QUARTERLY

Print ISSN 1815-4018
Online ISSN 2410-5422

# JIIMC

## Journal of Islamic International Medical College

RIPHAH
ISLAMIC INTERNATIONAL
MEDICAL COLLEGE

June 2018, Vol.13, No.2

**Indexed in:**
WHO-Index Medicus (IMEMR)

**Recognized by:**
Pakistan Medical & Dental Council (PMDC)
Higher Education Commission, Pakistan (HEC)

JIIMC JOURNAL OF ISLAMIC INTERNATIONAL MEDICAL COLLEGE

**Print ISSN 1815-4018** **PMDC No. IP/0059** **Recognized by PMDC & HEC**
**Online ISSN 2410-5422**



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**Journal of Islamic International Medical College**
**Quarterly**

June 2018; Vol.13, No.2
Print ISSN: 1815-4018
Online ISSN: 2410-5422

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# JIIMC

"Journal of Islamic International Medical College (JIIMC)" is the official journal of Islamic International Medical College Rawalpindi Pakistan. The college is affiliated with Riphah International University and located in Rawalpindi (Punjab) Pakistan.

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Published by IIMC, Riphah International University Islamabad, Pakistan
Web Site: jiimc.riphah.edu.pk

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**Recognized by:** Pakistan Medical & Dental Council ; Higher Education Commission (HEC) Islamabad (Category Y)
**Indexed in:** WHO - Index Medicus for Eastern Mediterranean Region (IMEMR), Scientific Journal Impact Factor (SJIF), International Scientific Indexation, Scopemed (ejmanager)
**Registered with:** International Serials Data System of France
**Covered by:** Pakmedinet, PASTIC inventory "Directory of Scientific Periodicals of Pakistan"- Pakistan Science Abstracts (PSA)

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# EDITORIAL

## Thalassaemia in Pakistan

Suhaib Ahmed

Thalassaemia is the commonest single gene disorder of globin chain synthesis all over the world .[1,2] It is found as thalassaemia minor when the defect is inherited from one of the parents and as thalassaemia major when the defect is inherited from both of the parents. Studies of thalassaemia in Pakistan have shown that 5% of the population have thalassaemia minor.[3] Based on this figure it is estimated that each year in Pakistan approximately 5000 children with thalassaemia major are born and the total number of children with thalassaemia major may be over 50,000.[4]

Thalassaemia major is a serious disorder in which the affected child is unable to synthesize its own haemoglobin. The child remains well for the initial three to four months but after this the signs and symptoms of anaemia start appearing. The child looks pale, becomes irritable, and fails to thrive. Diarrhoea and frequent respiratory tract infections are other frequent symptoms. Examination may show hepato-splenomegaly. The lab investigations show moderate to marked hypochromic microcytic anaemia, numerous nucleated red cells and markedly raised Hb-F.[5] An early diagnosis and blood transfusions may halt further deterioration. The child has to be put on lifelong blood transfusions. As a result of regular blood transfusions a large amount of extra iron starts accumulation in the body. This iron can be very harmful for many important organs like heart and endocrine glands. The extra iron must be removed from the body by using iron chelating medicines. Stem cell transplantation is a curative treatment of thalassaemia major. When done early in life over 80% disease free survival can be achieved.[6] The facilities for stem cell transplant are available at several places in Pakistan but the procedure is expensive and due to the limited capacity it can be offered to only a small number of patients.[7]

There are more than 50,000 patients of thalassaemia major in Pakistan. Unfortunately the treatment facilities for such large number of patients are not adequate. Most of the patients are treated at the centres run by nongovernmental organizations (NGO) who have limited resources. Consequently the outcome is also not good. A report published in this issue of the Journal gives a detailed description of the outcome in a large number of patients at a local treatment centre.[8] Most of the children were chronically under-transfused and were also markedly iron overloaded. Majority had growth retardation below the fifth centile for the Pakistani population. The median age at death was just ten years. Although this is a single centre study but the situation at the other centres in Pakistan, with few exceptions, is not expected to be different.

Thalassaemia minor (carrier) is an asymptomatic disorder and most people do not even know about their abnormality. Most people with thalassaemia minor are detected during blood testing for some other reason or when they get married to another carrier and give birth to a child with thalassaemia major. Thalassaemia carriers usually have haemoglobin within the normal range, MCV < 75 fl and MCH < 25 pg. The diagnosis can be confirmed by haemoglobin electrophoresis that typically shows Hb-$A_2$ above 4.0%. A small number (<3%) thalassaemia carriers are silent i.e. their red cell indices and Hb-$A_2$ levels are within the normal range. Such carriers can be missed on routine screening methods and PCR is required to detect them.[5]

Thalassaemia is an autosomal recessive disorder. A child with thalassaemia major is born only when both of the parents have thalassaemia minor. In a carrier couple there is a 25% probability in each pregnancy that the child may inherit abnormality from both of the parents. Marriage of a carrier to a non-carrier will not result in thalassaemia major. The births of children with thalassaemia major can be avoided by premarital carrier screening and avoiding

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*Received: May 12, 2018; Accepted: May 25, 2018*

marriage between two carriers. The carrier couples who are already married can be offered prenatal diagnosis and selective termination of pregnancy if the fetus is found to be affected. Based on these two approaches excellent results have been achieved in many high risk populations in the Mediterranean countries and lately in Iran.[9] The birthrate of children with thalassaemia major in most of these countries has been brought down to almost zero. The success of thalassaemia prevention programs in the Mediterranean region and Iran has largely been attributed to a political will by the government, public awareness through mass communication media, allocation of funds, and the provision of quality diagnostic services.[9,10] Pakistan has one of the largest numbers of thalassaemia major children in the world and providing treatment facilities to these children is far beyond the available health resources.[2] The burden of disease is likely to increase with the passage of time if no measures are taken to implement a thalassaemia prevention program. The facilities for carrier screening of thalassaemia are available since early eighties. Prenatal diagnosis for thalassaemia is also available in Pakistan since 1994.[11] A study on the extended families of children with thalassaemia major in Pakistan has clearly shown it to be the most cost effective method for large scale application.[12] However, in spite of the two basic facilities for thalassaemia prevention there has not been any appreciable reduction in the birth incidence of thalassaemia major in Pakistan. There are several impediments, including the lack of awareness and the high cost, to the use of these measures in Pakistan.[13] The government of Punjab has initiated a thalassaemia prevention program (PTPP) that aims at providing free of cost carrier screening in the extended families of children with thalassaemia and the general public. PTPP also offers free of cost prenatal diagnosis (https://ptpp.punjab.gov.pk/). The program is in its early stages and it would take several years to see its long term benefits. There is need to initiate such programs in the other provinces of the country and to establish a central coordination cell in the federal ministry of health.[5,14]

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ORIGINAL ARTICLE

# Clinical and Haematological Picture of Multi-Transfused Thalassaemia Major Patients at a Center in Pakistan

Suhaib Ahmed[1], Zohra Jabeen Wazir[2], Ishrat Abdul Qayyum[3]

**ABSTRACT**

**Objective:** To describe the clinical and haematological picture of multiply transfused patients of thalassaemia major at a treatment center in Pakistan.

**Study Design:** Descriptive cross-sectional.

**Place and Duration of Study:** The study was conducted at Thalassaemia Treatment Centre Rawalpindi. The study was done between Jan 1994 and May 2017.

**Materials and Methods:** A total of 383 patients of thalassaemia major (TM) on regular blood transfusions were examined for various clinical and haematological parameters. In addition case records of 101 patients of TM who died during treatment were also studied.

**Results:** In the 383 patients on treatment 328 (86%) were born to consanguineous parents, 246 (64%) were from lower socio-economic group, 85 (22%) had one or more affected siblings, 145 (38%) had hepatomegaly, 191 (50%) had splenomegaly, 42 (11%) had undergone splenectomy and 187 (49%) had never received iron chelation therapy. Height and weight in the 383 patients on treatment showed marked stunting. Median age of the 383 patients on treatment was 104 months as compared to 119 months in the deceased group of patients (p=0.0263). Pre-transfusion Hb in the alive patients (7.1 g/dL) was higher than in the deceased patients (6.4 g/dL) (p=0.0142). Ferritin level in the patients on treatment (3698 µg/L) was lower than in the deceased patients (4616 µg/L) (p=0.0069). In the 383 patients on treatment 141 (36.8%) were HCV positive.

**Conclusion:** Majority of the patients at a thalassaemia treatment center are chronically under-transfused and have moderate to severe growth retardation. They get inadequate iron chelation resulting in high mortality before tenth year of life.

**Key Words:** *Blood Transfusion, Complications, Pakistan, Survival, Thalassemia.*

## Introduction

Genetic haemoglobin disorders are the most common single gene disorders in the world. It is estimated that about 250 million people carry the gene for thalassaemia or abnormal haemoglobin.[1] The disease has high prevalence in a broad belt including Mediterranean countries, Middle East, Indian Subcontinent and South East Asia.[2] Successful preventive programs in the developed countries have reduced the new births of thalassaemia major (TM) to almost zero.[3] However, in most developing and under developed countries the picture is very different.[4] Approximately 80% of the new births of children with genetic haemoglobin disorders take place in the under-developed or developing countries that have very limited resources for management and prevention. These have been largely ignored by governments of countries with a high-frequency of these disorders and by the international funding agencies.[5] As the infectious diseases are getting under control the mortality due to genetic diseases like thalassaemia is becoming obvious.[1]

Pakistan has a population of nearly 200 million and approximately 5% of the people carry the gene for β-thalassaemia.[6, 7] It is estimated that each year over 5000 new births of TM take place and the total number of children with TM may be well over 50,000.[8] Most of the children with TM in Pakistan are treated at centers run by Non-Governmental Organizations (NGOs). There are at least 50 NGOs

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*Funding Source: NIL; Conflict of Interest: NIL*
*Received: Jan 01, 2018; Revised: Mar 10, 2018*
*Accepted: Mar 18, 2018*

that provide blood transfusion services to over 25,000 children with TM. The NGOs work on a charitable basis and are mostly facing paucity of funds. Lack of funds and voluntary blood donations pose great challenge for providing quality treatment to children with TM.[9] There are almost no published data on the outcome of treatment of thalassaemics in Pakistan. This study is the first of its kind that describes the clinical and haematological features of children with TM getting treatment at a center run by a NGO in Pakistan.

## Materials and Methods

This descriptive cross-sectional study was done after approval by the ethical review committee of the executive council of the Society. All available children with TM registered at the center between Jan 1994 and May 2017 were examined. The patients of TM registered at the center but not available for examination due to various reasons were excluded. The study variables included age, sex, consanguinity, socio-economic status, other affected siblings, age at diagnosis, age at first transfusion, height, weight, hepato-splenomegaly, splenectomy, pre-transfusion haemoglobin, mean transfusion interval, iron chelation, serum ferritin levels and HCV status. In addition, the case records of children who died while on treatment between Jan 1994 and May 2017 and had adequate documentation were also studied. The deceased patients with incomplete documentation were excluded. The deceased patients were studied for age at death, age at diagnosis, age at first transfusion, mean pre-transfusion haemoglobin, mean transfusion interval, iron chelation, and serum ferritin. The results were analyzed by Stats Direct version 2.5.5 statistical package. Frequency of occurrence of the categorical variables in the alive and the deceased patients was compared by Chi square test and the numerical data were compared by t-test. Survival estimates of the deceased children were made using Kaplan-Meier method.

## Results

A total of 383 patients of TM on treatment were examined. Their median age was 104 months that ranged from 6-429 months, the male to female ratio was 1.1:1, 328 (86%) were born to consanguineous parents, 246 (64%) were from lower socio-economic group, 85 (22%) had one or more affected siblings, 145 (38%) had hepatomegaly, 191 (50%) had splenomegaly, 42 (11%) had undergone splenectomy and 187 (49%) had never received iron chelation therapy. Height and weight in the 383 patients on treatment showed marked stunting (Fig 1).

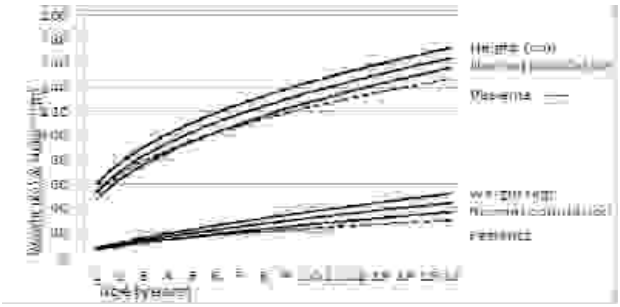

**Fig 1: Comparison of Height and Weight of Male Children of TM Aged 1-16 Years and the Normal Population of the Same Age in Pakistan.[10]**

It was more marked in the male patients and most of them were below the fifth centile of the Pakistani population.[10] Almost all of the patients were underweight that became more obvious with increasing age (Fig 1). The case records of 101 patients who died during treatment and had adequate documentation were studied. Their median age at the time of death was 119 months that ranged from 6-324 months.

