability purposive sampling, irrespective of their number of antenatal visits and booking gestational age. Patients were prospectively

followed till delivery to find out pregnancy outcome. Comparison of perinatal outcome was done between group A which constituted patients who had ≤3 visits and group B which constituted patients who had >3visits.

OPERATIONAL DEFINITIONS

High risk pregnancy was defined as the one with risk factors such as high parity ≥5, severe anemia, previous caesarean section, high blood pressure, diabetes, other medical disorders with pregnancy, recurrent miscarriages or preterm deliveries, maplresentation, postpartum haemorrhage and placenta previa.

Main outcome measures were still births, perinatal mortality rate, intrauterine growth restriction, preterm births, constitutionally small babies and admission to neonatal intensive care unit (NICU). **Stillbirth** was defined as birth of babies weighing 500gram or more who did not show sign of life.[24] (heart beat, respiration, umbilical cord pulsation) **Perinatal mortality rate** was defined as number of stillbirths and neonatal deaths within seven days of life per 1000 live births and stillbirths.[25] **Intrauterine growth restriction** was defined as birth weight less than 10th centile for gestational age due to pathological factors.[26]

Preterm birth was defined as birth after the age of viability (24weeks) and before 37 weeks of pregnancy.[27]

Constitutionally small babies were defined as those with birth weight less than 10th centile for gestational age due to their normal genetic influences, in the absence of pathological factor.[26]

Data Collection

Data was collected by a semistructured proforma through details of antenatal record and prospective follow up on patient's admission in antenatal period and at delivery. Data was analyzed by SPSS version 10.Means, percentage and frequencies of relevant variables were calculated , tests of statistical significance applied. Continuous variables were analyzed by frequency and percentages, whereas categorical variables were analyzed by Fischer's exact test and Z test, where applicable. Level of significance was taken as <0.05.

RESULTS

Age of the patients ranged from 18-40 years.(Fig.1) **Mean age** of patients was **27.4years** ±4.83 S.D. The most frequent age group was 18-25 years **44.16% n=53**

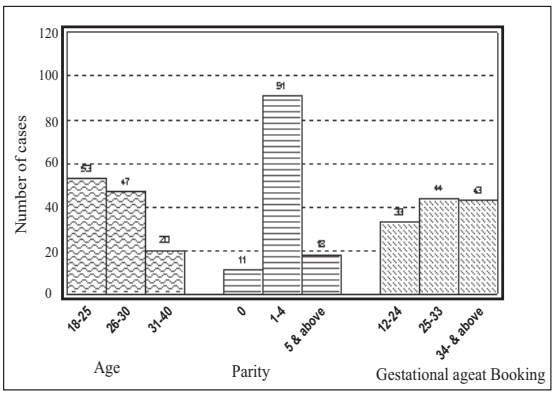

Figure –1 : Age, parity and booking gestation groups in the study population

Patients' **parity** ranged from 0 to 10. **Mean parity** was **2.5±1.94** S.D. **Primi**gravida were **9.17% n=11** and the most frequent parity was **para 1to 4** i.e**, 73.3% n=91**. Grandmultipara (parity 5 or more) 15 % n=18(Fig. 1)

Mean gestational age at first antenatal visit was **28.7 ±7.97 S.D**. 35.83% n=43 attended for first antenatal visit after 34 weeks of gestation(Fig. 1) **Mean gestational age at delivery** was **38.15 ±5.83SD**

Table 1: Comparison of perinatal outcome between the two groups

OUTCOME MEASURES	Group A* n=49(40.83%)	Group B** n=71(59.16%)	P value
PNMR*	61.22/1000	14.08/1000	0.101◊
Stillbirths	6.12% n=3	1.40% n=1	0.185•
IUGR	6.12% n=3	5.63% n=4	0.602•
Preterm birth	6.12% n=3	2.81% n=2	0.329•
Constitutionally small	-	1.40% n=1	0.592•
N.I.C.U admission rate	12.24% n=6	8.45% n=6	0.000144◊

*PNMR: Perinatal mortality rate NICU: Neonatal intensive care unit
* ≤ Three visits ** > Three visits
◊P value calculated by Z test •p value calculated by Fischer's exact test

Table 2: Comparison of Perinatal mortality rate of study population with annual PNMR of the year 2008

Category	PNMR of Study population	Annual PNMR for the year 2008∔	p value◊
Overall PNMR	33.33/1000*	79.10/1000	0.0417
Group A**	61.22/1000	79.10/1000	0.238
Group B***	14.08/1000	79.10/1000	0.0043

∔Annual perinatal mortality rate of the department for the year 2008
* Perinatal mortality rate of all cases included in the study
** ≤ three visits. *** > three visits
◊p value calculated by Z test

Overall Perinatal mortality rate was **33.33/1000** which was significantly lower than our unit's PNMR for the year 2008($p<0.05$) Comparison between two groups on the basis of number of antenatal visits to three or less against more than three visits was done.(Table i) In group **A i.e three or less than three antenatal visits(n=49,**40.83%) the **perinatal mortality** rate was **61.22/1000,** while **in group B i.e more than three visits,** it was **14.08/1000** ($p>0.05$) When compared with perinatal mortality rate of Gynaecology and Obstetrics Unit II, C.H.K for the year **2008 (79.10/1000)** PNMR was significantly decreased in group **B** ($p<0.05$) whereas though lower, but it was not significantly decreased in group **A** (Table 2).

The number of stillbirths in group **A** was 03, Among these, in the first case, patient had only single visit at 36 wks and previous CS due to cephalopelvic disproportion and she was presented with obstructed labour, while in second case, only one visit in 32 weeks with missed follow-ups and the cause of stillbirth was severe IUGR. In the third, early onset of severe PIH diagnosed on booking visit at 29 weeks and severe IUGR caused Intrauterine death(death of fetus in utero prior to onset of labour) at 32 weeks of pregnancy. There was one stillbirth in group **B,** patient had bad obstetrical history and gestational diabetes, booked at 14 weeks of pregnancy, had regular visits but she stopped taking insulin at 34 weeks and missed her follow up till 36 weeks; she reported with intrauterine death. **Other adverse perinatal outcomes** in these two groups were also not statistically different except NICU admission(Table 1), There was no neonatal death in either group but there was significant difference($p<0.05$) in the neonatal intensive care admission rate between group A and B. Maternal outcome was not significantly different between the two groups ($p=0.397$) In group **A,** one patient had caesarean hysterectomy for uncontrollable haemorrhage at C.S for placenta previa major (2.04% n=1) while in group **B,** it was 1.40% n=1. In this group also caesarean hysterectomy was done due to placenta accreta at caesarean section in a patient with previous 4 C.S.

DISCUSSION

Civil hospital Karachi is a public sector tertiary care hospital. Patients coming here belong to the lower socioeconomic class or lower middle class with majority of women being illiterate. Around 1200-1500 new cases enroll to seek antenatal care each year in our unit and majority of them having high risk pregnancy. The number of deliveries at our unit was 2236 in the year 2008 as the number of unbooked

cases and referred cases were also quite high. **Mean age** was 27.4 years but the most frequent age group was 18-25 yrs, which correlates with early childbearing, being a norm in our society. **Mean parity** was **2.5±1.94SD**.Whereas the most frequent parity was 1 to 4, while generally it is considered to be the safest parity group but they were all having high risk pregnancy. As shown in our data that the **mean booking gestational age** was **28 weeks**. Patients included in group A who first time attended clinic **after 34 weeks** were **35.83% (n=43).** Out of them 41.86% (n=18) first time attended clinic after 36 weeks of pregnancy. These also include 5 patients (27.27%) of previous 2 or 3 caesareans and 3 patients with sever PIH.

This high percentage of first attendance at term, shows lack of awareness and obstacles in seeking antenatal care.

Comparison according to the number of antenatal visits revealed higher perinatal mortality(61.22/1000) among group **A** i.e three or less than three visits as compared to **group B** with more than three antenatal visits having perinatal mortality rate of (**14.08/1000)** but this difference was not statistically significant ($p > 0.05$), which may be due to smaller sample size of group A as compared to group B. In group A majority were late attenders & non compliant 93.87% (n=93) visited first time after 34 weeks of gestation whereas 12.24% (n=6), did report before 34 weeks but did not return for follow up after one or two visits. As compared with department's PNMR of 79.10/1000, PNMR of group A, though lower was not statistically different ($p>0.05$). The number of still births in group **A** was three. One with severe early onset PIH who was booked at 29 weeks but had intrauterine death at 32 weeks of pregnancy because of severe IUGR and this patient had only two antenatal visits. Second still birth was in a patient with previous CS due to cephalopelvic disproportion with single visit at 38 weeks who declined admission for CS on same visit and later came with obstructed labour. Third still birth was also in a patient who had two visits prior to 32 weeks but failed to return thereafter and had stillbirth due to IUGR. In these three cases the cause of stillbirth was IUGR due to severe PIH in 33.33% (n=1), IUGR due to unknown cause in 33.33% (n=1) and IUGR due to mechanical factor in 33.33% (n=1).

Comparison of PNMR of group B with department's PNMR of 79.10/1000 revealed it to be significantly lower ($p < 0.05$) In group **B** there was only one stillbirth**,** the patient had bad obstetrical history, along with asthma and gestational diabetes. She was booked at 14 weeks of pregnancy, had regular visits but she stopped insulin at 34 weeks and missed follow up till 36 weeks, when she was diagnosed to have intrauterine death, one week prior to her planned CS. In group B, the cause of stillbirth was Diabetes as previously discussed. This unfortunate happening reflects the lower level of risk appreciation in our illiterate pregnant women. In overall study population, in 50% cases cause of stillbirth was severe intrauterine growth restriction where as diabetes and mechanical factor (obstructed labour) causes were 25%, each. In group **A,** IUGR was found in 6.12% of cases (n=3), the cause was severe PIH in 66.66% and in 33.33%, it was unexplained, while in group **B** the cause was Severe PIH in 25%, Monochorionic twin pregnancy in 25%, and unexplained in 50 % of cases. Preterm births were higher in group A than in group B, however this difference was not statistically significant. The causes of preterm delivery in group A were iatrogenic, preterm delivery with severe PIH, grandmultipara with prelabour rupture of membranes along with breech presentation and previous 3 CS

during labour at 34 weeks. Whereas preterm labour in group B were due to twin pregnancy and previous single C.S with impeding rupture. In group **B** one baby was constitutionally small as maternal weight was 38 kg at delivery and height was 148 cm. Neonatal intensive care unit (NICU) admissions showed significant difference between the two groups($p<0.05$). In group **A**, out of 6(**12.24%**) admissions; n=2(4.08%) babies had birth weight less than 2Kg, n=2(4.08%) had severe IUGR, n=1 (2.04%) due to meconium aspiration, as patient had bad obstetrical history and missed her date for elective CS and presented with PROM and n=1(2.04%) due to birth asphyxia in patient with severe PIH who presented with abruptio placentae. In group **B**, out of 6 (**8.45%**) NICU admissions, n=3 (4.22%) were due to IUGR, n=1(1.40%) was due to low birth weight, n=1(1.40%) was due to meconium aspiration and n=1(1.40%) was due to neonatal sepsis following prolonged rupture of membranes.

In our data 75% n=3 of all still births occurred in patients who had less than three visits and 66.66% of these didn't comply to medical advices.Similarly in regular attenders (with more than three visits) in group B, one patient had perinatal mortality and she also failed to comply medical advice at the crucial time of pregnancy. Thus excluding these cases only 1 patient among the study population had perinatal mortality who was compliant but booked at 29 weeks, had only 2 visits and presented with severe PIH and IUGR causing perinatal mortality causing a rate of 8.33/1000. Caesarean hysterectomy was the only maternal morbidity among the two groups . The patient in group **A** was admitted on her only visit at 38 weeks due to diagnosis of placenta previa major and severe haemorrhage who had caesarean section and lead to hysterectomy. While in group B patient had previous 4 CS and was antenatally diagnosed as having placenta accreta. Our data shows a trend that those women who had less than three visits are also late attendants & non compliant and they do not appreciate the risks in pregnancy. Prompt and relevant management at tertiary hospital even with late attendance results in relatively better perinatal outcome than general perinatal outcome in public sector tertiary hospitals (as shown by PNMR of 61.22/1000 vs 79.10/1000) but with late and minimal antenatal attendance and non compliance there is little time for management of significant multiple problems as undue increase workload, may result in deficient care. Overall Perinatal mortality rate was significantly lower i.e (33.33/1000) than the perinatal mortality rate of our unit in the year 2008 which was 79.1/1000 (p value 0.00417) and depicted an almost similar grim picture of other tertiary care hospitals of Pakistan which have shown perinatal mortality rates of 97.2/1000 to 100.7/1000 · There were 4 still births but no neonatal death. There was no maternal mortality but two patients had caesarean hysterectomy due to placenta previa major with morbid adherence. Our study's limitation is that it did not take into account those regular or minimal attendants of our antenatal clinic who did not deliver at our unit and thus their outcome is unknown which might have influenced the results.

CONCLUSION

Minimal or late antenatal attendance & non compliance are major contributors to adverse pregnancy outcome despite best possible care provided at a tertiary care hospital. Two out of three stillbirths in minimal attendance goup (A) and only one stillbirth in group B were avoidable. This study reveals the issue that it is illiteracy & poor level of

understanding among patients attending public sector hospitals that does not yield expected beneficial results of comprehensive care provided to them at tertiary hospital. It reaffirms the dire need that antenatal services coverage is maximized with emphasis on quality training programs for midwives to enable them to deliver effective antenatal care and to identify obstetric problems such as hypertension, antepartum haemorrhage, severe anemia, malpresentations and cephalopelvic disproportion and early referral in complicated cases.