Pre-transfusion Hb in the patients on treatment (7.1 g/dL) was higher than in the deceased patients (6.4 g/dL) (p=0.0142). Average interval between transfusions was four weeks in both of the groups. Ferritin level in the patients on treatment (3698 µg/L) was lower than in the deceased patients (4616 µg/L) (p=0.0069) (Table 1).

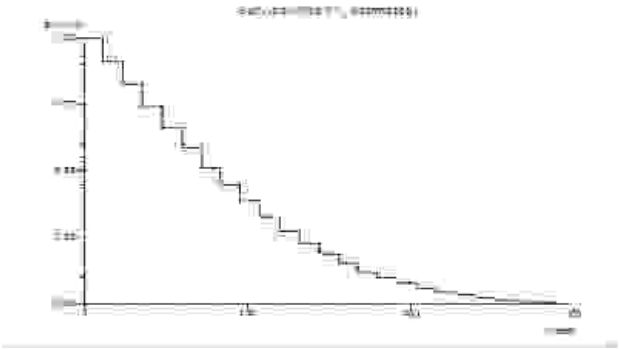

**Fig 2: Kaplan-Meier Survival Estimates of 101 Patients of TM Who Died While on Treatment**

Out of the 383 TM patients on treatment 141 (36.8%) were HCV positive.

Kaplan-Meier median survival estimate of 101 patients of TM who died while on treatment was 84

months (95% CI 79.21-88.8) (Fig 2). Median survival in the 60 male patients was 72 months (95% CI 65.6-78.4) whereas in the 41 female patients it was 84 months (95% CI 76.2-91.8).

## Discussion

Thalassaemia is the commonest single gene disorder in Pakistan.[6] It is estimated that each year over 5000 new children with TM are born and the total number of TM patients may be over 50,000.[8] Treatment of such large numbers of patients is a gigantic task. Unfortunately there are very few public sector institutions that take care of patients with TM. Most of these children are treated at centers run by over 50 NGOs working under the umbrella of Thalassaemia Federation of Pakistan (TFP).[9] In the absence of adequate resources and a mechanism to ensure implementation of treatment guidelines the outcome of treatment at the centers of NGOs is unlikely to improve. There are almost no published data on the outcome of treatment of TM at any of these centers.[9] This study is the first of its kind in Pakistan and it clearly shows a picture of mismanagement. It may not be representative of all thalassaemia treatment centers in Pakistan but it provides a fair idea about the overall scenario.

**Table I: Comparison of Age and other Haematological Parameters between Alive and Deceased Patients of TM**

| Parameter | Alive (n=383) | | | Deceased (n=101) | | | P value |
|---|---|---|---|---|---|---|---|
| | Mean | Median | Range | Mean | Median | Range | |
| Age at diagnosis (months) | - | 8 | 3-160 | - | 7 | 2-180 | 0.1845 |
| Age at 1st Transfusion (months) | - | 6 | 3-240 | - | 8 | 2-180 | 0.1675 |
| Age at examination/death months)<br>All ages:<br>Male:<br>Female: | -<br>-<br>- | 104<br>105<br>104 | 6-429<br>7-429<br>6-429 | -<br>-<br>- | 119<br>111<br>132 | 6-324<br>10-324<br>6-286 | 0.0263<br>0.2022<br>0.0269 |
| Pre-transfusion Hb (g/dL) | 7.1 | - | 2.1-12.8 | 6.4 | - | 3.0-10.8 | 0.0142 |
| Transfusions interval (weeks) | | 4 | 1-156 | - | 4 | 1-16 | 0.1896 |
| Ferritin (µg/L) | 3698 | - | 840-14900 | 4616 | - | 856-14500 | 0.0069 |

The Kaplan-Meir estimates showed median survival of 84 months. Survival was longer in the female patients as compared to the male. The longer survival in female patients has also been reported in a previous study.[11] Although it is a cross-sectional study that is not comparable to longitudinal studies on survival but these data are no match for over 68% TM patients surviving at 35 years in an Italian study.[12] High mortality in these patients appears to be due to chronic under transfusion and iron overload due to lack of iron chelation.[13]

Chronic anaemia with pre-transfusion haemoglobin around 7.0 g/dL is clearly reflected by hepato-splenomegaly in nearly half of the patients and stunting of growth in almost all of them. The main reasons for low pre-transfusion haemoglobin include shortage of blood due to insufficient voluntary blood donations and lack of awareness amongst the parents to get timely blood transfusions. The problems can be addressed by creating awareness. It is important for the treating doctors and the parents to understand that maintaining pre-transfusion haemoglobin above 9.0-10.5 g/dL would not only improve the overall health of the child but would also reduce the annual consumption of blood. When a child with TM remains chronically under transfused erythropoietin is constantly released and stimulates bone marrow. The resulting marrow expansion and hepato-splenomegaly cause haemodilution and worsening of anaemia. This vicious circle can be broken only by correcting anaemia through blood transfusions.[13] The treating doctors should make an extra effort to guide the parents about benefits of high transfusion regimens. The shortage of blood can be met by public awareness through mass media about voluntary blood donations.

Iron overload is an invariable complication of TM. It is mostly caused by regular blood transfusions. The extra iron is carried in plasma through transferrin and when the latter is fully saturated iron travels as non-transferrin bound iron (NTBI).[14] NTBI is preferentially taken up by myocardium, endocrine glands and hepatocytes and is responsible for growth failure, hypogonadism, hypothyroidism and diabetes etc. Myocardial haemosiderosis is another life threatening complication of iron over load that causes conduction defects and cardiac failure.[13] The age at which iron chelation is started is the key factor because starting it late in life is much less effective.[12]

Cardiac complications resulting from iron deposition are the commonest cause of death in TM.[12] The peak mortality observed around ten years of age in this study also appears to be related to cardiac haemosiderosis. Stature of the vast majority of

patients in this study was below 5th centile of the Pakistani population.[10] Growth retardation is common in TM after the first decade of life. It is mostly because of chronic anaemias, endocrinopathy due to iron overload, malnutrition, zinc deficiency, chronic liver disease and psychological stress.[13]A previous study on endocrine abnormalities in 131 children from the same center showed growth hormone deficiency in 30%, hypoparathyroidism in 17.5%, hypothyroidism in 8%, diabetes mellitus in 1.5% and impaired fasting glucose metabolism in 4% of TM patients.[15] In this study growth retardation in the male patients became obvious after the fifth year of life whereas in female patients it was less significant. In the female patients it usually appears after the tenth year of life.[16] Growth retardation at a very early stage appears to be related to chronic anaemia, malnutrition and marked iron overload.

Nearly half of the patients in this study had never used iron chelation and even those who were using it were getting it infrequently and at suboptimal doses. This is also reflected by grossly elevated serum ferritin levels. The high cost and lack of awareness are the two main limiting factors in the wider use of iron chelation. Nearly 2/3rd of the patients in this study are from the lower socio-economic group and are unable to bear the high cost of iron chelation. Low-cost oral iron chelators are an urgent requirement and efforts should be made to facilitate their local production.

Hepatitis C virus (HCV) infection is another problem area in the management of TM in Pakistan. Nearly 40% of the patients in this study were positive for HCV. Previous studies have also shown that 40-50% of the TM patients are HCV positive.[17,18] Poor screening facilities at many centers are the major cause of high HCV prevalence in TM. Many patients keep visiting different treatment centers in search of blood. Since the screening facilities at all centers are not uniform the patients can easily get HCV infection by one wrong transfusion. The HCV point of care testing devices are known to give false negative results due to their low sensitivity.[19,20] These devices are in common use by majority of the blood banks in Pakistan and are partially responsible for the high prevalence of HCV in multiply transfused patients of TM in Pakistan.

Voluntary carrier screening and prenatal diagnosis of thalassaemia are available in Pakistan since 1994.[21] It is unfortunate that most of the children with TM in this study and elsewhere in Pakistan were born during the period when prenatal diagnosis was available. This study also showed that 22% of the TM patients had at least one affected sibling. Lack of awareness amongst the doctors and the parents and high cost of testing are the two major limiting factors in the use of prenatal diagnosis in Pakistan.[22]

Consanguinity, also seen in this study, is an important contributing factor in causation of recessive genetic disorders in Pakistan.[23] The best way to tackle this sensitive issue would be to offer premarital carrier screening in the close family setting or to offer prenatal diagnosis where marriage between two carriers is unavoidable.[6]

## Conclusion

Majority of the patients at a thalassaemia treatment center in Pakistan are chronically under-transfused and have moderate to severe iron overload. This results in severe growth retardation and high mortality around ten years of age. The outcome of treatment can be improved by creating awareness and providing adequate amount of blood and iron chelating drugs.

## Acknowledgements

Authors are grateful to the management of Pakistan Thalassaemia Welfare Society for allowing access to the case records and the children with thalassaemia major being treated at their center.

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ORIGINAL ARTICLE

# Feto-Maternal Outcome in Pregnancy with Anemia

Zubina Adnan[1], Ayesha Nayyar[2], Shaista Nayyar[3], Adnan Mehraj[4]

## ABSTRACT

**Objective:** To determine the frequency of women having pregnancy with severe anemia, its maternal complications and feto-maternal outcome.

**Study Design:** Descriptive Case Series study.

**Place and Duration of Study:** This study was conducted at Obstetrics and Gynecology department, CMH / SKBZ Al-Nayhan Hospital Muzaffarabad from 15th April 2014 to 20th Oct 2014.

**Materials and Methods:** A total of 186 pregnant women admitted for delivery with gestational age ≥ 34 weeks were included in the study. Demographic information regarding age, gestational age and parity was taken on predesigned Performa. Intra-partum and post-partum observations were made for maternal complications like pregnancy-induced hypertension and postpartum hemorrhage. Perinatal complications like low birth weight and APGAR score < 7 at 5 minutes were noted.

**Results:** Mean age of women in the study sample was 26.76±3.36 years. Mean gestational age of women was 38.46±0.63 weeks. Thirty-five (19%) women were diagnosed with anemia among which 12(6.5%) women had severe anemia, 4(2.2%) had mild, and 19(10.2%) had moderate anemia. The frequency of PIH (37.14%), PPH (17.14%), Low birth weight (62.68%) and APGAR score < 7 (60%) was noted higher in anemic patients.

**Conclusion:** Severe anemia during pregnancy significantly increases the chance of adverse perinatal and maternal outcomes in terms of PIH, PPH, Low birth weight and APGAR score.

**Key Words:** *Apgar Score, Fetomaternal Outcome, Induced Hypertension, Low Birth Weight, Pregnancy, Post-Partum Heamorrahge, Severe Anemia.*

## Introduction

Pregnancy is a vital part of a woman's life but it is period of greater risks of different complications for mother and fetus. One of the most prevalent complication is anemia, which is being faced throughout the world. This challenge is more commonly faced in developing countries due to poor nutritional status.[1]

According to the estimates of (WHO), anemia has a prevalence of 23% in developed countries with almost double in developing countries.[2] The average prevalence rate is 56% in developing countries with a great variation with respect to different regions of the world ranging from 35% to 100%.[3]

Some women have iron deficiency anemia at start of pregnancy, which begins to exacerbate due to physiological changes of mother due to pregnancy. This complication begins in first trimester and increases with passage of pregnancy. According to WHO definition the women having hemoglobin level of 11 gm/dl or less is considered as anemic during pregnancy.[4]

There are many risk factors which contribute to pregnancy anemia including iron deficiency which is considered main cause of anemia. Other contributing factors are deficiency of B12 or thalassemia trait, which are also a very common causes of anemia. The prevalence of anemia is very high especially in third trimester and have a very significant adverse impact on maternal health during pregnancy and fetal outcome.[5]

Very severe adverse consequences are related with anemia in pregnancy for mother and fetus. These adverse effects are not bounded during pregnancy, neonatal and infant period only, but this increases

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*Funding Source: NIL; Conflict of Interest: NIL*
*Received: Oct 06, 2017; Revised: May 28, 2018*
*Accepted: June 01, 2018*

the chances of non-communicable diseases during adulthood as well. Studies have also shown relationship of low birth weight in next generation with anemia during pregnancy. Anemia is preventable complication of pregnancy and it can easily be diagnosed and treated with easily available techniques and tools which are affordable and can be implemented in even primary health care settings. [6,7]

The frequency of maternal complications is very high among sever anemic pregnant women in comparison with normal pregnant women. In a study it was noted that in anemic pregnant patients the maternal complications like pregnancy induced hypertension (36% vs. 10%), infections (16% vs. 5%) and Post-Partum Heamorahhge (14% vs. 5%) was significantly higher in contrast to normal pregnant women. [5] Similarly the fetal outcome like low birth weight (57.62% vs. 42.37%), and apgar score < 7 (57.57% vs. 42.42%) was also significantly distressed in Anemic patients. Some studies have shown significantly very high difference in low birth weight (64% vs 10%) in anemic and non-anemic pregnant women.[8] Antenatal iron-folic acid supplementation reduces low birth weight and preterm in both developed and developing country settings.[9]

Studies conducted in Pakistan show a high percentage of anemia during pregnancy so this study had been planned to find out the adverse effects of anemia during pregnancy on maternal complications (like pregnancy induced hypertension and post-partum hemorrhage) and fetal outcome in terms of low birth weight in our local target population in comparison with normal controls. The aim of this stud is to determine the frequency of women having pregnancy with severe anemia, its maternal complications and feto-maternal outcome.

## Materials and Methods

In this Descriptive Case Series study, a total of 186 pregnant women were included from Obstetrics and Gynecology Dept. CMH / SKBZ Al-Nayhan Hospital Muzaffarabad. This study was conducted in a period of seven months from 15th April to 20th Oct 2014. The sample size was calculated by using WHO sample size calculator taking Confidence level of 95 %, Anticipated population proportion P = 14%[2], and Absolute precision required = 5%.

All the women were recruited for the study by non-probability consecutive sampling method. The study was started after taking formal written permission from hospital ethical committee. Informed written consent was taken from all patients who fulfilled the inclusion and exclusion criteria. All women admitted for delivery with gestational age ≥ 34 weeks were included in the study. Pregnant women with known history of thalessemia and sickle cell anemia, women with ante-partum hemorrhage and no available previous report of Hb were excluded from the study.

Demographic information regarding age, gestational age and parity were taken on predesigned Performa. Blood sample was taken from each patients and sent to the laboratory for hemoglobin level. The WHO's anaemia classification and categorization was adopted for functional definition of haemoglobin conditions; anaemic (serum Hb 5-11g/dL), and non-anaemic (serum Hb>11g/dL) the anemic group was further divided into Mild (9-10.9 g/dL), Moderate (7-8.9 g/dL) and severe anemia (< 7 g/dL). Intra-partum and post-partum observations were made for maternal complications like hypertension and postpartum hemorrhage. Perinatal complications like low birth weight and APGAR score < 7 at 5 minutes were noted.

All data was analyzed using SPSS Version 21. Mean and standard deviation was calculated for numerical variables. Frequency and percentages were calculated for categorical variables. Effect modifiers like age, parity and gestational age was controlled by stratification. Post stratification Chi square test was applied to compare pregnancy induced hypertension, PPH, Mode of delivery, APGAR score (< 7) at 5 minutes, low birth weight in anemic pregnant and non-anemic pregnant women. P-value <0.05 was considered significant.

## Results

In this sample of 186 women the mean age was 26.76±3.36 years with minimum and maximum age of 22 and 37 years respectively. Parity status showed that most of the 62(33.3%) women had 2 children, followed by 61(32.8%) women with parity 3, and 21(11.3%) women had parity 1, there were 17(9.1%) women whose parity was 0, and 16(8.6%) & 9(4.8%) women had parity 4 & 5 respectively. Mean gestational age of women was 38.46±0.63 weeks, ranging from 37 to 40 weeks. In our study sample,

35(19%) women diagnosed with anemia. As per WHO criteria there were 151(81.2%) women who had normal Hb level, among anemic women 4(2.2%) had mild, 19(10.2%) had moderate and only 12(6.5%) women had severe anemia. (Fig 1).

There were 29(16%) women with pregnancy induced hypertension. Post-Partum heamorahhge was seen in 17(9%) women. There were 62(33%) fetus who had low birth weight. APGAR score <7 was observed in 84(45%) babies. (Fig 2).

In our study sample 35 women were anemic and among these 13 (37.14%) women had pregnancy induced hypertension. While Among 151 non-anemic women only 16 (10.59%) had pregnancy induced hypertension. A statistically significant (p-value=0.000) association was present between anemia and pregnancy induced hypertension. Among anemic women 6 (17.14%) suffered from Post-Partum Hemorrhage, while among non-anemic only 11 (7.29%) suffered from Post-Partum Hemorrhage. Which shows statistically insignificant *(p-value=0.068)* association between anemia and Post-Partum Hemorrhage. (Table I).

The results showed that among anemic women the fetal outcome was significantly poor as compared with non-anemic women. Among women with anemia 22 (62.86%) had low birth weight babies as compared to non-anemic mothers in which 40 (26.49%) had low birth weight babies showing a statistically significant *(p-value=0.000)* association between mother's anemic status and low birth weight babies. Similarly, among babies of anemic mothers, APGAR score <7 at 5 minutes was observed in 21 (60%) babies as compared to 63 (41.72%) babies of non-anemic mothers who had APGAR score <7 at 5 minute. There was no statistically significant *(p-value=0.05)* association between anemia and APGAR score <7 at 5 minutes. (Table II).

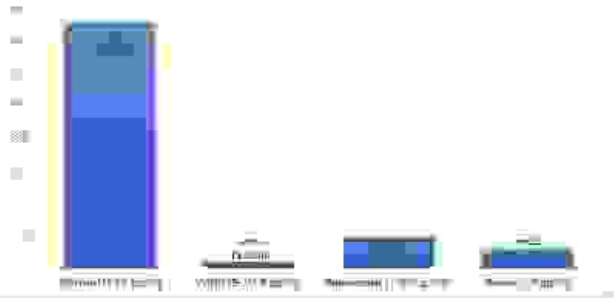

**Fig 1: Bar Chart Showing Frequency Distribution of Different Categories of Anemia**

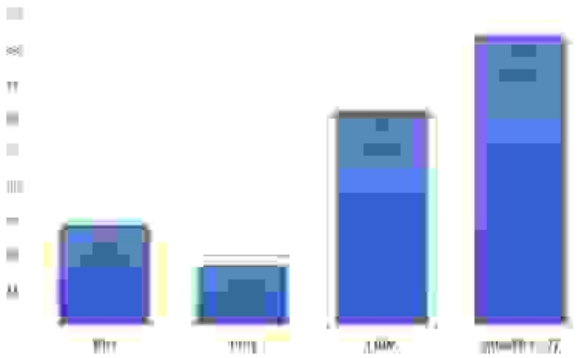

**Fig 2: Bar Chart Showing Distribution of Maternal Complications & Fetal Outcome**

**Table I: Maternal Complications in the Cases of Pregnancy with Anemia (n= 35)**

| Complications | Anemia | | Total | P-Value |
|---|---|---|---|---|
| | Pregnancy with Anemia (n=35) | Pregnancy with Normal Hb (n=151) | | |
| Pregnancy induced Hypertension | 13(37.14%) | 16(10.60%) | 29 | 0.000 * |
| Post-Partum Hemorrhage | 6(17.14%) | 11(7.28%) | 17 | 0.068 ** |

* Difference is significant at 5% level of significance

** Difference is not significant at 5% level of significance

**Table II: Perinatal Complications like Low Birth Weight and Apgar score < 7 At 5 Minutes in Pregnancy with Anemia**

| Complications | Anemia | | Total | P-Value |
|---|---|---|---|---|
| | Pregnancy with Anemia (n=35) | Pregnancy with Normal Hb (n=151) | | |
| Low Birth Weight | 22(62.86%) | 40(26.49%) | 62 | 0.000 * |
| APGAR Score (<7) | 21(60%) | 63(41.72%) | 84 | 0.050 ** |

* Difference is significant at 5% level of significance

** Difference is not significant at 5% level of significance

## Discussion

Anemia is a pregnancy complication which can be prevented easily and fetal and maternal morbidity and mortality related to anemia can be minimized.

The prevalence of anemia is very high with great variations in different regions around the globe. It varies from 15% in western countries to 75% in developing countries of Africa and Asia. In developing countries its prevalence ranges from 33% to 75% in different regions. [5,10,11]

The incidence of iron deficiency anemia in Pakistan has been report quite high by different studies. A high incidence of 50% was found despite routine iron therapy in a study conducted in Karachi. Similar high incidence of iron deficiency anemia among pregnant women has been reported from other developing countries of Africa and India. [12-14]

Women having anemia during pregnancy often feel body aches and fatigue. There are many causes of anemia in Pakistan. The main contributing causes are poor economic conditions, repeated pregnancies with short interval, gender bias, worm infestation and lack of health seeking behavior. The major causative factor for anemia in Pakistan is iron deficiency during and at the start of pregnancy. [15] The anemia a common pregnancy complication increases the risk of low birth weight and intra uterine growth retardation.[16]

In this study 35(19%) women had anemia. As per WHO criteria 151(81.2%) women had normal Hb level, 4(2.2%) had mild, 19(10.2%) moderate and only 12(6.5%) women had severe anemia.

Two local studies one from Sindh and the other from Multan reported (52%) of patients presented with moderate anemia, 12% with severe anemia requiring blood transfusions, and 36% of pregnant women were mildly anemic. 3 While study from Multan showed severe anemia in 8%, mild anemia in 44% and moderate anemia in 48% of patients.[12] This prevalence of anemia in this study 19% was comparable to studies conducted in Trinidad and Tobago (15.3%), Thailand (20.1%), Zurich (18.5%), Hawassa (15.3%), and Gondar town (22%).[17-21]

In this study pregnancy induced hypertension was present in 37.14% women who were anemic and present in 10.60% in women who were not anemic. Post-Partum Heamorahhge was seen in 17.14% women who were anemic and in 7.28% women who were not anemic. Among 62.86% anemic mothers the babies were low weight while 26.49% among non-anemic women babies had low birth weight. There were 60% women whose fetus had APGAR score <7 at 5$^{th}$ minute and 41.72% non-anemic women's babied had APGAR score <7 at 5minute.

Ram Hari Ghimire in his study explored the association between anemia and maternal and perinatal complications. In his results he reported that pregnancy induced hypertension and Post-Partum Hemorrhage was significantly high is women who were anemic (PIH =36%, PPH= 14%) as compared to (PIH=10%, PPH=5%) in non-anemic women. Results regarding fetal complication among anemic and non-anemic mothers showed that APGAR score <7 was 18% and 5% among anemic and non-anemic mothers and low birth weight of fetus was observed as 22% and 9% in anemic and non-anemic mothers.[5]

Lone *et al*, in a multivariate analysis of their study population showed that the risk of low birth weight babies in the anemic population was 1.9 times higher.[22] A local study from Rawalpindi reported the number of low birth weight infants (64%) was highly significant in the anemic mothers than the non-anemic (10%).[23]

The anemia during pregnancy increases the chances of obstetric haemorrhage, infection rate and obstetric shock and trauma due to labour complication etc. This increases the chance of maternal mortality by five times as compared with non-anemic women. Similarly, in severe anemic patients having hemoglobin less or equal to 6 gm/100ml the chance of high cardiac output failure rises significantly specifically in cases of hypertension and pre-eclampsia. These complications are less likely to occur in patients having mild or moderate anemia. But in these patients having mild or moderate anemia the child can come up with some speech learning or behavioral problems.[24]

Anemia in pregnancy has been found as a significant contributing risk factor for adverse perinatal and maternal outcomes. Special attention should be given to minimize the anemia especially during pregnancy.

## Conclusion

In this study anemia was observed in 19% pregnant women, in which 2.2% women had mild, 10.2% moderate and 6.5% women had severe anemia. There were significantly higher maternal complications among anemic women as compared

with non-anemic women. The fetal complications that is Low Birth Weight and APGAR <7 at 5 minute has also been found significantly associated with maternal anemia.

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ORIGINAL ARTICLE

# Clinico-Pathological Study of Hysterectomy at Pak Red Crescent Medical and Dental College

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**ABSTRACT**

**Objective:** To determine the common pattern of leisons identified in hyterectomy specimens and to correlate the histopathological findings with clinical indications.

**Study Design:** A Descriptive study.

**Place and Duration of Study:** Study was conducted in the Department of Obstetrics and Gynaecology of Red Crescent Medical College Hospital from 1st July 2015 to 1st July 2017.

**Materials and Methods:** In the period between 1st July 2015 to 1st July 2017 data including Age, Parity, Presenting complaints and indication for hysterectomy was obtained from patients. The type of hysterectomy was also reported. Specimens were preserved in 10% Formalin. Histopathology results of all hysterectomy specimens were collected.

**Results**: During two year study a total of 100 hysterectomies were performed. The patient age ranged between 35-60 years with an average of 45 years old and peak parity was 4-8. The most common presenting complaint was menstrual irregularities followed by lower abdominal pain. Hysterectomy with bilateral salpingo-oophrectomy was performed in 75% of cases. Hysterectomy alone was performed in 15% and in 10% of cases vaginal hysterectomy was performed. In 35% cases the indication of hysterectomy was fibroid uterus followed by dysfunctional uterine bleeding in 15% of cases. The commonest histopathological diagnosis made was chronic cervicitis i.e in 42% cases, which was an incidental finding followed by fibroid uterus in 40% of cases.

**Conclusion**: This study confirms that benign diseases are more common than their malignant counterparts and the most common pathology identified is chronic cervicitis.The clinical and histopathological correlation is 100% in case of leiomyoma, cervical and endometrial polyps. Seventy four cases were correlated clinically with histopathological diagnosis.