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ORIGINAL ARTICLE

Analysis of Causative Factors in Oral Cancer

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ABSTRACT

Objective: To analyse different causative factors leading to oral cancer.

Study Design: Descriptive type of study.

Setting: This study was conducted at the department of E.N.T, DUHS, Karachi.

Study Period: From Jan 2008, to October 2008.

Subjects & Methods: Fifty histopathologically proven patients of oral cancer were selected and analyzed regarding predisposing and causative factors. Patients having premalignant oral disease, post operative, post radiotherapy patients and patients with inconclusive reports of histopathology were not included in this study.

Results: The analyses of 50 cases of oral cancer revealed that the majority of the patients were indulged in the habit of Paan, Betel nut, Naswar, Tobacco Chewing and smoking. 42 out of 50 patients (84%) had history of indulgence into these habits. Only eight out of 50 cases (16%) had no habit of Pan, Betel nut, Naswar and smoking. Early tertiary care hospital presentation was only 36% and patients presented with stage II or I. Sixty four percent (64%) patients who reported at Civil Hospital Karachi (CHK) had stage III or IV.

Conclusion: The analysis of fifty patients of oral cancer revealed that majority of the patients were indulged in different chewing and smoking habits. Betel nut, Tobacco, Naswar and Paan Chewing were different causative and predisposing factors of oral cancer.

Key Words: Oral cancer, causative factors.

INTRODUCTION

Since the beginning of last century, it has been well recognized that the oral cancer is one of the commonest cancers in subcontinent. In western world, oral cancers constitute 10% of all malignant tumors, standing second only to bronchogenic carcinoma in males and breast cancer in females and is the sixth most common cancer in the world [1]. The developing world had a different pattern, showing higher incidence of cancer of mouth, it is more common in the subcontinent than the western world. Another report describes oral cancer as number one amongst men and number three among women in the subcontinent.[2]

Oral cancers constitutes 20-35% of all cancers seen in various public sector hospitals in Karachi and

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slightly less in other regions of Pakistan. Nevertheless it is a major killer in our population[3] It has been identified that the habit of Naswar, betel nuts and tobacco chewing and smoking are the major contributing factors in the occurrence of this malignancy. The (IARC) regards the chewing of betel quid to be a known carcinogen. Certain studies have tried to prove that regular chewers of betel nut have a high risk of damaging their buccal mucosa and acquiring cancer of the mouth.[4] However, it is not scientifically proven that chewing of the nut alone can be carcinogenic.[5] Studies have shown that chewing the nut along with tobacco (which is a known carcinogen), lime and betel leaf might be carcinogenic. Studies have found tobacco and caustic lime increase the risk of cancer from betel nut preparations.[6]

Studies exist of the use of a "pure" *paan* preparation: areca nut, betel leaf, and lime. While a single recent study claimed that unprocessed areca nuts, at high doses, displayed a very weak carcinogenicity.[7] In contrast, since 1971 many studies have found betel nut extracts to cause cancer in rodents.[8] In 2003 the (IARC) concluded that the habit of chewing betel quid with or without tobacco is carcinogenic to humans. [9] Support to this is provided by a recent study which found that paan even without tobacco is a risk factor for oral cancer. They found that paan with or without tobacco increases oral cancers risk, 9.9 and 8.4 times, respectively.[10]

According to several studies, the ingredients of Betel nut, Naswar and Tobacco act as oral carcinogens. Lack of education and awareness prevent people from reporting to a tertiary care hospital at an early stage. The delayed presentation to a specialized hospital poses a challenge to manage this disease. The spectrum of oral cancer varies from place to place irrespective of country. Places within our country have various trends which gives chance to the causative and predisposing factors, causing oral cancers.

Cigarettes contain many cancer-causing agents (carcinogens). These include, tar, arsenic, benzene, cadmium, formaldehyde and polonium-210. Tar, the brown residue that stains smoker's teeth and fingers is a collection of solid particles. The risk of oral cancer increases depending since how long a person has been smoking and how often he or she smokes. [12]

Long term exposure to cancer-causing agents in cigarettes alters normal functions of oncogenes and tumor suppressor genes in oral tissues. These changes will eventually lead to uncontrolled cell growth and the onset of oral precancer. Several studies have found links between chemicals in cigarette smoke and cancer, by looking at the footprints, these chemicals leave on our genes for example, one study showed that benzo [a] pyrene, a chemical in cigarette tar, damages a key gene called p53.[11] The researchers found that p53 in cancer patients was damaged in exactly the same way as in their experiments.[12]

PATIENTS & METHODS:

This study was conducted at the department of E.N.T Head & Neck Surgery, DUHS and CHK, which is a 1800 beds' tertiary care hospital. Fifty (50) histopathologically proven cases of oral cancer were selected during the period of 10 months. Patients having premalignant oral disease and patients with inconclusive reports of histopathology were not included in this study. A detailed and comprehensive history including the details and duration of symptoms were taken. A comprehensive

past history including surgical and medical illnesses, radiotherapy, chemotherapy and medication history was also taken.

Special emphasis was given on the personal history like habits and addiction. Betel nuts, Paan, Tobacco chewing, Smoking and Naswar addiction were inquired. Quantity of pan, tobacco, betel nuts and Naswar per day was also asked. In smokers, number of cigarette smoked per day and duration of indulgence into these habits were also recorded. Finally we took family and socioeconomic history. Complete clinical examination including Ear, Nose, Throat, Neck, Systemic CVS, CNS, respiratory and abdominal examinations were performed. Baseline routine hematological, biochemical and serological laboratory tests including CBC,Urine D/R,BT,CT, Hepatitis B and C viral screening, LFT and UCE were performed for every patient. Imaging studies included x-ray chest, P.A View, ultra sound and MRI from skull base upto the root of neck were also done. Ultimately a panendoscopy was done under general anaesthesia especially for second primary. Neck palpation was also done.

Table 1: Risk factors for squamous carcinoma

· Betel nut and tobacco chewing
· Naswar
· Submucosal fibrosis
· Smoking
· alcohol intake
· Malnutrition
· Avitaminosis
· Chronic glossits
· Plummer-Vinson syndroms
· Hot spicy foods
· Chronic dental diseases
· Alcoholic mouth washes
· Leucoplakia
· Syphilis
· Cirrhosis
· Lichen planus
· Chronic hyperplastic candidiasis
· Human immunodeficiency virus (HIV)
· Xeroderma pigmentosa
· Dyskeratosis congenita

RESULTS

A total of fifty (50) patients with oral cancer during 6-months period were analyzed.

Forty two patients out of fifty cases (84%) were habituated to either betel nuts, tobacco, naswar and smoking. Only eight, out of fifty cases (16%)there had no habit of these things.

Ten out of fifty cases (20%) were addicted to two things Paan(betel nuts & tobacco) and cigarette smoking. This study also shows dose-response relationship with respect to duration (time period in years) and frequency (number of times per day) of betel nut with tobacco chewing.

Majority of cases belong to low socioeconomic status (80%) Thirty one (31) out of fifty (50) cases i.e. 62% chew betel nut with tobacco with or without paan. Seven (14%) chew betel nut with tobacco for less than or upto 10 years. While twenty four cases (48%) chew for more than 10 years.

Fourteen percent (14%), chew for not more than 10 years, while Forty eight (48%) chew betel nut with tobacco for more than 10 years. This shows strong dose-response relationship to duration of betel nut with tobacco chewing (with / without paan) Only one oral cancer patient (02%) chew betel nut with tobacco for not more than 5 times a day, while 20 cases(40%) chew more than 5 times a day. This also shows very strong dose-response relationship with respect to frequency of betel nut with tobacco chewing (with / without paan). Urban (70%) to rural (30%) ratio was 7:3.

Table 2: Habits

Habits	Number of patients	Percentage
Betel nuts, Chewing, paan chewing with or without tobacco chewing	31	64%
Naswar	10	20%

Table 3: Sex Incidence.

Gender	Number of patients	Percentage
Male	38	76%
Female	12	24%

Table 4: Site Involvement.

Site involved	Number of patients	Percentage
Buccal mucosa	31	62%
Tongue	15	30%
Lips	5	10%
Floor of month	10	20%
gums & alveolus	5	10%
Hard palate	5	10%

Table 5: Histopathological variants of squamous cell Carcinoma N=50

Variant	No: of patients	Percentage
BWell differentiated	41	82
Moderately differentiated	06	12
Poorly differentiated	01	02
Carcinoma in situ	02	04

Table 6: Frequency Of Betel Nut With Tobacco Chewing No: Of Cases = 50

No:	Frequency (times per day) of betel nut with tobacco chewing (with / without paan)	No: of cases	%
1	≤ 05 times per day	02	04
2	> 05 times per day	29	58

Table 7: Betel Nut And Tobacco Chewing (With Or Without paan) No: Of Cases = 50

No:	Betel nut and tobacco chewing	No: of cases	%
1	Yes	21	42
2	No	10	20

Table 8: Duration Of Betel Nut With Tobacco Chewing No: Of Cases = 50

Duration (time period) of betel nut with tobacco chewing (with / without paan)	No: of cases	%
≤ 10 years	07	14
> 10 years	24	48

Table 9: Frequency of betel nut with tobacco chewing No: of cases = 50

No.	Frequency (times per day) of betel nut with tobacco chewing (with / without paan)	No: of cases
1	≤ 05 times per day	01
2	> 05 times per day	20

DISCUSSION

Karachi is the largest city of Pakistan and Civil Hospital is the largest government tertiary care hospital of this city. Patients from the neighboring regions also approach for treatment at this institute. Oral cavity cancer is one of the commonest malignancies at Karachi. Chewing of paan containing betel nut and tobacco, sweet supari and "gutka"an indigenous preparation of tobacco with slaked lime in and around Karachi is a very common social habit.

Betel nut chewing is one of the major factors for this high incidence of oral malignancy. Number of betel leaf (pan) chewed per day by an individual is also high (15-25/day) in Karachi, which acts as a continuous irritant to the oral mucosa.[13] Use of smokeless tobacco (Paan Parag, city gutka etc) is on the increase in Karachi. A male to female ratio of 2.3:1 was observed.Smokeless tobacco, also known as chewing tobacco or snuff, is popular in different communities of Pakistan. Many studies have shown that smokeless tobacco can also cause oral cancer.[14] One study found that people who used smokeless tobacco had almost 50 times higher oral cancer risks than those who didn't.[15]

Most dangerous chemicals in smokeless tobacco are called tobacco-specific nitrosamines (TSNAs). One

review found that people who use smokeless tobacco expose themselves to thousand times more TSNAs than non-smokers, and up to 50 times more than smokers.[16]

Smokeless tobacco is also addictive as cigarettes. Some studies found that the amount of nicotine absorbed from smokeless tobacco is 3-4 times greater than that from a cigarette.[17] The nicotine is also absorbed more slowly and stays in the bloodstream for a longer time. Tobacco use is known as a major risk factor for oral and other cancers. All tobacco products, including cigarettes, cigars, pipe tobacco, chewing tobacco, and snuff, contain toxins (poisonous substances), carcinogens (cancer-causing agents), and nicotine (an addictive substance). Each tobacco product is linked to an increased risk for specific cancers. Smokers are six times more likely than nonsmokers to develop some form of oral cancers. Cigarettes contain more than 60 cancer-causing agents.[17]

The study confirmed that commonest histological variety is squamous cell carcinoma. Well differentiated i.e. Grade 1 squamous cell carcinoma was the highest in our group and this was similar to the carcinoma. Findings of type who reported 52.6% of patients who had well-differentiated tumors.

The buccal mucosa was found to be the most common site involved by the malignant process and was observed in 42.57% of our patients followed by the tongue in 58(19.14%) cases.

Interpretation of data from a single Institution do have limitations. The data reflects our specific patient population reporting to the hospital and not the community as a whole. Most of the patients had similar smoking or tobacco chewing habits. The highest rate of oral cancer is found in the developing world where oral cancer alongwith that of pharynx is the third commonest site of cancer. In Pakistan, India, Bangladesh and Srilanka, oral cancers are most common and accounts for one third of all cancers.[18] Cultural differences in the use of tobacco lead to the variation in the geographic and anatomic incidence of oral and pharyngeal cancers in accordance with dose response and other factors. There should be an intense public education plan and appropriate prevention and cessation strategies for smoking and smokeless tobacco products along with a social war against alcoholism to revert back the present trend of preventable oral cancers. Primary prevention measures including education and awareness programs are on the anvil. It is high time now that an early detection scheme will be started at this institution utilizing oral self-examination, toluidine blue staining, brush biopsy and scalpel biopsy as need be.

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ORIGINAL ARTICLE

Serum Vitamin D in Common Non-Metabolic Disorders: Results of Patients' Survey at Public Hospitals of Karachi

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ABSTRACT

Objectives: To determine serum vitamin D level in common non-metabolic medical and surgical disorders.

Study design: Cross sectional study.

Place and duration of study: Study conducted from March 2006 to January 2009 at multiple centres as per author's affiliation.