**Key Words:** *Leiomyoma, Menorrhagia, Total Abdominal Hysterectomy, Vaginal Hysterectomy.*

## Introduction

Abdominal hysterectomy means complete removal of uterus through abdominal route. Hysterectomy is the most commonly performed major gynaecological surgery throughout the world. It is performed in 560 / 100,000 women per year in the US[1] and 414 / 100,000 women per year in Finland.[2] Hysterectomy rate varies from place to place depending upon patient and clinician related factors.[3]

Hysterectomy is a successful operation in terms of symptom relief and patient satisfaction. It provides definitive cure to many diseases involving uterus as well as adnexa, eg, fibroids, DUB, adenomyosis, endometriosis, pelvic inflammatory disease, pelvic organ prolapse and malignancy.

Histopathological examination of surgical specimens carries ethical, legal, diagnostic and therapeutic significance. A variety of conditions in gynecological practice require removal of a uterus that may show no gross or microscopic pathology when examined by the pathologist. Removal of a normal uterus may be indicated and permitted in the treatment of ovarian, fallopian tube and vaginal cancer, pelvic inflammatory disease, endometriosis, DUB, pelvic organ prolapse, pelvic pain and pelvic tuberculosis.

The diagnostic value of histopathological examination is well explained in patients with genital cancer where adjuvant treatment is dependent upon grade and extent of invasion of disease. Similarly diagnosis of adenomyosis is only established by

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*Funding Source: NIL; Conflict of Interest: NIL*
*Received: Oct 27, 2017; Revised: Feb 07, 2018*
*Accepted: Feb 12, 2018*

histopathological examination, while DUB is a diagnosis of exclusion. Conversely, many patients may be suspected of having a malignancy on pre-operative assessment e.g. those with postmenopausal bleeding and histopathological examination may aid to rule out this suspicion.

The purpose of this study was to correlate various indications of abdominal hysterectomy with the histopathological findings of the specimens, thus determining the percentage of the pre-operative clinical diagnoses that were confirmed on histopathological examination. We also wanted to determine the frequency of unexpected disease, thus high lighting the need for subjecting each specimen for histopathological examination. Failing this may result in sub-optimal care or treatment and over treatment of certain diseases, in particular the malignant conditions.

## Materials and Methods

This descriptive study was conducted in the department of Gynae/Obs Department at Red crescent teaching hospital. It was retrospective analysis of 100 patients who had hysterectomy as an elective procedure had specimen including the appendages examined histopathologically over 2 year between 1st July 2015 and 1st July 2017. Study protocol was approved by institutional ethical committee.

The study included all women undergoing planned abdominal hysterectomy. Data was recorded on proformas, including demographic characteristics and clinical features. Only one dominant diagnosis was considered and documented as an indication for the procedure. Hysterectomy specimens were saved in 10% formalin and sent for histopathological examination. Abdominal hysterectomy for uterine malignancies and for emergency conditions e.g obstetrical haemorrhage were excluded. Histopathology reports were analysed and compared with the clinical indications of surgery. Data were analysed using SPSS version 22. Frequency and percentage were calculated for categorical variables.

## Results

The age group of patients ranged from 35 to 65 years with mean age of 50 years (Table I). 50 % of patients belonged to the age group between 45 -55 yrs. 35% between 41 - 44 yrs . 8 % were <40 years . 7% were >55 yrs. 63 .8% had parity of >5 . 4 % were nulliparous."

In this study 100 cases of hysterectomy were studied at Red Crescent Medical College Dina Nath. The various indications for hysterectomies are depicted in Table I. All were for benign indications. The most common indication for hysterectomy was leiomyoma (35%). This was followed by adenomyosis (15%) and dysfunctional uterine bleeding (15%). The most common clinical presentation was menorrhagia. Some of the cases presented with dysmenorrhea, low backache and dyspareunia. The most common type of hysterectomies were Total Abdominal hysterectomy with Bilateral saplingo-oophrectomy followed by Total Abdominal hysterectomy. Histopathology reports of all hysterectomies were reviewed (Table II).

Seventy four cases were correlated clinically with histopathological diagnosis. Twenty three had a different diagnosis than the clinical one (Table III).

**Table I: Clinical Indication for Hysterectomy and Age Distribution of Study Population (N= 100 )**

| Clinical Indication | No of Cases | Percentage |
|---|---|---|
| Uterine Leiomyoma | 35 | 35% |
| Dysfunctional Uterine bleeding | 15 | 15% |
| Endometrial Polyp | 5 | 5% |
| Adenomyosis | 10 | 10% |
| Endometrial Hyperplasia | 15 | 15%pp |
| Uterine Prolapse | 7 | 7% |
| Chronic Pelvic Pain | 1 | 1% |
| Post Menopausal Bleeding | 2 | 2% |
| Ovarian Cyst | 2 | 2% |
| Cervical Polyp | 3 | 3% |
| **Age in Years** | **No of Cases** | **Percentage** |
| 35-40 | 8 | 8% |
| 41-44 | 35 | 35% |
| 45-55 | 50 | 50% |
| >55YRS | 7 | 7% |

**Table II: Pattern and Frequency of Uterine Histopathology Identified in 100 Hysterectomy Cases**

| Histopathological Diagnosis | Number | Percentage |
|---|---|---|
| Leiomyoma | 40 | 40% |
| Adenomyosis | 7 | 7% |
| Endometrial Hyperplasia | 20 | 20% |
| Endometrial Polyp | 5 | 5% |
| Chronic Cervicitis | 42 | 42% |
| Benign Serous Cyst Adenomas | 2 | 2% |
| Cervical Polyp | 4 | 4% |
| Atrophic Endometrium | 7 | 7% |

**Table III: Histopathological Reports Inconsistent with Pre- Op Diagnosis**

| Disease | Pre op Diagnosis | Histopathological Report |
|---|---|---|
| Dysfunctional Uterine Bleeding | 15 | 7 ...DUB<br>8..chronic cervicitis |
| Adenomyosis | 10 | 7..adenomyosis<br>3..fibroid |
| Endometrial Polyp | 5 | 8 |
| Fibroid Uterus | 35 | 40 |
| Endometrial Hyperplasia | 15 | 11...endometrial hyperplasia<br>3.....endometrial polyp<br>1.....chronic cervicitis |

## Discussion

Hysterectomy offers a definitive cure for women with heavy bleeding associated with fibroids who have completed child bearing.[4] Although expensive in the short Term and not without risk[5],it may provide a cost effective option for women who are less likely to benefit from more conservative approaches. The Abdominal route has been most commonly used for large uteri although the vaginal route can be used by experienced operators,[6] usually after GnRH pre treatment.

A large cohort study of 37,298 hysterectomies performed in UK for benign indications reported an operative complication rate of 3.5 %, A post operative complication rate of 9% and an overall mortality rate of 0.38 per 1000.[7] Mortality was 0.25 per 1000 in women undergoing hysterectomy for menstrual problem. The role of subtotal hysterectomy remains unclear. Women undergoing subtotal hysterectomy should be warned about a 7% risk of occurrence of ongoing menstrual bleeding.[8] Hysterectomy may have long term implications for bladder function; a systemic review[9] estimated a long term increase in the odds of developing urinary incontinence following hysterectomy. A recent Scottish study found an increased risk of pelvic floor or urinary incontinence surgery following hysterectomy for heavy menstrual bleeding compared with endometrial ablation.[10]

Few studies have been done in our community regarding histopathological analysis of hysterectomy specimens and relationship between the preoperative clinical diagnosis and histopathological diagnosis[11]The commonest type of surgical resection was Total Abdominal Hysterectomy with Bilateral salpino-oophrectomy (TAH with BSO) (58%)followed by TAH (Total Abdominal Hysterectomy)(38.3%).[12] The aim of our study is to analyse the common pathologies identified in hysterectomy specimens and to correlate the findings with the clinical indications. The commonest estimated age range of hysterectomy in our study is 41-50 years which is similar to that reported by Gousia Rahim Rather and Prveen S Tayyab S.[13,14] The commonest presenting complaints in our study were menorrhagia followed by polymenorrhagia. The Rashmi Verma study also revealed that menstrual disturbance was the most important indication for hysterectomy.[15] This was also seen by Shergill SK and Riffat Jaleel, who found that abnormal menstrual flow was the commonest complaint in 66% of cases.[16,17] In this study main indication for hysterectomy was leiomyoma 35 (35% ) cases. Similar is found in studies by Sumatra et al and Leung PL followed by endometrial hyperplasia (16%), DUB(10%)cases.[18,19]

Only few studies have compared pre-operative clinical diagnosis with histopathology of hysterectomy specimens. We have found that 74% of our pre operative diagnosis were confirmed on histopathology like fibroid uterus, uterine polyps and utero vaginal prolapse and cervical polyp have 100% diagnosis confirmed on histopathology and same was reported by G Gupta etal.[20]Chronic cervicitis is an extremely common condition in adult female, at least at the microscopic level, chronic cervicitis was commonest finding in our study 42% which was an incidental finding. Same results were obtained by Ghousia Rahm Rather etal.[13] Leiomyoma was the second most common histopathological diagnosis. Fibroid was most common indication for hysterectomy.[21] 35 cases has preoperative indication of Leiomyoma in our study and was confirmed in 40 cases (100%). Adenomyosis was an indication for hysterectomy in 10 cases. Adenomyosis is rarely diagnosed pre operatively and is still largely under diagnosed as it has no specific symptoms of its own[22,23] In our study only 7 cases were diagnosed. The clinicopthological correlation between preoperative and histopathological examination was more than

90% especially in benign conditions in Dr Vandana study.[24]

Hysterectomy is one of the most frequently performed major surgical procedures in women worldwide.[25] There is high incidence of benign conditions in our study .Strong clinicopathological correlation has been found in the cases. Seventy four cases were correlated clinically with histopathological diagnosis. The result of the study is in concordance with the previously published data as regards to the commonest pathologies identified in hysterectomies and the commonest surgical route for hysterectomy.

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ORIGINAL ARTICLE

# Blowing Balloons, A Novel Way for Reducing Stress and Improving Pulmonary Function Tests

Noman Sadiq[1], Humaira Fayyaz Khan[2], Arif Siddiqui[3]

**ABSTRACT**

**Objective**: To determine the effect of blowing balloons on pulmonary function tests and perceived level of stress in medical students.

**Study Design:** Pretest-posttest quasi-experimental study.

**Place and Duration of Study:** This study was conducted at Department of Physiology, Islamic International Medical College from 1st April 2016 to 31st May 2016.

**Materials and Methods:** Sixty students of Islamic International Medical College participated in the study after fulfilling the DASS (Depression Anxiety Stress Scale) Questionnaire Performa. Pulmonary function tests of the participants were measured according to the standard guidelines published by American Thoracic Society. This was recorded by using Power lab and the data was then analyzed by using software, Lab chart 8 pro. The participants were then subjected to a supervised blowing balloon exercise using commercially available balloons. After completion of exercise, pulmonary function tests of the participants and the DASS Questionnaire was filled again by the participants.

**Results:** After blowing balloon exercise DASS Score of the participants was reduced from 21.87 $\pm$ 2.01 to 13.41 $\pm$ 4.29 ($p < 0.001$). Tidal volume was increased from 517.72 $\pm$ 48.57 ml to 638.65 $\pm$ 86.02ml ($p < 0.001$), Vital capacity was increased from 3.51 $\pm$ 0.56 L to 4.83 $\pm$ 0.77 L ($p < 0.001$) and the ratio of Forced Expiratory Volume in one second to the Forced vital capacity was improved from 89.36 $\pm$ 4.54 % to 92.66 $\pm$ 4.27 % ($p < 0.001$) after blowing balloon exercise.

**Conclusion:** Blowing balloon exercise is an effective way of improving pulmonary function tests and alleviating stress levels in medical students.

**Key Words:** *Depression Anxiety Stress Scale, Lab Chart, Pulmonary Function Tests, Stress.*

## Introduction

Stress is defined as the inability of an individual to respond sufficiently towards a physical, emotional or mental demand. It affects Psychological wellbeing of students, which is an area of increasing concern worldwide.[1,2] Medical students are more prone to experience level of depression, anxiety and stress. Studies have shown that low level of stress was present in 7.5%, moderate level of stress was present in 71.67%, and high level of stress was present in 20.83% of the medical students.[3,4] Perceived level of stress in an individual can be measured by using validated and reliable DASS (Depression Anxiety Stress Scale) questionnaire. On the basis of the score an individual can be labeled as stress free or suffering from mild, moderate, severe or extreme severe stress.[5]

Pressure of Academic workload, competitive environment, financial pressure, inadequate relationship with peers and teachers, physical illness, worries about future, poor food quality, overcrowding of students in hostel rooms, minimal opportunities for the students to relax are among the major stressors faced by the medical students.[6-8] Inability of the medical students to cope with these stressors leads to poor scholastic performance, interpersonal relationship difficulties, reduction in attention, concentration and decision making skills. It causes dropout from medical course and even

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*Funding Source: NIL; Conflict of Interest: NIL*
*Received: Dec 09, 2017; Revised: May 17, 2018*
*Accepted: May 24, 2018*

development of suicidal behavior in these medical students.[9]

Students employ many techniques to cope with stress, for example discussion with seniors, use of internet chat and cell phone texting[5], attending meditation, hypnosis, group discussions, counseling sessions[10],cycling, jogging and yoga.[11,12]

The main goal of breathing exercise is to improve pulmonary function tests (PFTs) which provides measurable feedback about the function of the lungs by assessing flow rates, lung volumes, and capacities.[13,14] The practice of breathing exercises can decrease stress and strain through the reduction in sympathetic activity and enhancement of parasympathetic activity. Pattern of discharge via vagal neurons is influenced by the respiratory rhythm of the body. Vagal neurons receive powerful excitatory input from pulmonary stretch receptors as a consequence vagal nerve activity i.e. parasympathetic activity is increased.[14] Breathing exercises include various forms of yoga exercise, slow lip pursuing exercise, blowing balloon exercise etc.[15]

Blowing balloon exercise has been documented in improving pulmonary function tests of smokers, for treating obstructive sleep apnea syndrome, in rehabilitation of stroke patients and in oral gymnastics.[16,17] The blowing balloon exercise strengthen the respiratory muscles and results in the increase of Tidal Volume (TV), Vital Capacity (VC), Forced Vital Capacity (FVC), Forced Expiratory Volume in one second (FEV1) and Forced Expiratory Volume in one second to the ratio of Forced Vital Capacity (FEV1/FVC).[18]

To combat stress encountered in a medical school environment, students are advised to get themselves involved in stress reducing activities like counseling sessions, physical exercises including gym, running, swimming, yoga etc.[10,11] However, all these activities are time consuming, a major barrier for medical students to practice them regularly.

In order to enhance students' performance and academic achievement in inherently stress full medical college environment, provision of cost effective, less time consuming fun activities for coping stress is the need of hour. The role of blowing balloons in reducing stress has not been studied yet. The objective of the current study was to assess the effectiveness of blowing balloon therapy in relieving the perceived level of stress in medical students.

## Materials and Methods

This pretest-posttest quasi-experimental study was conducted at Department of Physiology, Islamic International Medical College from 1st April 2016 to 31st May 2016.

A total of sixty participants were selected by simple random sampling through balloting method with an equal contribution from both genders. The study was conducted after getting approval from institutional ethical review committee. Initially DASS Questionnaire was distributed and filled by 200 students of 1st and 2nd year MBBS after written informed consent. Healthy subjects having age between 18-25 years, who scored 19 – 25 on DASS Performa (having moderate level of stress) were labelled as eligible. Subjects who were smokers, having oral lesions, using bronchodilator medicines or practicing any form of breathing exercise were not included.

Study participants were asked to reach physiology department between 8.00 - 9.00 am. They were made to relax for five minutes and then height, weight and blood pressure of the participants were recorded.

Pulmonary function tests comprising on tidal volume, vital capacity, forced vital capacity, forced expiratory volume in one second and the ratio of forced expiratory volume in one second to the forced vital capacity of the participants were measured by performing Spirometry by using Power lab 4/25T. Pulmonary function tests of the participants were measured according to the standard guidelines published by American Thoracic Society and European Respiratory Society.[19] Recorded data in the Power lab was analyzed by using Lab chart 8 pro software.

The study participants were subjected to a supervised blowing balloon exercise for a period of three days a week for six consecutive weeks using commercially available balloon.[15] During blowing balloon exercise participants were asked to obtain an upright position, breathe in maximum, maintain their state for one second then breathe out in balloon mouth. Then participants were requested to close the balloon mouth with their fingers. Participants were then again asked to breathe in

maximum, maintain their state for one second and breathe out in balloon. This completed their one mini set. They were asked to perform three mini sets in order to complete one set. A total of three sets were performed by them in a day.

To prevent fatigue participants were provided with a 2-3 minute break between consecutive sets. Subjects were advised to stop exercise whenever they felt dizzy. To prevent valsalva subjects were advised to not hold their breath for more than five seconds after they breathed out. After completion of blowing balloon exercise, pulmonary function tests of the participants were again measured by performing Spirometry through powerlab. Study participants were again provided with the DASS Questionnaire Performa and were requested to fill it.

The parametric data was analyzed in term of Mean ± Standard deviation by using statistical software SPSS version 21. Difference in the DASS score and Pulmonary Function Test of the study participants before and after the blowing balloon exercise was determined by using Parametric Paired t test. p value of <0.05 was considered as statistically significant.

## Results

Our study included sixty participants, all of the participants completed all the sessions of blowing balloon exercise and there was no drop out. Difference in the DASS score and pulmonary function test indices before and after performing blowing balloon exercise were compared.

The mean age of the participants was 20.5 ± 1.2 years. Descriptive statistics of the study participants are given in table I.

Table II shows the pulmonary function test indices.

**Table I: Descriptive Statistics of Study Participants (N= 60)**

| Characteristics | Participants N = 60 Mean ± S.D |
|---|---|
| Age ( years) | 20.50 ±1.24 |
| Height (cm) | 167.98 ± 9.29 |
| Weight (Kg) | 61.31± 11.94 |
| BMI ( Kg/$m^2$) | 21.67 ± 3.70 |
| Systolic Blood Pressure (mmHg) | 118.17 ± 4.69 |
| Diastolic Blood Pressure (mmHg) | 77.83 ± 6.13 |

All the indices of pulmonary function test i.e. tidal volume TV, VC, FVC, FEV1 and FEV1/FVC were significantly improved after performing blowing balloons exercise.

Table III shows the DASS Score of study participants. The mean DASS Score of the participants was 21.87 ± 2.01 before the commencement of blowing balloon exercise. This score was significantly decreased to the value of 13.41 ± 4.29 after performing six weeks blowing balloon exercise.

**Table II: Comparison of Pulmonary Function Tests before and after Blowing Balloon Exercise**

| Pulmonary Function Test Parameter | Values before Blowing Balloons Exercise Mean ± S.D N = 60 | Values After Blowing Balloons Exercise Mean ± S.D N = 60 | p value |
|---|---|---|---|
| Tidal volume ( milliliters ) | 517.72 ± 48.57 | 638.65 ± 86.02 | .000* |
| Vital capacity (liters) | 3.51 ± 0.56 | 4.83 ± 0.77 | .000* |
| Forced Vital Capacity (liters) | 3.09 ± 0.57 | 4.45 ± 0.78 | .000* |
| Forced Expiratory Volume in one second (liters) | 2.76 ± 0.54 | 4.13 ± 0.77 | .000* |
| Forced Expiratory Volume in one second / Forced Vital Capacity ( percentage) | 89.36 ± 4.54 | 92.66 ± 4.27 | .000* |

*p value < 0.05 is significant

**Table III: Comparison of Participant's Dass Score before and after Blowing Balloon Exercise**

| Measuring variable | Participants before Blowing Balloons Exercise Mean ± S.D | Participants after Blowing Balloons Exercise Mean ± S.D | p value |
|---|---|---|---|
| DASS Score | 21.87 ± 2.01 | 13.41 ± 4.29 | .000* |

*p value < 0.05 is significant

## Discussion

Medical colleges are notoriously famous for being stressful and this stress often exerts negative effect on the physical, psychological and academic

wellbeing of the students. The current study was conducted with an aim to find an effective way to alleviate stress among the students of medical college.

The current study shows that blowing balloons for six weeks resulted in significant improvement in TV, VC, FVC, FEV1 and FEV1/FVC indices of pulmonary function tests. Moreover blowing balloons significantly reduced the perceived stress score of medical students.

Findings of the present study are in accordance with the study conducted by Antte Kjellgren et al which showed that practice of yogic breathing exercise for 3 hours/day for 6 days/week for seven weeks resulted in reduction of depression, anxiety and stress levels in students. The compliance rate of yogic exercise was 94.17%.[20] However, the participants of the present study used blowing balloons exercise as a stress coping strategy and performed exercise for ten minutes in a day for 3 days/week for six consecutive weeks and the compliance rate was 100%.

Results of our study also support the findings of study conducted by Shapiro et al which reported that use of yoga, meditation and group discussion as stress coping strategy for 2.5hours/week for seven weeks resulted in significant reduction in stress levels of students. The compliance rate of the study participants were 93.5%.[11] In present study we used blowing balloon exercise as a stress coping strategy instead of yoga, meditation and group discussion. The compliance rate of our participants was 100%. Moreover our participants performed exercise for just ten minutes in a day for 3 days/week for six consecutive weeks instead of long sessions done by the participants of Shapiro, et al.

During blowing balloons in order to keep balloon inflating, the resistance of inflated balloon rubber is overcome by forceful expiration this results in the active contraction of Rectus abdominus, Transversus abdominus and intercostal muscles. Expiratory muscles get trained through this exercise.

Kim, J et al conducted a study on young adult smokers which concluded that blowing balloon exercise resulted in a significant improvement in several parameters of pulmonary function tests (VC, FVC, FEV1, FEV1/FVC).[15] Our study also concluded that blowing balloon exercise resulted in a significant improvement in pulmonary function tests including tidal volume. However, in the study by Kim, J et al, tidal volume was not measured. Results of this study is also in accordance with a study conducted by Jun, H et al, which showed that blowing balloon exercise for four weeks by elderly smokers resulted in an increase in VC, FVC, FEV1, FEV1/FVC, PEF.[21] In comparison with these studies this study was conducted on stressed otherwise healthy nonsmoker medical students.

V Blessy et al conducted a research on healthy subjects and performed Bhastrika pranayama exercise for five times a day for three months. In this exercise participants took slow and deep inhalation followed by forceful exhalation from the nose, resulted in improvement in TV, FVC, FEV1/FVC of study participants ($p < 0.02$).[22] Pulmonary function indices (TV, VC, FVC, FEV1, FEV1/FVC) of the participants of this study also showed significant improvement ($p < 0.000$). However, the current study participants performed blowing balloon exercise in which exhalation was done against a resistance which could be the possible reason for the marked improvement of pulmonary function tests as compared to the improvement in pulmonary function tests due to Bhastrika pranayama exercise used by V Blessy et al in which simple forceful exhalation was done.

A study conducted by Mauch AD on young healthy adults showed that practicing of Iyengar yoga for two weeks lead to an increase in TV, FVC FEV1/FVC of participants ($p < 0.01$). Participants were asked to take deep breath and then they were instructed to hold breath for few seconds in triangular standing posture.[23] Our blowing balloon exercise also resulted in the significant improvement in pulmonary function test of the participants however our exercise can be performed at without any posture restriction.

In spite of the fact that the study was conducted under the available resources and facilities the exercise period could have been extended from 6 weeks up to 6 months. In future effect of blowing balloon exercise can be compared with other stress coping strategies like yoga, cycling or treadmill exercise etc.

## Conclusion

Our study concluded that blowing balloons exercise improves pulmonary function tests and reduces the

level of stress in medical students. It is a cost effective and less time consuming method which can be used by the students as a stress coping strategy.

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ORIGINAL ARTICLE

# Cystatin C versus Creatinine as Early Stage Diabetic Nephropathy Marker

Atteaya Zaman[1], Amena Rahim[2], Muhammad Afzal[3], Abdul Khaliq Naveed[4]

**ABSTRACT**

**Objective:** To compare the levels of serum cystatin and creatinine as the markers of early stage of diabetic nephropathy.

**Study Design:** Case Control study.

**Place and Duration of Study:** The study was conducted at Railway Hospital, Rawalpindi for a period of one year from March 15th, 2016 to March 16th, 2017.

**Materials and Methods:** A total of 77 diabetics and 77 healthy controls were selected. These included adults above 40 years of age. The levels of Serum cystatin C and creatinine were measured using IMAGIN Specific Protein Analyzer. Both tests were done by applying standardized laboratory protocols. The study outcome was determined in terms of detection of diabetic nephropathy which was finalized on the basis of albuminuria status derived as three categories; normoalbuminuric, microalbuminuria and macroalbuminuria.

**Results:** Male cases were more than females (58.4% vs 41.6%), and were equally distributed in patients and control groups. The levels of Cystatin C (4.7 $\pm$ 3.9 mg/l), and creatinine (1.0 $\pm$ 0.13 mg/dl) were found significantly high in the macroalbuminuria group as compared to controls and normoalbuminuric group (p-value <0.001). Serum cystatin C was significantly raised in microalbuminurics as compared to serum creatinine proving its worth for detecting early stage diabetic nephropathy (p-value <0.001).