Patients and methods: Patients having a common non metabolic symptomatic disease were selected. Patients with rickets and osteomalacia were excluded. Serum vitamin D, serum calcium, phosphorus, alkaline phosphatase level and routine blood investigations were obtained in all patients. Normal range of serum vitamin D level was defined and lower values were divided into mild, moderate and severe . One sample t test was used for determination of significance. All data were collected and processed on SPSS version 10.

Results: Out of 205 patients, serum vitamin D level was found to be low in 169 (82.4%) patients. The vitamin D level was normal in 36 (17.6%) patients; low levels were categorized into three, mildly low in 33 (16.1%) patients, moderately low in 55 (26.8%) patients and severely low in 81 (39.5%) patients. Chi square test and one sample test (P value = < 0.001) with all type of deficiencies in all groups of diseases was highly significant.

Conclusion: Low serum vitamin D level with highly significant value seen in all groups of diseases and in all types of deficiencies. Its prognostic value, role in the development of the pathology and pathogenesis in different diseases has to be evaluated, and require further research in individual sets of diseases.

Key words: Vitamin D, calcium metabolism, alpha calcidol.

INTRODUCTION

During the past decade, important advances in the study of vitamin D had been made. In addition to its important role in skeletal development and maintenance, evidences suggests that vitamin D produces beneficial effects on extra-skeletal tissues and that the amounts needed for optimal health were probably higher than previously thought. At the same time, numerous reports[1-3] have shown that relatively high proportions of people have inadequate levels of vitamin D. The extra skeletal health benefits of vitamin D and high prevalence of inadequate levels

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of vitamin D have been largely unrecognized by both physicians and patients.

Vitamin D is one of the misnomer biochemical agent. Fundamentally its active component is 1alpha, 25-dihydroxyvitamin D, which is a hormone. It is indispensable for health. Chemically it is a steroid hormone, like estrogen, progesterone, testosterone and cortisol. It works through the specific receptors called VRDs (vitamin D receptors).[3] VRDs are present in nearly every tissue of body including bones and soft tissues. So deficiency of this vitamin can involve roughly any tissue in every disease process.[4] VRDs are proteins in nature and present on the surface of the nuclear membrane. Vitamin D enters the target cell and interacts with its nuclear VDRs, causing augmentation or depression of transcription of vitamin D-responsive genes in the respective tissue cells.[5] Thus the effects of vitamin D are wide spread and long lasting.

The recommended dosage of vitamin D for different age group is 200 IU in most of the patients. However in old age and in pregnancy, the requirement is increased to 400 - 600 IU.[6] Though, in older patients with osteoporosis, for the prevention of fracture, the estimated blood level of vitamin D should be 70-80nmol/l. That can be achieved by a dose of 20-25µg/day or 800-1000 IU,[7] especially in women and old age men. This roughly doubles the suggested dose of WHO.[7] Similarly the required dose of vitamin D has to be increased, if sunlight exposure is limited.[8]

There is a growing evidence that deficiency of vitamin D is involved in many diseases. [9-10] Inadequate levels of vitamin D are very common in old age.[11] In a European based study, serum level of vitamin D in patients, not taking supplements was, 19.3 ng/ml with standard deviation of 11.0. In patients taking supplements it was 21.6 ng/ml with standard deviation of 8.7.[11]

There is a distinct relationship between fracture healing and vitamin D. Virtually all patients hospitalized for fracture have vitamin D inadequacy.[12] In experimental study on animals, the plasma concentration of vitamin D rapidly decreased on day 3 and continued to decrease up to 10 days, after fracture. This rapid disappearance of 1, 25(OH) vitamin D3 in the early stages after fracture was not due to either increased degradation or decreased synthesis of 1, 25(OH) vitamin D3, but rather to increased utilization. This was confirmed by radioactive labeled vitamin D, as it was concentrated in the callus.[13] In another human study, comparative decrease in 24, 25(OH)vitamin D3 values was also noted in all patients. These changes reflect the consumption of these metabolites during healing at the fracture site.[14] No data is available of local population, for Vitamin D levels in diseases except the observational study of author[15] which showed deficiency of vitamin D upto 92% in non metabolic conditions. This study was conducted, to have a base line idea in general randomly selected patients. The objective of this study was to determine serum vitamin D level in common non-metabolic medical and surgical conditions.

PATIENTS AND METHODS

This cross sectional hospital based study was conducted from March 2006 to January 2009. Data for surgical conditions were collected of out patients as well as in patients from orthopedic and surgical units of Civil Hospital and Lyari General Hospital Karachi. Data for non-surgical conditions were collected from Medical OPD and ward of Abbasi

Shaheed Hospital Karachi. Surgical patients were defined as those requiring surgery as a definitive remedial measure. While non surgical patients were those in which no surgical procedure was required, however they were diagnosed to have an organic disease requiring medical therapy. Patients selected for elective or emergency surgery for one or other reason was grouped as pre surgical patients, they were 30 in number. In this group two samples of blood were taken, one before surgery and the other 48 hours after surgery. Every 5th patient was selected with organic disease supported by signs & symptoms and laboratory confirmation. Patients who were taking vitamin D in any form since last 3 months, or had chronic renal disease & taking steroids (corticosteroids or sex steroids anabolic steroids) in any form and those with rickets & osteomalacia were excluded from study. Verbal consent of the selected patients were obtained. Serum vitamin D levels of all patients were obtained alongwith serum calcium, phosphorus, alkaline phosphatase and routine blood investigations. For collection of serum calcium, blood samples were collected without application of tourniquet. The normal range of serum vitamin D was taken 31-100 ng/ml, 21-30 ng/ml as mildly low, 10-20 ng/ml as moderately low, and levels below 10ng/ml were labeled as severe deficiency.[16] Data was collected and processed on SPSS version 10. Results were described as percentages. One-sample t-test and Chi- square were used to determine the significance, which was taken as $p < 0.05$.

RESULTS

A total of 205 patients were included in this study. Out of those 126 (61.5%) were females and 79 (38.5%) were males. The mean age of patients was 42.8 ± 16.3 years. (R: 2-80)

Table 1: Age distribution

Age group	Female	Male	Total
1-10	1 (0.4%)	2 (0.9%)	3 (1.4%)
11-20	10 (4.8%)	4 (1.9%)	14 (6.8%)
21-30	28 (13.6%)	13 (6.3%)	41(20.0%)
31-40	23 (11.2%)	16 (7.8%)	39 (19.0%)
41-50	23 (11.2%)	18 (8.7%)	41 (20.0%)
51-60	23 (11.2%)	16 (7.8%)	39 (19.0%)
61-70	10 (4.8%)	8 (3.9%)	18 (8.7%)
over 70	8 (3.9%)	2 (0.9%)	10 (4.8%)
Total	126 (61.5%)	79 (38.5%)	205 (100%)

Majority of patients were house wives and with good socio economical status and only 25.0% belonged to poor socio economical group. Socio eonomical grouping were made according to their monthly income.

Table 2: Frequency of distribution for occupation of the studied group

Occupation	Female	Male	Total
Banker/ accountant / Businessman	2 (0.9%)	5 (2.4%)	7 (3.4%)
School teacher/Clerk	1 (0.4%)	7 (3.4%)	8 (3.90%)
Doctor	2 (0.9%)	5 (2.4%)	6 (2.9%)
Domestic servant/ cook/ driver	2 (0.9%)	7 (3.4%)	9 (4.3%)
Engineer	0	3 (1.4%)	3 (1.4%)
Govt officer/ Executive officer	0	5 (2.4%)	5 (2.4%)
Non working ladies.	108 (52.6%)	0	108 (52.6%)
Industrial worker/farmer	1 (0.4%)	11(5.3%)	12 (5.8%)
Retired	1 (0.4%)	14 (6.8%)	15 (7.3%)
Sales girl/man	1 (0.4%)	12 (5.8%)	13 (6.3%)
Student	7 (3.4%)	6 (2.9%)	13 (6.3%)
Paramedic	1 (0.4%)	4 (1.9%)	5 (2.4%)
Total	126 (61.5%)	79 (38.5%)	205 (100%)

Serum vitamin D levels were done in 205 patients. Low levels were found in 169(82.4%) patients, with a mean of 17.2 ± 13.81. Vitamin D level was normal

in 36 (17.6%) patients. It was mildly low in 33 (16.1%) patients, moderately low in 55 (26.8%) patients and severely low in 81(39.5%) patients. The deficiency was highly significant in patients with diabetes, infection and pre surgical conditions (P<0.001) and it was significant in other four groups of diseases (P<0.05) as seen in table 3.

Table 3: Vitamin D deficiency/normal with respect to diagnosis.

Diagnosis	Vitamin D deficient	Normal vitamin D	Value of Significance	Total/ percentage
Diabetes	24 (11.7%)	5 (2.4%)	0.001	29 (14.1%)
Infections	40 (19.5%)	5 (2.4%)	0.001	45(21.9%)
Muscular weakness	25 (12.1%)	9 (4.3%)	0.006	34(16.5%)
Osteoporosis	26 (12.6%)	8 (3.9%)	0.002	34(16.5%)
Obstructive. Lung diseases/Allergic sinusitis	13 (6.3%)	2 (0.9%)	0.005	15(7.3%)
Pre surgical patients	27(13.1%)	3 (1.4%)	0.001	30(14.6%)
Rheumatoid arthritis	14 (6.8%)	4 (1.9%)	0.012	18(8.7%)

Amongst infections 03 patients had chronic osteomyelitis of tibia,02 patients had hepatitis C, 01 patient had hepatitis A, 01 patient had resolving pneumonia, 03 patients had septic arthritis of hip and 34 patients had tuberculosis. In tuberculosis, 17 patients had intestinal tuberculosis, 14 patients had pulmonary tuberculosis, 03 patients had spinal tuberculosis and 01 patient had tuberculous meningitis. Serum calcium and vitamin D were low in all patients with tuberculosis. However, both the serum phosphorus and alkaline phosphatase levels did not show any relation with type and severity of vitamin D deficiency in tuberculosis. Where as serum calcium, phosphorus and alkaline phosphatase were disturbed in other diseases. Serum calcium was low in 80 (39.0%) patients and normal in 125 (61.0%) patients. Serum phosphorus level was low in 10 (4.9%) patients, normal in 183 (89.3%), and raised in 12 (5.9%). Serum alkaline phosphatase was low in 1 (0.5%), raised in 18(8.8%) and normal in 186 (90.7%) patients.

Table 4: Frequencies with percentage of vitamin D deficiencies in different groups.

Diagnosis	Normal vitamin D Level		Low vitamin D Level	
	Male	Female	Male	Female
Diabetes	4 (1.9%)	1 (0.4%)	11 (5.3%)	13 (6.3%)
Infections	3 (1.4%)	2 (0.9%)	11 (5.3%)	29 (14.1%)
Muscular weakness	3 (1.4%)	6 (2.9%)	12 (5.8%)	13 (6.3%)
Osteoporosis	0	8 (3.9%)	4 (1.9%)	22 (10.7%)
Obs. Lung diseases /Allergic sinusitis	1 (0.4%)	1(0.4%)	5 (2.4%)	8 (3.9%)
Pre surgical patients	3 (1.4%)	0	18(2.4%)	9 (4.3%)
Rheumatoid arthritis	1 (0.4%)	3 (1.4%)	3 (1.4%)	11 (5.3%)
Total	15(7.3%)	21(10.2%)	64 (31.2%)	105(51.2%)

DISCUSSION

Deficiency of vitamin D is a worldwide problem.[17] About 59% of world population is suffering from this vitamin deficiency.[17] In America minimum deficiency has been seen in Latin America (51%). Where as Europe (52%) Australia (59%) and Asia (63%) are in middle zone. The most severe deficiency is seen in the Middle East (82%).[17] Deficiency in this part of the world is found to be around 82.4 %, slightly higher than Middle East due to the factors of urbanization and social reasons, including less exposure to sun, mostly seen amongst females. In addition, majority of our dietary products are not fortified with vitamin D. Another reason for vitamin D deficiency, seen world over is the smaller dose of only 400 IU of vitamin D, which was previously recommended. Recent study by Heaney, *et al*[18] conclude that healthy men utilize between 3,000–5,000 IU of cholecalciferol a day. This intake is enough to maintain the blood level of vitamin D between 30-50 ng/ml.[18-19]

This study showed significant deficiencies with lowest value of vitamin D, irrespective of concomitant diseases included in the study, which correlates with

the finding of Holick,[16] in a recent article Fair amount of research has shown that vitamin D deficiency plays a role in the development of Diabetes.[20] This study also showed that 24 (11.7%) patients with diabetes had deficiency of vitamin D. It has also been observed that correction of serum level of vitamin D makes the diabetic control easier to achieve. This is probably due to the fact that absence of adequate levels of vitamin D causes impaired release of insulin.[20-21]

Chronic infection like tuberculosis is also known to be associated with low serum vitamin D.[22-23] In this study almost all tuberculous patients showed highly significant deficiency which is in agreement with Sasidharan et al and Wilkinson et al.[22-23] Patients with soft tissue rheumatism, a fibromylgia syndrome, are often misdiagnosed as chronic fatigue syndrome. Low levels of vitamin D has been demonstrated in patients with fibromyalgia. [24-25] This study showed 34 (16.5%) cases of muscular weakness and 18(8.7%) of soft tissue rheumatism, who had significant vitamin D deficiency.