**Conclusion:** Serum cystatin C is a better predictive marker of diabetic nephropathy than serum creatinine.

**Key Words**: *Diabetic Nephropathy, Serum Cystatin C, Serum Creatinine.*

## Introduction

Type 2 diabetes is on a continuous rise worldwide owing to a steady increase in obese and aging population. The global prevalence of diabetes was estimated at 171 million (2.8%) in the year 2000 which is expected to reach 366 million 4.4% figure by the year 2030.[1]

The current prevalence of diabetes in Pakistan is around 10.0%.[2] As per WHO estimates, Pakistan is ranked 7th largest country suffering from diabetes mellitus and it is expected that by 2030 this rank will climb the ladder to 4th position which is an alarming statistics and situation.[3]

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*Funding Source: NIL; Conflict of Interest: NIL*
*Received: Jan 18, 2018; Revised: May 18, 2018*
*Accepted: May 24, 2018*

Diabetic nephropathy is one of the most common complications of diabetes. Diabetic nephropathy by definition is macroalbuminuria (albumin excretion rate $\geq$ 300 mg /24 hours) and deteriorating renal function is a known fact in diabetics. Previous reports confirm that approximately one third to one half of diabetic patients develop renal complications.[4]

Albuminuria is a significant prognostic factor for risk stratification of diabetic nephropathy and monitoring of its progression. There was a belief that microalbuminuria is predictive of future overt diabetic nephropathy in 80% cases, however, on the contrary, it has been proposed that around 30% of microalbuminuria cases progress to overt nephropathy after 10 years follow-up.[4,5]

Presently, the phenomena of normoalbuminuric diabetic nephropathy is well established and portrays that diabetic patients may present with a decreased GFR without progression from normal to microalbuminuria.[4] Gold standard method for determining GFR in research settings are inulin and Cr –EDTA plasma clearance. These techniques are time consuming, laborious and requiring expertise making them unfit for clinical practice. Hence the

used index for GFR is serum creatinine (mg/dl). Moreover, its sensitivity is poor in early renal damage and by the time its levels are detectable, significant decrease in GFR has already occurred.[5]

Putting these facts together, there is a ground for identification of alternative biomarkers to predict diabetic nephropathy early so that timely management and maintenance can be exercised. Cystatin C a 13.3k Da plasma protein is relatively new marker in the prediction of renal impairment and it correlates positively with other renal tests like GFR. Serum creatinine also a proven marker of nephropathy is relatively weaker test and easily changes by different maneuvers and circumstances like a person's muscle mass.[5] Cystatin C has been found constant and unaffected and an alternative with high sensitivity for diabetic nephropathy using a cut off of >60 ml of GFR.[5,6]

The focus of research by endocrinologists and other investigators is to find out new and better biomarkers for the diagnosis of early diabetic nephropathy. The aim of the study was to compare cystatin C and creatinine in the screening of diabetic patients on risk of early stage diabetic nephropathy.

## Materials and Methods

A case control study was conducted at the Railway Hospital, Rawalpindi from March 15th, 2016 to March 16th, 2017 for one year duration. A measured study sample of 77 diagnosed cases of diabetes were enrolled along with seventy seven normal controls. Convenient sampling technique was utilized. The study was conducted after obtaining permission from ethical review committee. A written informed consent was taken from all the patients. Demographic data was collected via questionnaires. Seventy seven diabetes cases and seventy seven non-diabetics of both genders and adults age (above 18 years) were included in the study.

For study purpose, Albuminuria was divided into three standard operational groups; i) Normoalbuminuria with ACR< 30mg/day, ii) Microalbuminuria with ACR 30 to < 300mg/day and iii) Macroalbuminuria with ACR $\geq$ 300mg/day.

Blood was drawn from peripheral veins, transferred to EDTA tube, gently mixed and made to stand upright. The blood samples were centrifuged at 2200 RPM for 10 minutes. The separated serum was stored at -20°C till completion of sample collection. The urine samples were collected in the jars provided to the patients and centrifuged at 1000 RPM for 10 mins, these were also stored at -20°C till analysis.

The estimation of cystatin C levels (mg/l) was carried out on IMAGIN Specific Protein Analyzer for quantitative determination of human cystatin C in serum. Similarly, the estimation of urinary albumin levels were carried out on IMAGIN Specific Protein Analyzer for quantitative determination of human Microalbumin[MALB] in urine by immunoturbidimetry.[4,5]

Data was analyzed using SPSS 20.0 version. First, descriptive statistics was applied to measure frequency and percentages for categorical variables like gender, and mean and standard deviations for continuous variables. Secondly, using student's t-test the means and standard deviation levels of serum cystatin C, serum creatinine and clinical measurements of blood pressure were compared among patients and controls. Categories of albuminuria were created as per operational definitions. The mean levels of serum cystatin C and creatinine were compared among these categories using T-test. For further analysis the renal status glomerular filtration (GFR) rate was categorized as GFR < 60, GFR 60-89.9 and GFR $\geq$ 90.[5,7] A p-value of <0.05 was considered significant difference. Parametric tests were applied as majority of the continuous numerical data was found equally distributed and dispersed.

## Results

In 154 study subjects the mean age was found similar in controls (55.7 years) and patients (56.5 years). Male gender was predominant and found equally distributed in patients and controls. (Table I).

The urinary albumin and creatinine was analyzed and it was found that there were 15 (19.4%) normoalbuminurics, 53 (68.8%) microalbuminuric and 9 (11.6%) cases with macroalbuminuria (Figure 1).

There was a gradual increasing trend of age and urinary albumin in the study subjects. The mean age of macroalbuminuric (59.3 $\pm$ 5.5 years) cases was significantly higher than controls (55.5 $\pm$ 5.1) and rest of albumin categories i.e. normoalbuminuric (56.2 $\pm$ 5.4) and microalbuminurics (56.5 $\pm$ 5.4). Male gender was predominant in the study and also in all albumin categories and controls, however, they were not

significantly different among categories (p-value, 0.58). (Table II).

Serum creatinine, serum cystatin C and other laboratory parameters were compared between albuminuria categories as well as controls. The levels of urine albumin (332.7 ± 30.1), creatinine (1.0 ± 0.13 mg/dl) and cystatin C (4.7 ± 3.9 mg/l) were found significantly high in the macroalbuminuria compared to controls and normoalbuminuric groups (p-value <0.001). Moreover, cystatin C was also found significantly associated with microalbuminuria than serum creatinine (2.6 ± 2.2 versus 0.94 ± 0.13 respectively).

GFR levels were significantly low in the micro (76.9 ± 15.1) and macroalbuminuria (74.0 ± 7.0) groups compared to normoalbuminuric and controls. Moreover, blood pressure was found significantly higher in the patients compared to controls (p-value <0.001). (Table III).

A selective analysis of cystatin C and serum creatinine levels was done according to GFR categories. The mean cystatin C was significantly high (1.7 ± 1.2) in patients with moderate to high kidney damage (GFR < 60), and mean cystatin C was also very high (2.6 ± 2.4) in patients with mild kidney damage (GFR 60-89). Serum creatinine was also found significantly deranged (1.2 ± 0.21) in GFR < 60 category, whereas in GFR 60-89 it was found border line deranged (0.94 ± 0.11). (Table IV).

**Table I: Demographic, Clinical and Pathological Characteristics of Patients and Controls**

| Age (years) | Patients (n=77) | Controls (n=77) | p-value |
|---|---|---|---|
| 40 to 50 | 19 (24.6%) | 21 (27.2%) | 0.91 |
| 51 to 60 | 41 (53.2%) | 40 (51.9%) | |
| 61 or above | 17 (22.1%) | 16 (20.7%) | |
| Mean ± SD | 56.5 ± 5.5 | 55.7 ± 5.1 | 0.89 |
| **Gender** | | | |
| Male | 45 (58.4%) | 45 (58.4%) | 0.51 |
| Female | 32 (41.6%) | 32 (41.6%) | |
| **Laboratory Parameters** | | | |
| Urine albumin | 113.2 ± 106.8 | 5.2 ± 3.8 | <0.001 |
| Creatinine (mg/dl) | 0.95 ± 0.14 | 0.56 ± 0.26 | <0.001 |
| Cystatin C (mg/l) | 2.5 ± 1.9 | 0.45 ± 0.27 | <0.001 |
| GFR (ml/min/1.73m$^2$) | 77.8 ± 14.5 | 125.1 ± 9.6 | <0.001 |
| **Blood Pressure (mmHg)** | | | |
| Systolic | 148.0 ± 10.8 | 131.8 ± 8.2 | <0.001 |

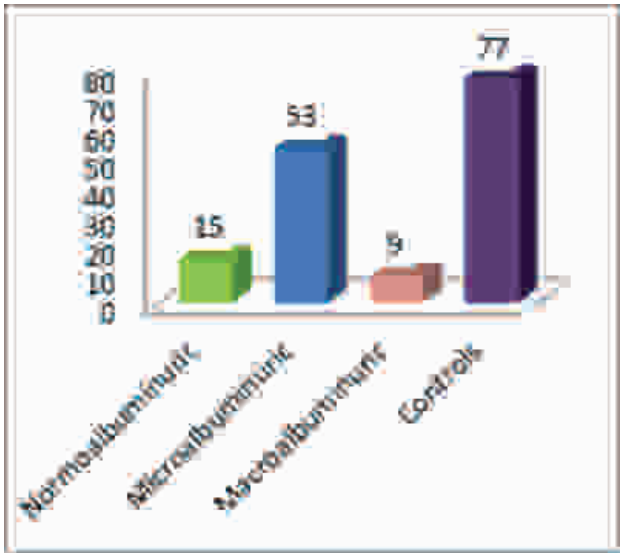


**Fig 1: Distribution of Albuminuria in the Study Patients**

**Table II: Association of Age and Gender with Urine Albumin Status of Patients and Controls**

| Age (years) | Control (n=77) | Diabetic patients | | | p-value |
|---|---|---|---|---|---|
| | | Normoalbuminurics (n=15) | Microalbuminurics (n=53) | Macroalbuminurics (n=9) | |
| Mean ± SD | 55.7 ± 5.1 | 56.2 ± 5.9 | 56.5 ± 5.4 | 59.3 ± 5.5 | <0.001 |
| **Gender** | | | | | |
| Male | 45 (58.4%) | 8 (53.3%) | 31 (58.5%) | 6 (66.6%) | 0.58 |
| Female | 32 (41.6%) | 7 (46.7%) | 22 (41.5%) | 3 (33.3%) | |

**Table III: Comparison of Biochemical and Clinical Parameters between Controls and Albuminuria Categories**

| Parameters | Controls (n=77) | Normoalbuminuric (n=15) | p-value* | Microalbuminuric (n=53) | p-value* | Macroalbuminuric (n=9) | p-value* |
|---|---|---|---|---|---|---|---|
| Creatinine (mg/dl) | 0.61 ± 0.21 | 0.69 ± 0.17 | 0.16 | 0.79 ± 0.38 | 0.701 | 1.0 ± 0.13 | <0.001 |
| Cystatin C (mg/l) | 0.41 ± 0.29 | 0.56 ± 0.39 | 0.10 | 2.6 ± 2.2 | <0.001 | 4.7 ± 3.9 | <0.001 |
| GFR ml/min/1.73m$^2$) | 121.6 ± 9.1 | 83.1 ± 15.4 | <0.001 | 76.9 ± 15.1 | <0.001 | 74.0 ± 7.0 | <0.001 |
| HbA1c (%) | 5.1 ± 2.8 | 7.1 ± 2.2 | 0.02 | 8.3 ± 2.7 | 0.01 | 8.9 ± 3.5 | 0.002 |
| Systolic BP (mmHg) | 130.6 ± 6.4 | 152.0 ± 9.0 | <0.001 | 147.1 ± 11.1 | <0.001 | 147.2 ± 11.5 | <0.001 |

** p-values based on comparison of controls with individual albuminuric category*

**Table IV: Relationship of Cystatin C and Creatinine with GFR Categories**

| Parameters | GFR < 60 (n=4) | GFR 60-89 (n=57) | GFR ≥ 90 (n=16) | p-value |
|---|---|---|---|---|
| Cystatin C | 1.7 ± 1.2 | 2.6 ± 2.4 | 1.2 ± 0.9 | <0.001 |
| Creatinine | 1.2 ± 0.21 | 0.94 ± 0.11 | 0.70 ± 0.18 | <0.001 |

## Discussion

The study findings reveal that cystatin C is significantly raised than creatinine in not only macroalbuminuria but also in cases of microalbuminuria. Microalbuminuria is most prevalent in the study, showing that two-third of patients were in the process of development of early diabetic nephropathy. Our study findings of raised cystatin C in early nephropathic derangement validate many previous reports on the topic. Lee BW witnessed that serum cystatin C is significantly lower in normoalbuminurics (0.83$\pm$ 0.22) than in microalbuminurics and macroalbuminurics (0.94 $\pm$ 0.33 and 1.05 $\pm$ 0.28 respectively; $p < 0.001$).[7] Jeon YK also witnessed a similar trend of relationship of cystatin C and diabetic nephropathy (micro and macroalbuminuria).[8,9,10]

Similarly in the current study the average serum creatinine and cystatin C are found high in micro and macroalbuminuric cases. Most of the study patients were in the early stage of diabetic nephropathy, however, 11.6% were proven cases of diabetic nephropathy (ACR > 300 mg/l). Cystatin C was found significantly high in micro and macroalbumin categories. Though serum creatinine was also found deranged in these cases, it is not that distinctive than cystatin C.