Table 5: Significant values of vitamin D in various diseases

Diagnosis	Testing value 30
Diabetes	<.001
Infections	<.001
Muscular weakness	.001
Osteoporosis	.005
Obstructive Lung diseases/Allergic sinusitis	.001
Pre surgical patients	<.001
Rheumatoid arthritis	.001
Types of deficiencies were:	
Mild deficiency	<.001
Moderate deficiency.	<.001
Severe deficiency.	<.001

Osteoporosis is a common disorder of this part of world.[15] In this study 34(16.5%) showed highly significant deficient level. This finding was also matching with findings of our contemporaries abroad.[7-8]

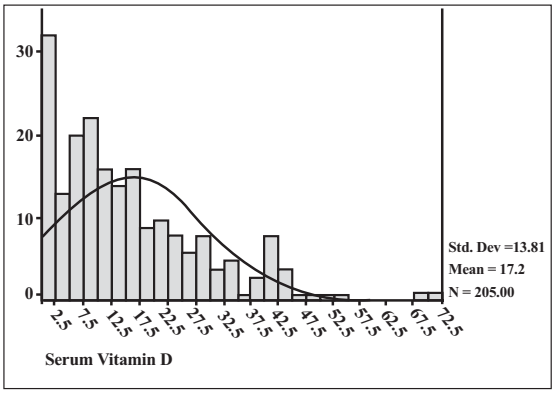

Figure – 1 : Histogram of vitamin D deficiency

Two samples of blood were taken from 30(14.6%) patients of pre surgical group, one before surgery and one after 48 hours of surgery. They also showed low serum vitamin D levels with highly significant P value with one sample T test and Chi Square test for both samples. In addition the difference between the means of two samples were (pre and postoperative) also significant(P value= .003), when paired T test was performed, indicating a significant drop in serum vitamin D after surgical stress. In literature, we were able to find that surgical stress can cause significant drop in vitamin D.[26-27]

In this study however serum level of all rheumatoid patients shows mild to severe deficiency. P value for t test was highly significant and Chi Square test was (0.012) significant in our study. Data from other studies also indicated that, the role of vitamin D is also important for prevention of some autoimmune diseases like multiple sclerosis, lupus, and rheumatoid arthritis.[28]

CONCLUSIONS

Low serum vitamin D levels with highly significant values is seen in all groups of diseases and in all types of deficiencies. Its prognostic value, role in the development of the pathology and pathogenesis in different diseases has to be evaluated, and require

further research in individual sets of diseases.

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ORIGINAL ARTICLE

Relation of Brain Natriuretic Peptide, Mean arterial and Pulse pressures among Normotensive, Pre-hypertensive and Hypertensive male cohort

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ABSTRACT

Background: Hypertension is an increasingly important medical and public health issue. Individuals prone to the development of hypertension often have a hyperdynamic circulation antedating the onset of hypertension by several years. Brain Natriuretic Peptide is a new promising cardiovascular risk marker due to its association with high blood pressure via its mechanisms of secretion and actions. Both pulse and mean arterial pressures are independent markers of cardiovascular diseases.

Objective: This study was designed to find out any relation between the rising values of pulse and mean arterial pressures among normotensives, pre-hypertensives and newly diagnosed hypertensives with the changes in plasma brain natriuretic peptide levels.

Methods: This was an observational, analytical cross-sectional study conducted in department of physiology at Basic Medical Sciences Institute, Jinnah Post Graduate Medical Center, Karachi. Study included 85 adult males, aged between 20-60 years, non- smokers, non- diabetic and having no other chronic illnesses. Pulse and mean arterial pressure values were found. Study participants were divided into three groups ranging from normotensive to hypertensive stages, as stated by Joint National Committee -7. Brain Natriuretic Peptide was assayed by AxSym technology.

Results: Brain Natriuretic Peptide developed a positive correlation with both pulse and mean arterial blood pressures and was also found out to be significantly raised in pre-hypertensive group.

Conclusions:This study concluded that Brain Natriuretic Peptide is positively related with increasing values of both variables i.e. pulse as well as mean arterial blood pressures. It also concluded that Brain Natriuretic Peptide is significantly elevated in pre-hypertensive stage and is not very different from the levels seen in sustained hypertension.

Key Words: Brain Natriuretic Peptide, Pre-hypertensive, Pulse pressure, Mean arterial blood pressure.

INTRODUCTION

The prevalence of increased blood pressure is increasing and there is no threshold of blood pressure that identifies cardiovascular risks. Hypertension experts have proposed a new definition of hypertension as "A progressive cardiovascular syndrome arising from complex and interrelated etiologies" which features early markers that are often present before blood pressure elevation is sustained. This revision of the definition of hypertension and the need to assess the blood pressure levels in the context of cardiovascular risks has

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guided for an earlier detection of patients at risk.[1]

Brain Natriuretic Peptide (BNP) is a new promising cardiovascular risk marker [2] that has been associated with high blood pressure.[3] Brain natriuretic peptide is found to be raised in hypertensives[4]and is related with increased incidence of cardiac events.[5]Volume overload increases mean arterial pressure (MAP)[6-7] and Pulse pressure (PP). Investigators have reported that individuals prone to the development of high blood pressure often have a hyperdynamic circulation antedating the onset of hypertension by several years.[8] BNP gene expression is one of the earliest responses to hemodynamic pressure overload and occurs before development of left ventricular hypertrophy.[9] BNP-dependent decrease in blood pressure results in part from a reduction in cardiac preload and partly after-load .BNP release is increased both in response to increased pre-load as well as after load.[10] So we speculated that increased plasma BNP levels may antedate or be closely related to subsequent increase in PP and MAP. However, the role of BNP in the clinical assessment of increasing blood pressure has not been fully investigated and actual meaning of a slight increase in BNP is still unclear. In view of above knowledge this study was designed to find out any existing relationship between plasma BNP levels, PP and MAP values.

MATERIAL AND METHODS

This study was carried out during February to October 2007 at Basic Medical Sciences Institute JPMC, Karachi. This study included a total of 85 apparently healthy males ranging between the ages of 20 to 60 years. The selected subjects had no history of diabetes, any hypertensive complication or any other chronic systemic illness. Exclusion was made on the basis of history and lab findings including TLC >10.9 x10^9/L or <3.9 x10^9/L, C-Reactive protein > 6 mg/L, Serum Creatnine >1.1 mg/dl, Fasting Blood Sugar>115 mg/dl .

According to JNC-7,[11] hypertensive subject was defined as a person having diastolic blood pressure or systolic blood pressure 140/90 mm Hg. All selected hypertensives were the newly diagnosed ones and had not yet started the treatment. Mercury sphygmomanometer was used for blood pressure measurements between 8-10 AM to avoid diurnal variations. Average of three readings was considered to be the needed observation. Blood samples were collected between 8 to10 AM after a fast of 12 to14 hours. Samples were preserved at –20C.BNP was determined by AxSYM technology based on microparticle enzyme immunoassay (MEIA) provided by Abbot Diagnostic Laboratories having kit Ref.No.8G82-20ABBL001/R4.

Systolic and Diastolic blood pressure values were recorded.Pulse pressure value was calculated by subtracting DBP value from SBP value.Mean arterial pressure was found out by adding 2/3rd of DBP value to 1/3rd of SBP value.The study participants were divided into three groups on the basis of PP and MAP values as normotensive(<120/<80mmHg),pre-hypertensive(120-139/80-89mmHg)and hypertensive (140/90 mmHg) according to JNC-7.[11]

RESULTS

In this study BNP value increased from a value of 12.39 to 27.85 pg/ml with the increasing values of MAP in all the three groups ranging from < 90 to >110 mmHg respectively. It showed a positive and statistically significant correlation on linear regression between MAP and BNP (P<0.251 r=0.25*)as shown in Table-1 and Figure-1.

Table 1: Values of Plasma Brain Natriuretic Peptide (BNP) Levels In Mean Arterial Pressure Groups

(All the values are expressed in Mean±SEM)

Mean arterial pressure (mmHg)	n	BNP (pg/ml) Mean±SEM	P value
< 90	24	12.39±4.52	*0.251
90-110	53	24.66±4.92	
>110	08	27.85±9.38	

n= Number of subjects.

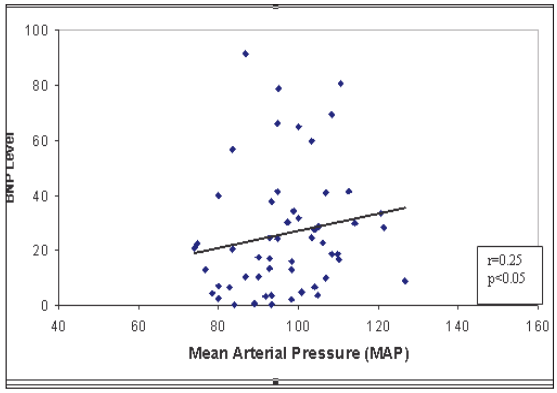

Figure – 1 : Correlation Between BNP And Mean Arterial Pressure (MAP)

We also found an increase in the BNP levels from 20.99 to 24.75 pg/ml with the increase in the PP values from ≤40 to ≥50 mmHg respectively. A positive but statistically non-significant correlation was found between PP and BNP (P<0.949 r=0.16)as shown in Table-2.

Table 2: Values of Plasma Brain Natriuretic Peptide (BNP) Levels In Pulse Pressure Groups

(All the values are expressed in Mean±SEM)

Pulse pressure (mmHg)	n	BNP (pg/ml) Mean±SEM	P value
≤40	52	20.99±5.08	0.949
41-49	24	21.37±5.15	
≥50	09	24.75±5.93	

n= Number of subjects.
r=0.16

Table 3: Descriptive Statistics of Study Population

Variables	n	Minimum	Maximum	Mean	S.D	SEM
Age (years)	85	22	60	41.06	9.629	1.04
BNP (pg/ml)	85	0.00	163.50	21.4938	31.94292	3.4647
Systolic BP (mmHg)	85	90	162	122.11	15.887	1.72
Diastolic BP (mmHg)	85	60	112	82.95	10.611	1.15
Pulse pressure (mmHg)	85	20	70	39.1529	9.94450	1.0786
Mean arterial pressure (mmHg)	85	73.33	126.67	96.0039	11.71379	1.2705

DISCUSSION

Hypertension is widely recognized as a major risk factor for cardiovascular disease.[12] Acknowledging the graded and continuous nature of the relations of blood pressure to vascular risk JNC-7 introduced "pre-hypertension" to describe people with SBP between 120-139 and DBP between 80-89mmHg.Framingham Heart Study indicated that BP values in the 130-139/85-89mmHg range are associated with a more than two fold increase in relative risk from cardiovascular disease compared with the BP levels below 120/80mmHg. A strategy of estimating cardiovascular risk and adjusting the intensity of blood pressure lowering to the absolute risk of cardiovascular disease is desirable in prehypertensive individuals.[13] With the knowledge of such discussion it is useful to have a bio-marker that can serve as a reliable indicator of the risks attributed to the progression of blood pressure above and beyond other clinical determinants. Plasma BNP was thought to be a candidate bio-marker based on cross-sectional associations with blood pressure measures.[14] Studies have extended the potential role of BNP measurements to risk stratification of the general population in which long term mortality increases in proportion to BNP concentration both in patients with or without evidence of cardiovascular

disease.[15-16]BNP has related itself positively with the pathophysiological conditions characterized by alterations of cardiac function and systemic hemodynamics as hypertension when compared with their controls.[17-10] A high BNP concentration may reflect the cardiac load based on the mechanism of its secretion[18] while Framinghm study demonstrated that an increase in BNP predicted the risk of death and cardiovascular events in community residents.[2] However there is little information about the role of BNP in subjects with the rising values of blood pressure and without any established overt cardiovascular disease.

Higher PP is associated with higher risk for cardiovascular mortality and adverse cardiovascular outcomes.[19] Zakopouls in 2001 found PP a marker of cardiovascular disease even in subjects without hypertension.[20] PP is mainly determined by stroke volume and arterial compliance.[21]A higher PP in patients with a normal cardiac function probably reflects more severe atherosclerosis as reduced compliance of the vessels leads to an increased systolic and decreased diastolic pressures and this scenario is thought to apply especially to hypertension.[22]

The rising values of both PP and MAP are independent markers of cardiovascular risks so we speculated that the increase in these pressures may correlate with the changes in plasma BNP levels. Shingo Seki et al[23] in 2008 found a positive relationship between PP and BNP but could not exclude the influence of aging on both variables.His study group had a mean age of 58 years and mean PP value of 65mmHg. Further, his study included untreated essential hypertensives only. Minora Yambe[24] in 2006 found the same results but in an older age group of mean 54 years with a mean PP value of 49mmHg.Our study also found a positive relationship between the two variables but in a graded manner with the rising values of PP(mean 39mmHg) among normotensive to hypertensive in a younger age group of mean 41 years.We could not find a statistically significant relation probably because of the limited number of study participants.

Cataliotti et al[25] in 2005 observed a decrease in MAP after oral administration of human BNP in normal conscious dogs. Kin vander zander et al[26] in 2003 also found a decrease in MAP after BNP infusion in his study group of advanced age(mean 60 years).Our study group comprising of younger age (mean 41 years) developed a positive relation between BNP and MAP in gradually increasing pattern among all the three groups and on linear regression a statistically significant relation was disclosed between the two variables as shown in Table-1and Figure-1.

CONCLUSION

Our study found that BNP is positively related with the increasing values of both variables i.e. pulse as well as mean arterial blood pressures. This study also found that BNP levels are significantly raised in the prehypertensive stage which may remain increased in the sustained hypertension. BNP may be valuable for risk stratification in primary care by general practitioners so it is suggested that BNP levels should not only be assayed in hypertensive but in prehypertensive preferably to decide all those measures which may prevent or delay the onset of hypertension.

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ORIGINAL ARTICLE

Prospective Evaluation of a Rapid Diagnostic Test Dot EIA (Typhidot) for Typhoid Fever

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ABSTRACT

Objective: A prospective evaluation of a rapid diagnostic test Dot EIA (Typhidot) for typhoid fever.

Study design & duration: This prospective study was undertaken in a tertiary hospital from April 2007 to July 2008.

Research Methodology: The patients were divided into three groups as I, II & III with typhoid fever, typhoid suspects & non-typhoid febrile illnesses respectively. Blood culture and typhidot tests were done for the subjects included in the study. The validity of the typhidot test was evaluated by determining the sensitivity, specificity, positive and negative predictive value.

Results: The mean age in years was 28±19 (SD). Out of 150 subjects, males were more than females. The typhidot IgM yielded very high sensitivity, specificity and a negative predictive value noted in group I = 93% & 98.80% 93% respectively and in group II= 79%, 98.80%, & 83% respectively where as in group III (non-typhoid febrile controls), the IgM antibodies were not detected at all (p=0.01). Sensitivity, specificity, and negative predictive value for typhidot IgG noted in group I = 73%, 63%, & 22.4% respectively and in group II = 82%, 37%, & 68% respectively (p=0.03) and a false positive reading of 63% was noted in group III (controls).

Conclusions: Typhidot test is a valid tool in the diagnosis of typhoid fever but a reliable and valid interpretation should be based on positive IgM.

Key words: Salmonella typhi Dot- EIA (typhidot)typhoid fever.

INTRODUCTION

Typhoid fever is an important cause of morbidity in many regions of the world, with an estimated 12 to 33 million cases occurring annually.[1] Pakistan is a hyper-endemic area for typhoid fever, & according to WHO 2008 report, the incidence of typhoid fever in 5-15 years aged children was 412 per million in 2002.[2] A definitive diagnosis of typhoid fever can be made by isolation of Salmonella typhi (S.typhi) from blood or bone marrow by culture, which is regarded as "gold standard method". However, bacterial culture facilities are often unavailable, expensive, time consuming & usually negative because of prior antibiotic usage. Despite improved methods of

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bacteriologic isolation, there is a real need for rapid serologic diagnostic test for typhoid fever. The Widal test has been used for almost more than 100 years. It is widely available in developing countries, and is still regarded as a useful test in endemic areas.[3] There is, however, considerable interest in newer methods of diagnosis of typhoid fever such as latex agglutination, coagglutination, and the polymerase chain reaction.[4] The dot-enzyme immunoassay (EIA) is a relatively newer serologic test based upon the presence of specific IgG and IgM antibodies to a specific 50-kD outer membrane protein (OMP) antigen on S. typhi strains[5] and has been commercially marketed as a dot-EIA (typhidot). The test incorporates nitrocellulose strips impregnated with the OMP antigen and separately identifies IgM and IgG antibodies. Although the test has shown promising results in preliminary studies from Malaysia[6] and Philippines.[7] The interpretation of IgG response in highly endemic areas remains problematic. There is a concern that in such endemic populations pre-existing IgG antibodies to S. typhi may increase rapidly following reinfection and potentially mask concomitant IgM response. A recent, commercially available, enzyme-linked immunoassay (typhidot) is reported to circumvent these blocking antibodies by inactivating IgG antibodies, followed by an immunoassay targeting specific IgM.[8] Preliminary data using the Typhidot and Typhidot-M in combination have shown sensitivity and specificity of 95% and 86%, respectively.[9] Although the tests have shown promising results in trials from Southeast Asia, given the genetic diversity and plasticity of S. typhi strains, it is not proved if the test would be of comparable sensitivity in other regions.[3] We prospectively evaluated the efficacy of the two dot- EIA test (typhidot).

RESEARCH DESIGN AND METHODS

This prospective study undertaken in a tertiary hospital, Muhammad Medical College Hospital, Mirpurkhas, covered the period from April 2007 to July 2008. Blood culture and typhidot tests for typhoid fever & suspects were performed for the subjects admitted to the hospital. The subjects were selected who fulfilled the criteria of; ages 18-40 years, fever =14 days, clinical manifestations suggestive of typhoid fever, & no history of typhoid immunization in the recent past. Patient's history, physical examination findings, diagnostic studies, and results of blood culture and typhidot test were recorded. The blood cultures and typhidot tests were analyzed at the end of study period. Patients were divided into three groups - those with diagnosis of typhoid fever & suspects as group I and II respectively and those without typhoid as group III (controls).

Group I – 50 patients with blood culture (+) typhoid fever

Group II – 50 patients with blood culture (-) typhoid suspect

Group III – 50 patients with non-typhoid febrile illnesses.

Fifty patients with non-typhoid febrile illnesses (Group III) included; patients with pneumonia (n=7), pharyngiitis (n=5), Cholecystitis (n=5), dysentery (n=3), otitis media (n=7), malaria (n=10), urinary tract infection (n=7) and acute viral hepatitis (n=6) The validity of typhidot test was evaluated by determining the sensitivity, specificity, positive and negative predictive values in the diagnosis of culture, culture positive typhoid fever, culture negative typhoid suspects and controls

RESULTS

A total of 150 patients who fulfilled the inclusion criteria were studied, out of them 50 had positive cultures (group I) for Salmonella typhi, 50 had negative blood cultures (group II) and fifty had non-typhoid febrile illnesses (group III). The mean age in years was 28±13 for all groups I, II and III. There were more males (n=115) than females (n=35). More patients (91%) were admitted before the seventh day of illness. Sensitivity, specificity, and a negative predictive value for typhidot IgM noted in group I were 93%, 98.80% and 93% respectively, in group II these were 79%, 98.80% and 83% respectively, where as in group III (non-typhoid febrile controls), the IgM antibodies were not detected at all (p=0.01). Sensitivity, specificity, and a negative predictive value for typhidot IgG noted in group I were 73%, 63% and 22.4% respectively, In group II these were 82%, 37% and 68% respectively, where as a false positive reading of 63% was noted in group III controls (p=0.03).

DISCUSSION

The results of our study showed the sensitivity, specificity and negative predictive values of typhidot IgM in the diagnosis of typhoid fever were ranging from 93% to 98.8% in blood culture proven cases and these were slightly lower in blood culture negative typhoid suspects. IgM antibodies were not detected in the controls. Our results for typhidot IgM is well in comparison with the studies done by Choo et al.[6] However, typhidot IgG yielded more variable results with lower sensitivity, specificity, negative predictive value and high rate of false positive results among controls. This phenomenon possibly reflects the high endemicity of typhoid fever in our region where as infected cases are not recognized because of the mild presentation of the disease. Our results are comparable with the studies conducted by Sherwal et al, [10] Bhutta et al [3] and Jesudason et al, [11] which showed sensitivity and specificity of 92% & 87.5%, 80% & 77% and 92.3% & 98.8% respectively. The effectiveness of typhidot test in early stages of typhoid fever was seen in two different studies of Malaysia. [12-13] Its sensitivity and specificity was reported as 90.3% & 91.9% respectively in the first study while in the second one it showed sensitivity & specificity of 98% & 76.6% respectively.[12-13] A study conducted in Manila by Collantes et al [14] has reported sensivity & specificity of 93% & 100% in blood culture positive typhoid fever patients. One study from Pakistan of Shaikh KR et al [15] has shown sensitivity and specificity of 72.4% and 93.3% respectively, which is nearly comparable with our study. Our values of sensitivity and specificity are higher than reported by Karamat et al from Northern Pakistan. These differences may be due to several factors including the genomic diversity among S.typhi isolates in the region and differences in antigenic epitopes. Other factors responsible for reported differences in areas of high endemicity include various stages of illnesses and the rate of IgG increase in relation to (OMPs, which may interfere with identification of concomitant IgM antibodies). Most of our patients presented in the first week of their illness, whereas information on duration of illness is lacking in other studies. The relative low sensitivity of the blood culture in diagnosing typhoid fever is understandable in the wake of widespread antibiotic use in Pakistan. Although bone marrow cultures significantly increase the yield from blood cultures, but it is a invasive procedure and is difficult to obtain. It must be emphasized that although cultures are associated with a lag period of at least 48 hours

for preliminary confirmation of infection but with the recent emergence of drug resistance amongst strains of S.typhi it remains an essential investigation. The diagnostic difficulties in partially treated cases, may be reduced if the blood cultures are combined with rapid serologic tests. Our data indicates that Typhidot IgM has significant diagnostic yield. The Typhidot offers an additional advantage amongst second-line serologic diagnostic tests for typhoid fever as the test strips do not require an ELISA reader for evaluation, and only minimum training is required. Combining the Typhidot and Typhidot-M tests, may improve sensitivity but it is an expensive proposition. Given the recent call for an essential diagnostic program in developing countries, it is important that the Typhidot and Typhidot-M tests may be evaluated on a larger scale in different parts of the world with epidemiologically diverse strains of S.typhi.

Table 1: Results of IgM typhidot

	Sensitivity	specificity	NPV†
Group I (n=50)	93%	98.80%	93%
Group II (n=50)	79%	98.80%	83%

† **NPV**= negative predictive value

Table 2: Results of IgG typhidot

	Sensitivity	specificity	NPV†	False +ve
Group I (n=50)	73%	63%	22.4%	—
Group II (n=50)	82%	37%	68%	—
Group III (n=50)	—	—	—	63%

† **NPV**= negative predictive value

CONCLUSION

Typhidot IgM is a highly sensitive specific tool for the diagnosis of typhoid fever with high negative predictive value. In contrast, typhidot IgG has low sensitivity, specificity and negative predictive value. A valid conclusion can be made from a single sample, based on results of IgM titer.

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ORIGINAL ARTICLE

Neuropharmacological Assessment of Sweet Potato Proteins in Mice

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ABSTRACT

Objective : To assess the neuropharmacological effects of *Ipomoea batatas* [L.] Lam tuberous proteins in male white albino mice.

Subjects: 72 adult male mice of NMRIstrain (weighing 25-30 gms) were used. Animals were divided into 12 groups (6 in each group). Each group of animal was treated individually with saline water (5 ml/Kg, p.o.), proteins isolated from *I. batatas* (1.5 and 3.0 mg/Kg, p.o.), standard drugs Diazepam (5.0 mg/Kg, i.p.) and Morphine (5.0 mg/Kg, i.p.).

Methodology: I) General behavior was assessed by

a) Undisturbed observation (awareness, alertness, spontaneous activity).

b) Response by least provoking stimuli (sound, touch, and pain). Pain nociception determined by small artery clamp at the base of tail & pain anti nociception by tail immersion test.

II) Exploratory behavior was determined by Hole Board test.

Statistical analysis : Statistical analysis of difference between groups was evaluated by One way ANOVA followed by post hoc Tuckey test for comparison between drugs (standard and test) and vehicle treated control groups.

Results: The results revealed that the proteins isolated from *I. batatas* (1.5 and 3.0 mg/Kg, p.o.) caused no significant change in exploratory behavior ($p > 0.05$), but demonstrated decrease in spontaneous motor activity, pain response and touch response in general behavior profile.

Conclusion: I. batatas tuber proteins have exerted CNS depressant and analgesic activities in the tested animal model.

Key words: *Ipomoea batatas proteins,* mice, general behavior, exploratory behavior, antinociception.

INTRODUCTION

Interest in medicine derived from herbs is growing nowadays because of its health beneficial properties. Sweet potato (*Ipomoea batatas* [L.] Lam) is actually a perennial, viney plant that is widely cultivated as an annual plant in the tropics, where it is grown for its edible tubers. These tubers contain storage reserves laid down by the plant and are utilized as a vital source of nutrition in developing countries. Sweet potatoes tubers are rich in complex carbohydrates, dietary fibers, beta carotene (a vitamin A equivalent nutrient), vitamin C, Vitamin B6 and vitamin E. Low amount of proteins and fat are also present along with copper, manganese, potassium, iron etc.[1] Research has reported health beneficial properties of *Ipomoea batatas* in past. It was reported that *I. batatas* fibers, crude extracts and anthocyanins played an

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important role in stabilizing plasma glucose as well as cholesterol levels in animals and humans, growth inhibition of several human colon carcinoma cell lines, in wound healing, and suppress the development of atherosclerotic lesions in mice.[2-6] Tubers contain proteins, which exhibit antioxidant, antimicrobial and trypsin inhibitor activities. [7-10]

I.batatas is a traditional counterpart and its consumption by human beings is considered to be safe. Food may contain toxic compounds with small safety margins between intake and obvious toxic effect level. Therefore, careful assessment of useful components isolated from edible plants must be carried out. So far neuropharmacological effects of *I. batatas* proteins have not been explored. Therefore present study is designed to assess the effects of *I. batatas* proteins on central nervous system of mice through behavioral studies (gross & exploratory) and by evaluating analgesic activity in these animals.