Previous literature on cystatin C suggests its superiority in detecting early diabetic nephropathy.[9,10,11] As patients on the risk of diabetic nephropathy can be recovered and early deterioration of renal function can be averted. This highlights the significance of an easy and feasible laboratory parameter like cystatin C.[4,9,12]

Serum cystatin C has proven its role as an alternative marker for estimating GFR. Moreover, the failure of creatinine to detect early decline in GFR is due to the fact that serum creatinine levels only start rising when almost 50% of renal function is lost, suggesting that GFR can change before serum creatinine becomes abnormal.[13,14] Cystatin C may rise faster than creatinine after a fall in GFR and is a reliable endogenous marker for assessing renal function in type 2 diabetic patients with renal impairment.[15,16]

It was found out that cystatin C and creatinine are significantly high in moderate to severe kidney damage and it is also high in mild kidney damage category (GFR 60-89). Our results have time and again proven that cystatin C was a highly useful marker of kidney damage in diabetic patients.

There are many advantages of the study which include; firstly, it was a comparative study comprising of diabetics and control groups, with a reasonable sample of seventy seven cases and seventy seven controls. Relationship of two commonly used markers i.e. cystatin C and serum creatinine were compared according to patient's albuminuria status and then also according to GFR status.

## Conclusion

Based on the findings of current study it is concluded that cystatin C is a significant predictive marker of diabetic nephropathy than serum creatinine.

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ORIGINAL ARTICLE

# Morphological Study and Demographic Survey of Abruptio Placenta Patients in Term Delivery. A Case Control Study

Saima Mumtaz[1], Manzoor Ahmad[2], Sumaira Abbasi[3]

**ABSTRACT**

**Objective:** This study was conducted to assess the gross morphological changes in abruptio placenta and its demographic prevalence in our set up.

**Study Design:** A case control study.

**Place and Duration of Study:** The study was conducted in Anatomy Department of Federal Medical and Dental College and data was collected from Gynaecology and Obstetrics department of Pakistan Institute of Medical Sciences, affiliated with Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU) Islamabad in a duration of eight months from 13th July 2015 to 20th February 2016.

**Materials and Methods**: Eighty pregnant women presented in the term pregnancy, forty having abruptio placenta, and forty with normal placenta already diagnosed by ultrasounds. Non-purposive sampling technique was done for the comparison between two groups. An organized data collection check list was used for the collection of data. The data was statistically analyzed using SPSS version 20 and MS Excel. The Chi-Square test and Student T test were applied, with *p* value ≤0.05.

**Results:** During the study period, eighty pregnant women with forty abruption placenta cases were included. Among these most frequent age was 26 to 30 years (50%) with mean± SD age of 28.1± 4.9. Majority, 70% were of low socioeconomic status and mode of delivery was C-Section (87.5%) in abruptio placenta group. The mean weight (grams) of abruptio placentae was found to be 396.4 ±49.9 as compared to 523.3 grams ± 38.8 in normal placentae. The diameter (cm) of abruptio placentae was 13.0±2.9 as compared to normal placentae16.4 ± 3.8. The number of cotyledons were reduced to 11.5±6.1 in abruptio placentae as compared to control 16.5 ± 6.8.

**Conclusion:** It is concluded that gross morphological changes in abruptio placenta include reduction in its weight, dimensions and number of cotyledon. Moreover, the demographics show that it is more prevalent in low socioeconomic women leading to high rate of Cesarean Section.

**Key Words**: *Abruptio Placenta, APH (Antepartum Hemorrhage), Socio-Economic Status.*

## Introduction

Abruptio placenta is the early detachment of normally located placenta from its uterine wall.[1,2] It was first defined by Edward, an English physician in 1776, as "accidental hemorrhage in placenta".[3] While in 1819, Baudelocque used the term "concealed accidental hemorrhage".[3] In 1901, Holmes studied 199 new cases and introduced the term "Ablatio placentae", which was later modified into abruptio placentae by Delee.[4] In 40% of cases, there is no etiology but in 60% of cases, it is associated with chronic and pregnancy induced hypertension(PIH),[5,6] maternal vascular disease, smoking, drug ingestion, nutritional deficiency, uterine anomalies, trauma, tumors, antiphospholipid antibody syndrome, hyperhomocystinemia, twin pregnancy etc.[7,8]

It is most common in 2nd and 3rd trimester with the incidence of 1% of all pregnancies. Its perinatal mortality rate is 119/1000 births.[3,7,8] Recurrence risk of abruptio placentae is 4-12% but increases to 25% in two consecutive pregnancies with fetal demise upto 7%. It can be sorted into grade I (mild) which is 40%, grade II (moderate)that is 45% and grade III (severe)which is 15%.[9] In grade I, there is slight vaginal bleeding and uterine irritability, while in grade II there is uterine hypertonicity with mild to moderate vaginal bleeding, hypofibrinogenemia, and fetal distress.[10] But in grade III, fetal death and heavy vaginal bleeding with maternal hypotension, hypofibrinogenemia and thrombocytopenia.[10] These cases are diagnosed by taking history and on clinical ground as tender uterus with increased resting tone and hypertonic or hyperactive uterine contractions

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*Funding Source: NIL; Conflict of Interest: NIL*
*Received: Sep 18, 2017; Revised: Jun 06, 2018*
*Accepted: Jun 07, 2018*

and on delivery passage of clots with hematoma or hemorrhage of placenta.[11]

On gross examination, discontinuity in placental tissue from maternal site is of diagnostic value. In 80% of patients external bleeding is present, but in 20% it is concealed and diagnosis is mostly delayed.[12] Important clinical parameters in assessment of severity are maternal hemodynamic status, coagulation profile, complete blood picture and fetal condition. Abruptio placenta may be due to hemorrhage into the decidua basalis of the placenta, leading to hematoma formation and increase in hydrostatic pressure resulting in separation of the adjacent placenta.[3,13] In severe detachment of placenta, blood accumulates in uterine muscularis and perimetrium layer, and even sometime blood enter into the broad ligament and under the peritoneum of pelvis with classical picture of uteroplacental apoplexy. This term first explained by Couvelaire.[14] Gross examination, revealed fissures on the perimetrial surface of the uterus and evidence of active bleeding and hemoperitoneum.[15,16] As a result of exposed hematoma, disruption and separation of the basal plate from the decidua increases until complete placental detachment occurs.[17] On naked examination, the weight of placenta may be normal but in most cases it is less than average range of 475-650gms.[18] Calcification and vascular dilation or congestion may be visible.[19,20] In 60% of cases, uteroplacental arterial insufficiency leads to ischemia and rupture of involved vessels can be evident, thus causing abruptio placenta.

It is important to identify morphological examination of births (placenta) occurring in public sector hospital in Pakistan because of its high recurrence rate and associated maternal and fetal mortalities. This study was conducted to assess the gross morphology of abruptio placenta and its demographic prevalence in our set up.

## Materials and Methods

A case control study, based on non-purposive sampling technique was carried out in anatomy department of Federal Medical and Dental College in collaboration with Gynecology and Obstetrics department of PIMS / Shaheed Zulfiqar Ali Bhutto Medical University Islamabad over a period of eight months (13th July 2015 with approval of ethical committee up to 20th February 2016). Sample size was calculated according to the WHO formula (prevalence ratio).[21] Eighty placentae were collected from labor room and Gynecology department of PIMS hospital, who delivered either vaginally or by caesarian section with the permission of ethical committee of SZAB Medical University Islamabad. Forty placentae from confirmed cases of abruptio placentae (case group) with complete medical and obstetric history were collected and recorded to identify the confounders (hypertension, smoking, twin pregnancy). Forty control groups were taken from normal and uncomplicated pregnancies. Mothers with the age of 15-40 years were included in the study. Pregnant women with other placental abnormalities (like placenta Previa, placenta accrete, and percreta) in the term pregnancy, twin pregnancy and age above 40 years were excluded. After delivery, the specimen were washed with tap water, labeled with numbers and preserved in 10 % formalin solution for 48 hours.[1]

A structured data collection check list was used to collect the required data. Chi-Square tests were applied for simple descriptive statistics (frequencies, percentages) and computed for each categorical variable such as age, socioeconomic status and mode of delivery. Whereas mean and standard deviation was calculated for numerical (continuous) variables which included placental weight, diameter, and number of cotyledons and were analyzed by Student T test. The data was statistically analyzed using SPSS version 20 and MS Excel. *P* value <0.05 was considered statistically significant.

## Results

### Demographic Data:

**Table I: Age of Patients in the two Study Groups (n= 80)**

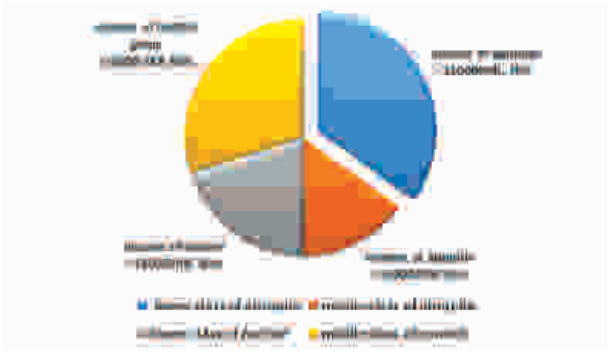
| Age in years | Abruptio (n=40) | Control (n=40) | *p*-value |
|---|---|---|---|
| 15- 20 | 3 (7.5%) | 2 (5.0%) | 1.0 |
| 21 to 25 | 8 (20.0%) | 11 (27.5%) | 0.62 |
| 26 to 30 | 20 (50.0%) | 18 (45.0%) | 0.20 |
| 31 to 40 | 9 (22.5%) | 9 (22.5%) | 1.0 |
| Mean $\pm$ SD | 28.1 $\pm$ 4.9 | 27.6 $\pm$ 4.8 | 0.08 |

Age was equal in both patients and control groups in the study. In the patients (abruption placenta) group the mean age was 28.1 $\pm$ 4.9, whereas in the controls it was 27.6 $\pm$ 4.8 years. This difference in mean age was not statistically significant (*p*-value = 0.08). Age stratification also showed no major variation in

patient's age in both groups. (Table I).

The above Pie Chart illustrates that 60% of control

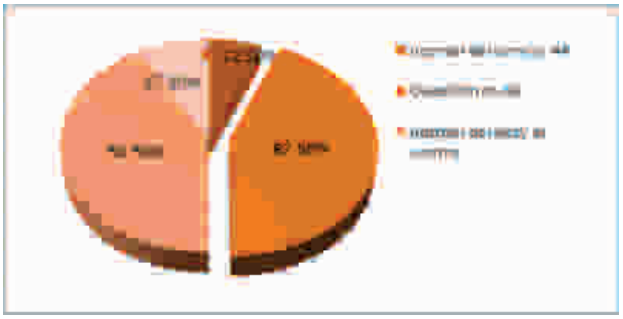
**Chart 1: Comparision of Socioeconomic Status in Abruption Versus Control Group**



group belonged to middle socioeconomic class while in case group 30% belong to middle class. Similarly it is revealed that lower socioeconomic class in control groups was 40% and in abruptio it was 70%.

This pie chart illustrates that C-section rate was high

**Chart 2: Comparison between the Mode of Delivery in Control Versus Patient Group**



among abruptio patient as compared to control group. But *p*-value=0.187 was not found statistically significant.

The placental weight and diameter were compared among the abruptio placenta patients and normal controls. The mean placental weight was 396.4 ± 49.9 grams in patients and 523.3 ± 38.8 grams in the controls and this difference in the means was statistically found highly significant (p-value = <0.001). Similarly the mean placental diameter was 13.0 ± 2.9 cm in patients and 16.4 ± 3.8 cm in the control and this difference was also highly significant (p-value = <0.001). The mean numbers of cotyledon in placenta were 11.5 ± 6.1 in patients and 16.5 ± 6.8 in the controls and this difference was also significant (*p*-value = <0.001). (Table II).

**Table II: Placental Weight in Grams and Diameter in Centimeters of Patients in the Two Study Groups**

| Placental weight (gms) | Abruptio (n=40) | Control (n=40) | *p*-value |
|---|---|---|---|
| Mean ± SD | 396.4 ± 49.9 | 523.3 ± 38.8 | <0.001 |
| Placental diameter (cm) | – | – | – |
| Mean ± SD | 13.0 ± 2.9 | 16.4 ± 3.8 | <0.001 |
| Numbers of cotyledon in placenta | – | – | – |
| Mean ± SD | 11.5 ± 6.1 | 16.5 ± 6.8 | <0.001 |

## Discussion

**Fig 1: Showing Measurement of Diameter of Abruptio Placenta**

**Fig 2: Showing Multiples Abruption in Placenta**

**Fig 3: Showing Single, Huge Abruptio with Large Hematoma Formation in Placenta**

Abruptio placenta is referred to as detachment of normally placed placenta before time. Its incidence range is 0.3% to 2.2% in the developed world, whereas in Pakistan its incidence is quite high reaching up to 7%.