MATERIALS AND METHODS

Animals

Adult male mice of NMRI strain (25-30g) were obtained from Department of Pharmacology, Faculty of Pharmacy, University of Karachi. These animals were maintained at controlled temperature (23°C ± 1°C) with 12 hrs. dark/light cycles and given free access to standard food and water ad libitum. Animals were divided into 12 groups (6 in each group). Each group of animal was treated individually with saline water (5 ml/Kg p.o.), proteins isolated from *I. batatas* (1.5 and 3.0 mg/Kg, p.o.), standard drugs Diazepam (5.0 mg/Kg i.p.) and Morphine (5.0 mg/Kg i.p.). Each animal was used once. All these experiments were performed in accordance with the guidelines of the National Institute of Health (NIH).

Drugs/Chemicals

Injection Diazepam (Sigma) was used as the standard drug in general behavior profile and exploratory behavior in mice. Injection Morphine was used as the standard drug in tail flick test. Other chemicals used for extract preparation and protein estimation were Tris-HCl buffer (pH 7.5, Scharlau), solid ammonium sulfate (Scharlau), 10 % polyacrylamide gel (SERVA), coomassie brilliant blue stain (BioRad) and Bradford reagent (BioRad).

Plant material and extract preparation

The tubers of *I. batatas* were collected in the month of February from Pharmacognostic Garden, Research Institute of Pharmaceutical Sciences, University of Karachi.

Tuber tissues were homogenized in 50 mM Tris-HCl buffer (pH 7.5, Scharlau). These homogenates were centrifuged at 10,000 x g for 10 min. The supernatants were brought to 35 % saturation with solid ammonium sulfate (Scharlau) and then resulting precipitate was collected by centrifugation at 10,000 x g for 10 min. [11] The *I. batatas* proteins were further purified by Batch adsorption technique.[12-13] and precipitated with ammonium sulfate as described earlier. All conditions were kept as cold as possible (temperature range between +0S°C to +4°C). Sample was suspended in distilled water just before use.

SDS-PAGE

SDS-PAGE was performed as described by Laemmli.[14] Protein samples were resolved by 10 % polyacrylamide gel (SERVA) under reducing conditions. Proteins were then visualized by

coomassie brilliant blue staining (BioRad).

Protein estimation

Protein concentration was determined according to the method of Bradford [15]; bovine serum albumin (SERVA) was used as standard.

General behavior

Evaluation of general behavioral profile was performed by the method of Irwin and Dixit *et al.*[16, 17] Experimental mice (6 in each group) were tested by *I. batatas* protein extracts (1.5 and 3.0 mg/Kg, p.o.), standard drug Diazepam (5.0 mg/Kg, i.p.) and saline water (5 ml/Kg, p.o.) as a vehicle for control group of mice. After drug/vehicle administration, each group of mice was placed individually into the observation cage and observed at 30 min. intervals in the first one hour and at the hourly intervals for the next 4 hours. The behavioral activities and changes were observed carefully in each mouse. In order to examine any toxic effect or mortality, animals were kept in observation for 15 days.

Awareness, alertness and spontaneous activity: The awareness and alertness was recorded by visual measure of the animal's response when placed in a different position and its ability to orient itself without bumps or falls. The normal behavior at resting position was scored as (-), little activity (+), moderate flexibility (+ +), strong response (+ + +) and abnormal restlessness as (+ + + +). The spontaneous activity of the mice was recorded by placing the animal in a bell jar. It usually shows a moderate degree of inquisitive behavior. Moderate activity was scores as (+ +) and strong activity as (+ + +). If there is little motion, the score was (+), while if the animal sleeps, the score was (–). Excessive or very strong inquisitive activity like constant walking or running was scored as (+ + + +). A similar test was performed with the same scoring, when the animals are removed from the jar and placed on a table. [18]

Sound response:

Albino mice normally utter no sound, so that vocalization may indicate a noxious stimulus.

Touch response:

The touch response was recorded by touching the mice with a pencil or forceps at the various part of the body (i.e. on the side of the neck, abdomen and groin).

Pain response:

The pain response was graded when a small artery clamp was attached to the base of the tail, and response was noted.

Exploratory behavior (Hole-board test)

The hole-board test was performed as described by Clark *et al*. [19] White wooden box, with 16 equidistant holes was used. Animals (6 in each group) were treated with *I. batatas* proteins (1.5 and 3.0 mg/Kg, p.o.), standard drug Diazepam (1.0 mg/Kg, i.p.) and saline water (5.0 ml/Kg, p.o.) as a vehicle for control group of mice. After 30 min. of drugs/vehicle administration, experimental mice were placed individually into the center of the box and allowed to move freely inside. The number of times, each mouse pushed its head completely through one of the holes was recorded for the period of 5 min.

Warm water tail immersion assay

Male albino mice were devided into four groups of 6 animals each. After stimulating thermal nociceptors, time dependant analgesic activity of *I. batatas* protein extract (1.5 and 3.0 mg/Kg, p.o.) and morphine (5.0

mg/Kg, i.p.), was determined and this activity was compared with saline (5.0 ml/Kg, p.o.) treated group. Antinociception was evaluated by measuring response latencies in the warm water (55°C ± 1°C) tail immersion assay. [20] Response latencies were measured as the time required by the animal to respond to the thermal stimuli. Mice were not permitted to exceed 10 sec. of exposure to the thermal source to prevent prolonged painful stimulation or tissue damage. Base line tail flick latencies were determined prior to any treatment. Antinociception response was evaluated 30 min. after administration of drugs or vehicle and every 30 min. for 2 hrs.

Statistical analysis

All these results are presented as mean ± SEM. Statistical comparisons were made by means of one-way analysis of variance (ANOVA) followed by post hoc Tuckey test for comparison between drugs (standard and tested) and vehicle treated groups. Differences between experimental groups were considered statistically significant when $p < 0.05$.

RESULTS

SDS-PAGE

General behavior

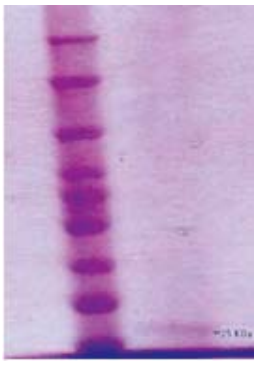

Figure – 1

SDS-PAGE showing ~25 KDa protein isolated from I. batatas (L.) Lam tuber (Right side) and protein ladder (Left side). SDS-PAGE was performed in reducing conditions. Test protein is indicated by line marker.

In the general behavior test, the group treated with *I. batatas* tuber protein exhibited passivity, mild decrease in spontaneous motor activity, mild decrease in reactivity to startle response, moderate depression of touch and pain responses but produced no influence on awareness and alertness as compared to those in the control group.

Symptoms of altered general behavior subsided after 1½ hr. of treatment. No mortality was recorded up till 15 days of observation of experimental mice. The results of the general behavior test are summarized in Table 1.

Table 1: Effect of I. batatas protein extract on general behavioral profiles in mice

Behavior type	Protein Extract 1.5 mg/Kg	Protein Extract 3.0 mg/Kg	Diazepam 5.0 mg/Kg	Saline water 5.0 ml/Kg
Spontaneous activity Alertness	++	++	++++	-
Awareness Sound response	-	-	+++	-
Touch response Pain response	-	-	+++	-
	+	++	++++	-
	++	++	++++	-
	++	++	++++	-

General behavioral test was performed on 4 groups of mice (6 animals in each group), -, no effect; +, slight depression; ++, moderate depression; +++, strong depression; ++++, very strong depression.

Exploratory behavior (Hole-board test)

In the hole-board test, *I. batatas* test proteins demonstrated no significant difference in the number of head dips when compared with the control group ($p > 0.05$). However standard drug Diazepam showed significant increase in head dips at dose that did not produce sedation compared with the control and *I.*

batatas protein extract treated groups of mice ($p < 0.05$). The results of the hole-board test are summarized in Table 2.

Table 1: Effect of of I. batatas protein extract in the hole-board test in mice

Groups	Dose	Number of head dips
Saline water (p.o.)	5.0ml/Kg	30.00 ± 0.51
I. batatas test protein (p.o.)	1.5 mg/Kg	29.26 ± 1.50
I. batatas test protein (p.o.)	3.0 mg/Kg	29.16 ± 2.23
Diazepam (i.p.)	1.0 mg/Kg	45.4 ±1.10*

Hole board test was performed on 4 groups of mice (6 animals in each group), * $p < 0.05$ as compared to control (ANOVA was followed by Tukey's post hoc test). Data are reported as mean ± SEM for the n=6 in each group where n = number of animals in each group.

Warm water tail immersion assay

I. batatas protein extract and morphine induced time dependent antinociception and test protein treated group of mice experience a significantly greater delay in tail withdrawal than their control counterparts (Table 3). Thus analgesic response of test protein was significantly different from control group ($p < 0.05$).

Table 1: Effect of of I. batatas protein extract in the hole-board test in mice

Groups	Dose	Tail immersion test (reaction time, seconds)
Saline water (p.o.)	5.0 ml/Kg	1.5 ± 0.28
I. batatas protein extract (p.o.)	1.5 mg/Kg	7.9 ± 0.71*
I. batatas protein extract (p.o.)	3.0 mg/Kg	8.4 ± 0.32*
Morphine (i.p.)	5.0 mg/Kg	4.6 ± 0.24*

Warm water tail immersion test was performed on 4 groups of mice (6 animals in each group), * $p < 0.05$ as compared to control (ANOVA was followed by Tukey's post hoc test. Data are reported as mean ± SEM for the n=6 in each group where n = number of animals in each group.

DISCUSSION

In past, research has been conducted on safety assessment of *I. batatas* tuberous plant and previous reports have demonstrated that terpenoids, isolated from stressed *I. batatas* produced toxic effects. The acute toxicity of ipomeamarone (IPM), a phytotoxin isolated from the injured *I. batatas* tubers was also evaluated in albino rats. [21-23] Therefore, it is very important to assess safety and toxicity of any therapeutically useful component isolated from this edible plant. In present study, general and exploratory behavior tests were performed on experimental mice for neuropharmacological assessment and safety evaluation of the protein extracts prepared from *I. batatas* tubers. These tests are classical screening of activities on central nervous system of animal models and provide information about anxiety and psychomotor performance. Our findings revealed that the *I. batatas* protein influences the general behavior profile and produced moderate reduction in spontaneous motor activity. Depression of parameters in general behavior of mice suggests central nervous system depressant action and potential sedative effect of test sample. [24-25]

In the exploratory behavior test (hole-board test), a useful tool for evaluating changes in various emotional states of animals, anxiolytics have been shown to increase the parameter of head dips [26-28] and decrease in head dips reveals a sedative behavior. [28] Mice treated with *I. batatas* protein extract showed non-significant difference in head-dips when compared with the control group ($p > 0.05$) indicating no anxiolytic or anxiogenic/sedative effect of sample proteins. In spite of moderate decrease in spontaneous motor activity, test proteins did not alter exploratory behavior of mice in head dip test. Perhaps *I. batatas*

protein extract has no effect on emotional depression or perhaps the doses of test extract in our study were not sufficient to alter this parameter as it is in the case of benzodiazepine, which acts as an anxiolytic at low doses and produce sedation at higher doses.[29] No mortality was observed up to 15 days after experiment in all treatment groups.

Up till now, very few proteins, either synthetic or natural, have known analgesic property. [30-31] In the tail immersion assay, proteins obtained from *I. batatas* tubers presented significant analgesic activity after stimulating thermal nociceptors as compared to control group. Our results demonstrated that our test sample is more potent than morphine. The behavioral tests and analgesic activity here employed, however do not allow discerning the underlying mechanism of action of *I. batatas* protein extract. Further research is required to clarify the mechanism of central activity of test sample.

CONCLUSION

I. batatas proteins possess CNS depressant and analgesic properties. Further studies are required to explore the underlying mechanism responsible for producing these effects to evaluate the toxicity and safety profile of this tuber protein.

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ORIGINAL ARTICLE

Morphological Study of Lead Induced Nephrotoxicity with Role of Zinc in Albino Rats

Naheed Khan[1], Khalida Perveen[2], Muhammad Rafique[2] and Najmus Sahar Syed[3]

ABSTRACT

Introduction: Damage to the kidneys is one of the primary toxic actions of the metals and nephrotoxicity by heavy metals has been the focus of much research. Lead is a heavy metal widely distributed in the environment. Its toxicity is a matter of concern as very low levels in the environment are found to effect under-nourished population. Entering the body through multiple routes it gets distributed in almost every organ including kidneys altering its structure and functions.

Objective: Heavy metals such as Lead are known to interact with the essential trace elements at the level of absorption and also during metabolism. The present study was designed to observe morphological changes in renal tissue with special reference to Proximal tubules following concomitant administration of essential micronutrient zinc with lead.

Design: Experimental study.

Materials & Methods: 45 young adult albino rats selected for the study were distributed into 3 main groups of 15 rats each. Group A served as control, Group B rats received Inj. Lead acetate 8 mg/kg intraperitoneally daily and Group C in addition to lead received Inj. Zinc chloride 0.21 mg/kg intraperitoneally daily. Each group was further subdivided into three sub-groups according to the period of treatment given i.e. 2,4 & 6 weeks, at the end of which animals were sacrificed. The kidneys after processing and staining (PAS-Haematoxylin) were subjected to detailed morphological examination of proximal tubules.

Results: The morphologic study in lead treated subgroups revealed changes indicating progressive distortion of renal cortical tissue with increasing time periods so that at six weeks a number of necrotic tubules with pyknotic nuclei were seen. Histological picture was close to that of Control and showed minimum distortion in rats co treated with zinc.

Conclusion: Based on the study, it can be stated that lead induced nephrotoxicity particularly damages the structure of proximal tubules and the damage is more pronounced with increasing time period. Concomitant treatment with essential micronutrient zinc reduces or delays the toxic effects of lead.

Key words: Heavy Metal, Proximal tubules, Nephrotoxicity, per oxidative damage, anti oxidant

INTRODUCTION

Over the last few decades, it has become increasingly obvious that kidney is adversely affected by an array of chemicals. Man is exposed to these nephrotoxic agents as medicines, industrial and environmental chemicals, and a variety of naturally occurring substances.[1]

Environmental chemicals such as lead are capable of inducing nephrotoxicity.[1] Many studies show a strong association between lead exposure and renal effects. [2-4] Proximal renal tubular cells are particularly vulnerable to the toxic action of chemicals; owing to their high energy demand such as re-absorptive and secretory functions.[1]Dose-related proximal tubular dysfunction has been observed in rats exposed to

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lead by researchers.[5,6] Lead is highly toxic and persistent in the environment and thus, a major concern for public health.[7]

Exposure to Lead can occur from multitude of sources. Lead used as an anti-knock during the manufacturing of gasoline is the main source of Tetraethyl lead, the end product found in the emission of vehicles getting into air.[8] These lead isotopes are very stable and do not decay for millions of years [9] contaminating air, water, soil and food.[10] Although lead has been removed from gasoline in western countries, leaded gasoline continues to be used in developing countries.[11] Exposure to lead may also occur from poorly controlled industrial emissions at metal refineries & battery recycling plants and demolition of old houses. Products that contain lead include pipes, solders, electric cables, paint, ceramics and ayurvedic medicines.[9]

Exposure to lead enhances per oxidation[12] of membrane phospholipids, accompanied by a concomitant decrease in the activity of antioxidant enzymes, such as superoxide dismutase[12] and glutathione reductase.[4,13] Superoxide dismutase is an enzyme responsible for detoxification of highly reactive and potentially toxic free radicals to less toxic hydrogen peroxide. The results of a study[4] indicated an inhibition of SOD in kidneys of lead treated animals. Lead accumulates in the mitochondria and causes both structural and functional alterations by inhibiting respiratory function and energy (adenosine triphosphate) production.[11]

Zinc is an essential element and a micronutrient with antioxidant effects, it plays a biochemical role in stabilizing membrane structure and thus reducing per oxidative damage to sulfhydryl group. [14] Moreover S.O.D which forms a major protective system against free radical injury is a zinc dependent enzyme.[15] In one study, co treatment with zinc greatly reduced mercury induced renal toxicity.[16] Zinc supplementation can effectively compete for and reduce the availability of binding sites for lead uptake.[17]

MATERIALS& METHODS

The study was conducted in the Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC) Karachi. A total of 45 active, young adult Albino rats with a fully developed urinary system were obtained from the Animal House of J.P.M.C.

The animals at the onset of experimental study, were divided into three groups comprising 15 rats; each group was further sub-divided into three sub-groups according to the period of treatment they received, i.e. 2 weeks, 4 weeks and 6 weeks; each subgroup comprised of 5 animals.

Ø Group-A rats with subgroups A1, A2, A3 served as control ,received injection normal saline 1 ml intra-peritoneally daily for 2,4 and 6 weeks respectively.

Ø Group-B rats with subgroups B1, B2, B3 received Injection Lead Acetate (Merck, Germany) at a dose of 8 mg/kg body weight intra-peritoneally daily for 2,4 and 6 weeks respectively

Ø Group-C rats with subgroups C1, C2, C3 received Injection Zinc Chloride (Merck, Germany) at a dose of 0.21 mg/kg body weight intra-peritoneally two hours before administration of Lead Acetate at a dose of 8 mg/kg body weight intra-peritoneally, daily for 2,4 and 6 weeks respectively.

Throughout the period of experimental study the animals were kept under observation to note any change in their general condition, behavior and their activities.

The animals were sacrificed under ether anesthesia at the end of experimental period (fig.1).

Figure – 1 : A photograph of opened and exposed abdominal cavity of group-A rat showing kidneys before excision

The abdominal cavity was opened by giving a midline incision and kidneys were dissected out, cut into two longitudinal halves and were fixed in Alcoholic Formalin for 24 hours. Later they were processed in ascending strengths of alcohol, cleared in xylene, infiltered and embedded in paraffin .After this process sectioning was performed in order to obtain 5µ thick longitudinal sections with the help of a rotatory microtome, which were then mounted on albumenized glass slides. The slides were stained with Periodic Schiff haematoxylin technique in order to visualize not only the cytoplasm and nuclei of the cells but also the structures containing a high proportion of macromolecules i.e the brush border and basement membrane. The morphological study of proximal tubules was done in detail under light microscope using 8x ocular and 100 x oil immersion objectives.

OBSERERVATIONS & RESULTS

The morphological examination of Periodic Schiff-Haematoxylin stained sections of kidneys belonging to Control groups-A1, A2 & A3 revealed a normal intact cortical and medullary architecture. The proximal tubules were closely packed and appeared circular, oval or elliptical in sections & were mostly confined to the cortex particularly in the vicinity of glomeruli .The cells appeared low, columnar with fine and granular cytoplasm. They were regularly arranged on an intact and well defined basement membrane. The spherical nuclei located centrally or towards the basal portion of the cell presented fine and evenly distributed chromatin .The luminal surface of these cells presented a distinct and regular brush border. The lumina of the tubules were devoid of any cellular or nuclear debris. The interstitium in the cortical area showed no signs of acute or chronic inflammatory cell infiltration. (fig.2).

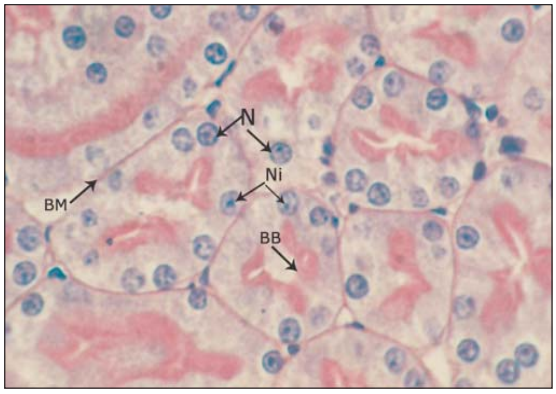

Figure – 2 : PAS-Haematoxylin stained, 5 µm thick, longitudinal section of kidney from group-A (control) rat showing proximal tubules with intact brush border (BB), basement membrane (BM), Nuclei (N).

The histological examination of lead treated rats revealed an irregular cortical architecture which was found highly distorted at 6 weeks in subgroup B3.The proximal tubules mostly confined to the cortex appeared dilated in all the lead treated subgroups(fig.'s 3&4) and this dilatation was accompanied by severe sloughing and degeneration in B3 (fig.5).

In subgroup B1(2 weeks lead treated) the lining cells of many tubules appeared enlarged while in B2(4 weeks lead treated) the enlarged cells were mostly vacuolated obscuring cytoplasmic details (fig.4) .In

subgroup B3 in addition to large vacuolated cells many cells appeared almost flattened (fig. 5).

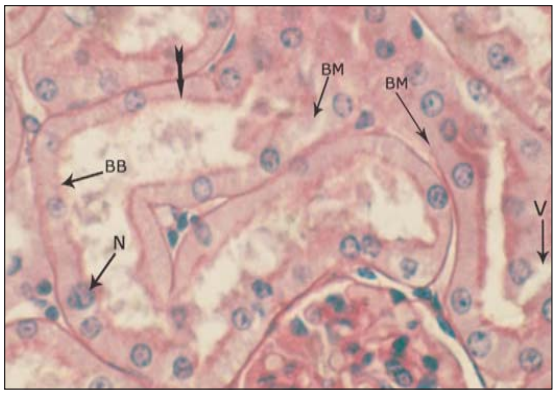

Figure – 3 : PAS-Haematoxylin stained, 5 µm thick, longitudinal section of kidney from group-B1(2 weeks lead treated) rat showing dilated proximal tubules with scanty brush border (BB), distorted basement membrane (BM), enlarged and displaced nuclei (N), and cytoplasmic vacuole (V).

The nuclei in many proximal tubular cells appeared enlarged and displaced from usual basal or central portion of cell. These enlarged nuclei (fig. 4) were more numerous in subgroup B2 which often presented a condensed and clumped chromatin. In subgroup B3 most of nuclei appeared pyknotic with condensed and darkly stained chromatin (fig.5). In some of the cells no nuclei were seen.

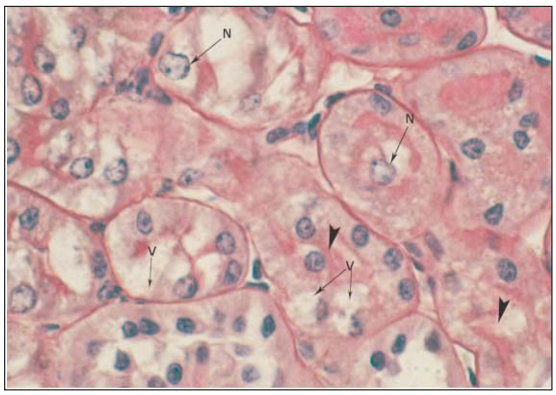

Figure – 4 : PAS-Haematoxylin stained, 5 µm thick, longitudinal section of kidney from group-B2 (4 weeks lead treated) rat showing enlarged nuclei (N), vacuolated cytoplasm (V), and epithelial debris (↑) in lumen.

The brush border on apical surfaces was found scanty at 2 weeks of treatment (fig.3) with few areas of complete loss at 4 weeks in B2. In subgroup B3 the distorted apical surface of cells presented almost a complete loss of brush border (fig. 5)

The underlying basement membrane was found disorganized in initial periods of treatment; few areas of distortion were seen in subgroup B2 while in subgroup B3 basement membrane was completely distorted in a number of tubules (fig.3-5).

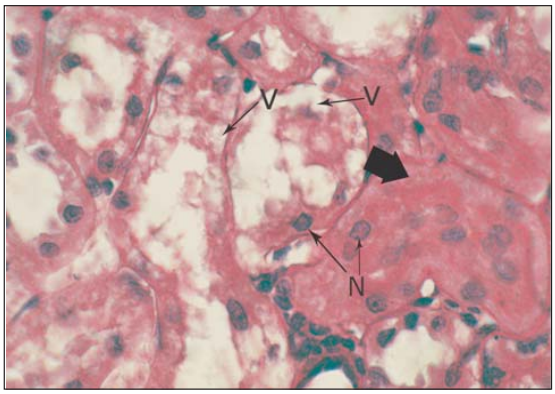

Figure – 5 : PAS-Haematoxylin stained, 5 µm thick, longitudinal section of kidney from group-B3 (6 weeks lead treated) rat showing necrotic tubules (↑) with pyknotic nuclei (N), severe vacuolar degeneration (V),distorted brush border and basement membrane,mononuclear infilteration .

The Lumina of tubules showed cast off materials in all the lead treated subgroups especially at 4 and 6 weeks in B2 & 3 (fig. 4) the epithelial and nuclear debris were found in numerous tubules. The interstitium surrounding the distorted tubules showed large areas of mononuclear cell infiltration (fig. 5). In 6 weeks lead treated subgroup B3 (fig.5) in addition to the tubules described above, groups of proximal tubules showing obvious necrotic changes were seen in the cortical region. They showed shrinkage in size and appeared as a homogeneous, glassy, eosinophilic material lying within the basement membrane. The cell boundaries were not recognizable and lumina were obscured. There were few pyknotic and irregularly arranged nuclei. Few tubules of same

features were also found scattered in renal cortices of subgroup B3.

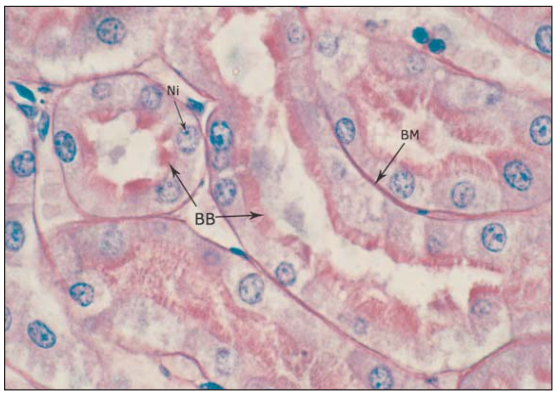

Figure – 6: PAS-Haematoxylin stained, 5 µm thick longitudinal section of kidney from group-C3 (6 weekslead + zinc treated) rat showing proximal tubules with regular basement membrane (BM) brush border (BB), nucleus (Ni) and occasional cytoplasmic vacuoles. Photomicrograph x1000

The study of the proximal tubules in subgroups C1& C2 (2&4 weeks lead+zinc treated respectively) showed a picture better than the corresponding lead treated subgroups. Few tubules showed epithelial casts in later weeks of treatment with lead + zinc. The study of sections belonging to subgroup C3 also showed a better picture than corresponding B3 as there were occasional cells with vacuolated cytoplasm and nuclei with darkly stained, clumped chromatin. The basement membrane and brush border were comparatively regular (fig. 6). Only a few tubules with scanty brush border were also seen.

DISCUSSION

Several chemicals both therapeutic and non-therapeutic have toxic effects on one or more anatomical elements of kidney. Proximal renal tubular cells are particularly vulnerable to the toxic action of chemicals.[1]

Environmental chemical such as lead is capable of inducing nephrotoxicity.[1] The kidneys are particularly exposed to the untoward toxic effects of lead as they form its major route of excretion.[3]

With increasing concerns about environmental pollution, the interaction of micro-nutrients with toxic metals is of great interest.[15] Zinc has been proved to have a protective role against the nephrotoxic action of many agents. In 2002, a study [16] concluded that co-treatment with zinc protected against mercury induced renal toxicity in mice.

It was thought worthwhile to carry out a study using experimental induction of nephrotoxicity in albino rats by administration of lead. Moreover attempts have been made to study the protective effects of concomitant administration of zinc on lead induced nephrotoxicity on different groups of animals at different time period. In that respect a detailed morphological examination of proximal tubular cells was done under light microscope in Periodic Schiff stain.

In the present study lead was used in a dose of 8 mg/kg body weight, while zinc was used in a dose of 0.21 mg/kg body weight. Both the lead acetate and zinc chloride were administered to the animals by means of intraperitoneal injections to allow accurately calculated doses of solutions to be administered to the animals and to enable uniform absorption.

The morphological examination of renal cortical tissue in lead treated group-B showed dilated proximal tubules as compared to the corresponding control groups. This could be attributed to inflammatory changes following lead exposure.[6]

The lining epithelial cells in the majority of tubules showed enlargement due to vacuolar degeneration of cytoplasm [2] which obscured cytoplasmic details and shifted the nuclei to atypical positions. Lead accumulation in mitochondria causes them to swell resulting in decreased activity of Na-pump & increased influx of Ca^{++}, H_2O and Na^{+} and an efflux of K^{+} causing cellular swelling & loss of microvilli. This results in scanty & indistinct brush border on the luminal surfaces of cells in all the lead treated subgroups. After 6 weeks of lead treatment there

was almost complete loss of brush border so that dilated tubules with flattened epithelia were observed. The basement membrane underlying the proximal tubular cells was found disrupted and discontinuous from the beginning of experimental period which can be correlated to the previous work [15] with disorganized basement membrane in the seminiferous tubules of rats treated with lead acetate. This can be attributed to decreased ATP which results in disruption of protein synthetic apparatus due to swelling of endoplasmic reticulum & detachment of ribosomes.[18]

There were enlarged nuclei with progressive clumping of chromatin in variable periods of treatment in group-B. The finding is in agreement with previous studies [5-6] and may be attributed to the presence of lead induced nuclear inclusion bodies and pseudo-inclusions or nuclear invagination of cytoplasmic contents.[11] The decrease in cellular ATP causes an increased rate of anaerobic glycolysis, which results in accumulation of lactic acid, reducing the cellular pH, which in turn causes clumping of nuclear chromatin.

In our study lead treated subgroups receiving lead for 6 weeks showed irregular & pyknotic nuclei, in contrast with the study [5] in which enlarged nuclei were observed even after two months treatment with lead acetate. However, pyknotic nuclei were seen in proximal tubular cells after exposure with other heavy metals such as cisplatin[19]at earlier stages of treatment. Presence of irregularly shaped pyknotic nuclei in our observations might be related to the degenerative changes found in renal tubular cells with continued lead treatment.

In such groups we also observed tubules showing shrinkage in size, with necrotic changes. Such changes were also described by other researchers[11] according to whom areas of dilated tubules alternate with necrotic & atrophic tubules.

The presence of sloughed material and epithelial casts and nuclear debris in the lumina of tubules in lead treated group-B indicates loss of cytoplasmic contents and nuclei from the distorted apical surface of the tubules which also explains the absence of nuclei in many cells.

The morphological examination of the renal cortical tissue in lead plus zinc treated group-C revealed a picture that was comparable to control group-A. The histology of the tubular cells with their apical and basal surfaces, their nuclei were all comparable to their corresponding controls, except occasional vacuoles observed in the cytoplasm of a few cells of subgroup C3. Moreover the tubules with obvious necrotic changes were not seen in group-C, as were found in late periods of treatment of group-B.

On the whole these results strongly suggest that the anti-oxidant role of zinc helps to ameliorate the effects of lead induced nephrotoxicity.

Several explanations have been clearly proposed to account for this effect of zinc in animals. Zinc supplementation could significantly compete for and effectively reduce the availability of binding sites for lead uptake.[17] Zinc has been clearly shown to have an anti-oxidant role and it acts by protecting the sulfhydryl groups against oxidation and prevent the production of hydrogen peroxide and super oxide radicals by transition metals.[20] Pharmacological doses of zinc by altering various biochemical pathways can induce proteins and enzymes, affect the metabolism of other metals and stabilize cellular membranes[20]

CONCLUSION

In the present study it was observed that administration of lead severely damages the proximal tubular cells indicating its nephrotoxic effects on renal cortical tissue. The concomitant administration of anti-oxidant zinc protects the proximal tubules by reducing or delaying the toxic effects of lead.

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CASE REPORT

Plexiform Neurofibroma of Face: A Challenge for Plastic Surgeons

Muhammad Ahmad[1] and Saleem A. Malik[2]

ABSTRACT

Plexiform neurofibroma is a benign tumour of peripheral nerves and connective tissue. They develop most often in patients with neurofibromatosis type 1 (NF1) and often grow continuously. Removal of plexiform neurofibromas is usually unsatisfactory because the network-like growth of these tumours often involves multiple nerve fascicles and other adjacent tissues. We present an interesting case of Plexiform Neurofibroma involving right half of the face. Surgical excision and debulking of the tissues were performed alongwith the correction of orbital skeleton and facial contours.

INTRODUCTION

Plexiform neurofibromas (PNF) are benign tumours originating from subcutaneous or visceral peripheral nerves and involves multiple fascicles. These tumours are either present at birth or develop within the first years of life. PNF occur almost exclusively in patients with neurofibromatosis type 1 (NF1), an autosomal dominant disorder caused by the genetic alteration of the tumour suppressor gene, NF1.[1] These lesions usually progress to adulthood, affecting skin, soft tissue, nerves and bone to varying degrees.[2] PNF can arise in various parts of body and may lead to severe clinical sequelae.[2] The course of the disease is unpredictable but the patients commonly seek medical care after a disfiguring mass and various facial soft tissue deformities appearing with or without dysplastic bony defects of the facial skeleton.[3] When extensive invasion of the facial soft tissue occurs, it is nearly impossible to radically excise all the involved facial soft tissues including the facial nerve.[3] Tumour rests may re-grow after the operation. The growth rates and patterns vary largely and are not predictable. Magnetic resonance imaging (MRI) enables distinction among 3 growth patterns in PNF: superficial, displacing and invasive.[4] For superficial PNF, total or sub-total resection may be possible. But invasive PNF infiltrates multiple tissue planes and cannot be completely resected without functional disturbances.[5] Plastic surgeons can be faced with facial deformity caused by deep or wide extensive involvement of plexiform neurofibroma on the craniofacial structures resulting in a debilitated social life for the patient.[6]

A rare case of plexiform neurofibroma with extensive facial involvement is presented.

CASE REPORT

A 26 years old woman visited the Plastic Surgery Clinic because of a disfiguring neurofibroma involving right half of the face, thereby affecting

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cheek, orbit, nose and lips (Fig. 1). Clinical examination of facial nerve paralysis was not possible because of the nature and extent of the involvement. Her baseline investigations were normal. X-rays of the facial skeleton revealed involvement of the underlying bony skeleton, i.e., outer orbital wall. A staged correction was planned to reconstruct the facial deformity. First of all, a diluted solution of adrenaline was injected carefully in the whole tissue. Excision of the mass was performed as far as possible. Complete excision was not practicable due to the wide spread and involvement of the underlying facial muscles. Enough tissue was left with the skin to avoid any necrosis. Faciocutaneous flaps were trimmed at the end of the procedure. Mass was also resected from the right nostril. Facial nerve was not found during the operation. Suspension of the angle of the mouth was performed. Resection of the lid and periorbital tissue was also performed. Debulking of the lid was combined with lateral canthopexy. Patient received blood transfusions during and after the operation. The drains were placed which were removed on 5-7 postoperative days. Compression dressing was applied. Postoperative course was satisfactory (Fig.2). There was no skin necrosis. The stitches were removed on the10th postoperative day. The patient was discharged after two weeks. Regular follow-up was done. The second stage was performed after 6 months in which the bony skeleton of the orbital wall was debulked and reconstructed. Healing was satisfactory with good postoperative result (Fig.3). Further suspension of the angle of the mouth with palmaris tendon graft was planned for the next stage of reconstruction.

DISCUSSION

Plexiform Neurofibroma is a major facial hamartoma and one of the most devastating, destructive, and debilitating disease involving the skin, muscle, mucosa and skeletal system.[3] Although benign in histological appearance, these lesions can be clinically malignant in their deforming and inexcisable growth. Facial drooping and other deformities frequently recur despite partial excision, leading to unsatisfactory function and poor aesthetic appearance. Total removal of facial neurofibroma and perfect correction of the facial soft tissue by conventional means or a single operation is impossible because of the invasion of superficial and deep facial structures, destruction of normal histological layers, and regrowth. Resection is further complicated by the absence of any encapsulating structure and, thus, any intermingling with normal facial layers from the skin, superficial fascia, muscles, nerves to, possibly, the cranial cavity.[1,7] Various factors such as patient's age, extent and location of the tumour have been discussed to correlate with surgical outcomes and post-operative tumour regrowth. Similarly loss of elasticity or integrity of the remaining soft tissue, regrowth or recurrence of a progressive mass, loss of skeletal and soft tissue support, and soft tissue detachment from skeletal structure by tumour infiltration, contribute to worsen the facial deformities.[7]

When viewed histologically, diffuse proliferation of compactly arranged spindle-shaped fibroblasts might infiltrate the surrounding soft tissues including the dermis, subcutaneous tissue, and blood vessels. Preexisting collagen bundles in the dermis and subcutaneous tissue forming a loose fibrous tissue separate the fibrous myxomatous dermis and skin. These changes cause soft tissue detachment from skeletal support and are aggravated by facial gravitational forces.[7,8]

Various techniques have been described including the netting operation in which Teflon mesh was used to suspend the inelastic drooping facial soft tissue [5,8,9] Similarly suspension of the tissue to the underlying bone with Mitek anchors may give more prolonged retention.[2] Plexiform neurofibroma is a

highly vascular lesion and the surgeon must be prepared for significant blood loss. This can be minimized with initial injection of vasoconstrictor agent combined with use of bipolar cautery. Since the skin as well as underlying tissues are involved, extensive thinning of the flap is dangerous. Malignant degeneration of a previously benign fibroma into a neurofibrosarcoma or malignant schwannoma is a constant threat. The occurrence of malignant change in neurofibroma differs among various reports but usually is 8 to 16 %.[10] A sudden change in size with pain should alert the physician to a malignant change. Although there is an obvious concern that surgical trauma and events of healing may provoke progression into an aggressive nature, the regular postoperative follow-up with serial computed tomographic scans can play an important role in detecting such a change. [2,4,8]

CONCLUSION

Surgical intervention is presently the only treatment option for plexiform neurofibroma. Debulking surgery is likely to lead to improvement in appearance and fewer clinical deficits, thus increasing the quality of life for these patients.

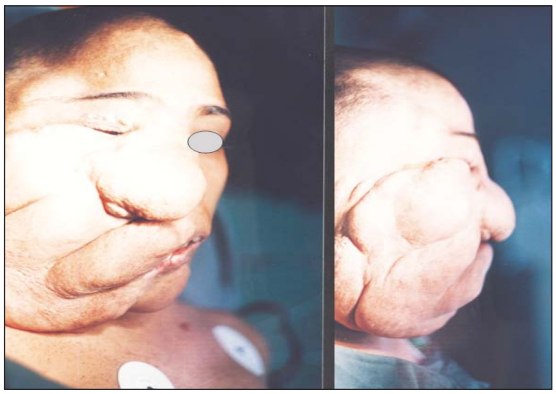

Figure – 2 : Huge Neurofibroma Face involving right half of the face

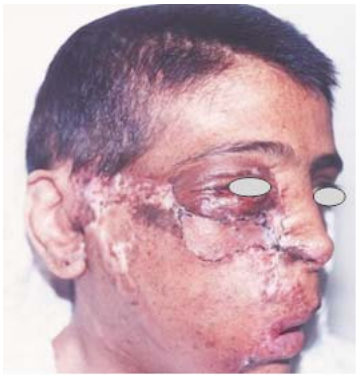

Figure – 2 : Two weeks after surgery with reconstruction of cheek, nostril, periorbital area and angle of mouth

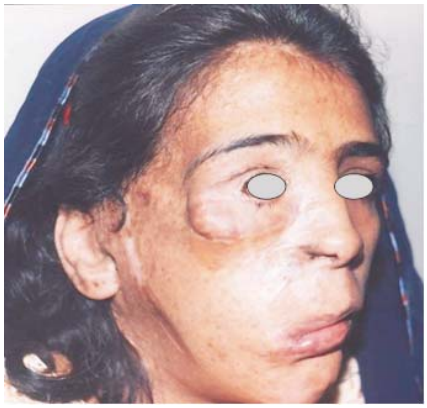

Figure – 3 : Nine months after surgery

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