In this study the mean age of patients was 28.0 years and a significant number (70.0%) of them was between 21 to 30 years. Abbasi RM et al in his work on fetomaternal outcome among cases of abruptio placenta asserted that a majority (60.0%) of their subjects were between 21 to 30 years of age. [22,23]

Similar work conducted by Shukur-ud-din S and colleagues witnessed an average age of 30.0 years in their cases of placental abruption.[24]A study conducted in India on morphological forms of placenta in normal and hypertensive cases reports mean age of 26.5 years.[25] Naseer-ud-din.A. In year 2010 shows a mean age between 20-30years in the study of placental abruption.[3]

The detailed categorization of socioeconomic status of abruptio patients indicate that most were from low socioeconomic class with income <15000 PKR, uneducated, and non-booked. Most of them presented to emergency and had no record of antenatal checkup as compared to control group. Moreover, the recent research proves that perinatal prognosis could be improved by regular antenatal visits, as the abruptio placenta is an acute clinical presentation of a chronic disease process.[3,14,17,26] Moreover the proportion of caesarean sections in abruptio placenta was higher than the control group. This also shows that delay in diagnosis and treatment by primary health care centers resulted in increased C-section ratio at tertiary.[22,27]

The weight of placenta is an important and functionally significant parameter as it is related to villous area and fetal metabolism. Low weight of placenta is good indicator of fetal hypoxia at term pregnancy. In the current study, placental weight and diameter was found significantly different among abruptio patients and control group.[1,22,28] The mean placental weight was significantly less in abruption group depicting loss of it during pregnancy or before delivery. Previous studies by Narasimha JV and Chandini et al found similar findings of decreased weight of placenta in patients compared to control group.[29,30] A local study from Bahawalpur also witnessed a similar trend of decreased weight of placenta in patient group when compared with the control group.[31] In abruptio placenta there is a breach in the tissue of placenta. Similarly, in this study the placental diameter were also found decreased in the abruptio patients compared to healthy controls. Similar results were found in previous studies by Zia-ur-Rehman et al. and Goswami P et al[1,28,31]

In the current study the number of cotyledon in the placenta was also found decreased in the abruptio patients. Agarwal GC et al also noted that mean number of cotyledons were less in patients than controls. Many other investigators also reported that mean number of cotyledons were less in women with pregnancy induced hypertension as compared to control group. [32]

## Conclusion

It is concluded that gross morphological changes in abruptio placenta include reduction in its weight, dimensions and number of cotyledon. Moreover, the demographics show that it is more prevalent in low socioeconomic women leading to high rate of Cesarean Section.

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ORIGINAL ARTICLE

# Role of Mobilization to Improve Cervicogenic Headache

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**ABSTRACT**

**Objective:** To determine the effect of Mobilization (headache SNAG and Reverse headache SNAG) to treat Cervicogenic headache.

**Study Design:** Randomized control trial.

**Place and Duration of Study:** The study was conducted from 1st January to 30th July 2015 in Riphah Rehabilitation Center, Riphah International University Lahore.

**Materials and Methods:** A sample of 42 patients with cervicogenic headache, 30-60year age were included through non probability purposive sampling techniques and randomly divided into two groups (headache SNAG and Reverse headache SNAG). The demographic data was recorded and informed consent was taken from all participants. Eight weeks of treatment session was provided to both group and assessment of improvement in cervicogenic headache was done at baseline, after 04, 06 and at the end of 8th week.

**Results:** The sample had 57 % male and 43% female distribution. Patients with acute cervicogenic headache were 48% and chronic cervicogenic headache were 52%. The p value ($p<0.05$) showed that there was a significant difference in the improvement of headache at 06 weeks and 08 weeks in patients treated with headache SNAG. There was no difference in outcome of headache scale in both treatment approaches (Headache SNAG and reverse headache SNAG) after 04 weeks.

**Conclusion:** The mobilization is very effective in the management of Cervicogenic headache. The headache SNAG is more effective as compared to the reverse headache SNAG in the reduction of pain and headache scale.

**Key Words:** *Cervicogenic Headache, Mobilization, Pain, Quality of Life.*

## Introduction

The Cervicogenic headache can be defined as the chronic semi-crainal headache and the etiology is the upper cervical vertebrae. The prevalence of chronic unilateral headache is 15-20%.[1] Globally it is estimated that prevalence of headache is 47% in adults which is symptomatic at least once in a last year. 1.7 to 4% adult population have headache on 15 or more days in a single month.[2] The Cervicogenic headache (CEH) is defined as "the pain that arises from cervical region to posterior head" It affects the quality of life of persons. The whiplash injury is one of major contributing factor for headache; this type of headache is short term.[3] The prevalence of CEH from the general population aged 30-44 year through self-reported questionnaire and it was 0.17% and the prevalence is more in females as compare to males in the general population.50% headache is co related with the use of medication and migraine was 42 %.[4]

It is characterized by dull pain and stiffness in the back of the head and neck and often radiate to the forehead. Pain is often on one side of head and may proceed to shoulder and arm on same side.[5] Cervicogenic headache has various symptoms including the refereed pain to the posterior side of head from the cervical region. The referred pain can be from the muscle and joints around the cervical region.[6] Risk factors may include two types of events like repetitive activities or whiplash injuries which can cause cervicogenic headache. Sedentary life style, stress, dehydration, bending forward and shoulder forward activities and slouched posture can also be a major risk factor for cervicogenic.[7]

The findings of CEH include the decreased range of motion, painful upper cervical joints, muscular tightness especially the upper back cervical muscles in the later phase of CEH.[8] The following techniques are used in the treatment of the CH: medical therapy,

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*Funding Source: NIL; Conflict of Interest: NIL*
*Received: Nov 18, 2017; Revised: Jun 08, 2018*
*Accepted: Jun 10, 2018*

acupuncture, local botulinum toxin injection, neural therapy[9], cervical epidural corticosteroid injection[10] greater occipital nerve (GON) block,[11,12] physical therapy, massage, traction, kinezitherapy and surgical treatment. It shows that the good results are obtained by a combination of physical therapy, manual therapy and kinezitherapy. [11-13] Patients group who are given SNAGs showed significantly greater improvement in neck disability index (NDI), when compared to the control group.[14] Upper cervical spine mobilization showed better results than massage therapy with regard to headache pain scale parameters and neck range of motion.[15]

The evidence suggested that the manual therapy, soft tissue mobilization and exercises for neck region have greater improvement as compared to other alternative strategies. The non-invasive management is also integral part of radiculopathy.[16] The data base showed the evidence based literature of cervical manipulation and mobilization and they reported that the mobilization of the cervical joints with appropriate strengthening exercises was more effective outcome in CEH in terms of pain intensity as compared to other treatment strategies.[17] The exercises have significant effects on the pain intensity, range of motion and activity of daily life in CEH but there are limited improvements in other secondary outcomes.[18]

The mobilization and mobilization with combination to other approaches have strong effect on the neck pain. The reviews reported that there is some evidence of improvement in the pain, functional disability, quality of life, global perceived effect on the CEH.[19] The main purpose of this study was to comapre the effects of Headache SNAG and Reverse Headache SNAG for treating Cervicogenic headache.

## Materials and Methods

This was a Randomized control trial and conducted from 1st January to 30th July in Riphah Rehabilitation center, Riphah International University Lahore. The non-probability sampling was used to collect the data and randomly divided into two groups. Group-A: Patients in this group were treated with Headache SNAG. Group-B: Patients in this group were treated with Reverse Headache SNAG. Sample selection was done on the following inclusion and exclusion criteria. The inclusion criteria include Age 30–60 years, both gender; cervicogenic headache (clinically diagnosed) and patient with radiculopathy, trauma and systemic illness were excluded from the study. Total 50 patients, who met the selection criteria, were enrolled for the study. The sample was calculated through software while considering the literature reference. Informed consent was taken from each patient stating about the safety of the study and their right to withdraw from the study at any time. Demographic details (name, age, sex,) were noted along with the necessary medical history. Then patients were divided into two groups by using card allocation method. In group-A patients were treated with Headache SNAG and in group-B patients with reverse headache SNAG. Each Patient received two treatment sessions per week with maximum eight treatment sessions over the period of four weeks.[15] Three patients were dropped from sample, one because of conveyance issue while remaining two moved out of city**.** The treatment procedure was done by the Researcher himself and all the information regarding the demographic data were gathered by using a pre-designed Performa. Improvement regarding the outcomes of the treatment was measured using Visual Analogue Scale and Headache Pain Scale. The measurement was taken before the study and then after 04, 06 and at the end of 8th week of treatment. SPSS version 21 was used for data analysis and T independent samples test was used to compare the two groups.

## Results

Total 50 patients were recruited including 27 male and 23 females. 48% of the sample was categorized as acute while 52% was chronic cervicogenic 33% patients had sedentary life style while 67% have active life style. 60% patients were computer user while 40% were not routine users. 86% used the hard pillow while 14% were users of soft pillow. The table shows that the mean age of sample was 40.17±9.42, mean computer use was 4.19 ±1.7 hours and mean sleeping hours were 6.21±0.951. There was no significant difference at baseline and 04 weeks ($p>0.05$) and it showed that both groups were homogeneous at the time of recruitment. There was a significant difference at 06weeks and 08 weeks ($p<0.05$) and it showed significant difference in the improvement of symptoms in two groups. Headache SNAG is more effective after 06 and 08 weeks of treatment than reverse headache SNAG. (Table I and II).

**Table I: Comparison of Groups for Headache Scale**

| Variable | Headache scale at Baseline | Headache after 04 weeks | Headache after 06 weeks | Headache after 08 weeks |
|---|---|---|---|---|
| Headache SNAG | 4.95±1.071 | 3.57±1.076 | 2.29±1.007 | 1.14±1.49 |
| Reverse Headache SNAG | 5.24±1.446 | 4.00±1.483 | 3.33±2.106 | 2.51±2.51 |
| *P* value | 0.471 | 0.291 | 0.046* | 0.018* |

**Table II: Comparison of Groups for Visual Analogue Scale**

| Variable | VAS scale at Baseline | VAS after 04 weeks | VAS after 06 weeks | VAS after 08 weeks |
|---|---|---|---|---|
| Headache SNAG | 4.23±.88 | 3.09±.094 | 1.80±.92 | 0.95±1.16 |
| Reverse Headache SNAG | 4.52±1.364 | 3.33±1.42 | 2.76±1.42 | 2.33±2.24 |
| *P* value | 0.426 | 0.527 | 0.054 | 0.016* |

## Discussion

The results show that headache SNAG technique is more effective as compared to reverse headache SNAG to treat Cervicogenic headache. Although initially there was no significant difference in outcome but continuous application of headache SNAG was effective and showed good results long term. The improvement was recorded in headache scale and visual analogue scale after 04, 06 and 08weeeks of application of both manual techniques.

SNAG is considered a comprehensive mobilization in reducing the pain intensity and improving the functional status of patients. A study conducted by Muhammad Khan to determine the effect of upper cervical Sustained natural apophyseal glide (SNAG) with posterior anterior mobilization showed that there was significant difference in disability index and pain scale. The results are similar with this study finding that the SNAG mobilization has more effective than other treatment approaches in reducing pain in Cervicogenic headache.[20] A systematic review was conducted by Stephanie Racick in 2013 to determine the evidence based and effective treatment approach in the treatment of Cervicogenic headache. They included the study related with mobilization, manipulation, strengthening and other treatment options and concluded that mobilization with other approaches is effective in reducing pain in patients of Cervicogenic headache. (54) Janusz Kocjan conducted a study in 2015 to determine the effectiveness of SNAG in CEH. They compared the cervical rotation in conjunction with SNAG mobilization. The result showed better improvement when compared to other mobilization techniques.[21]

The reverse SNAG is used to mobilize the cervical segments for mobility and improving the joint movement. A study conducted in 2014 by Susan A. Reid on the comparison of Maitland and mulligan SNAG for the treatment of Cervicogenic dizziness. They compared the both techniques 1 and 2 weeks and concluded that the both technique are effective in reducing the pain and frequency of dizziness in patients with Cervicogenic dizziness.[22] The literature showed that the different approaches are effective for the pain relief, dizziness intensity reduction and improvement of range of motion. The study finally concluded that there is limited evidence in the literature about the SNAG although there is relief in pain scale and other symptoms related with Cervicogenic headache.[23] The study conducted by Armed in 2014 on the effectives of SNAG glide and manipulation on the cervical disorder. They took measurement on neck disability index, ranges and visual analogue scale for the recording of improvement in the patients with cervical impairment. They used manipulation while in current study mobilization was used to assess the effects on pain. Finally they concluded that the mobilization SNAG with manipulation has good effects and showed the significant statistical difference as compares to the other treatment options like simple exercise alone in improving the pain and disability index in patients with cervical disorder.[24]

The limited sample size and lack of quantitative equipment for detecting change are major limitation in study. Further studies with physiological biomarker, radiological findings and with larger sample size are recommended.

## Conclusion

Mobilization is very effective in the management of Cervicogenic headache. The headache SNAG is more effective as compared to the reverse headache SNAG in the reduction of pain on headache scale. The mobilization should be included in the appropriate management of Cervicogenic headache.

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ORIGINAL ARTICLE

# Comparative Evaluation of CHROMagar and API 20C AUX in Isolation and Identification of *Candida Species*

Uzma Mussarat Malik[1], Abdul Bari Khan[2], Muhammad Luqman Satti[3]

**ABSTRACT**

**Objective:** To evaluate the performance of CHROMagar and API 20C AUX for the documentation of different *Candida* species.

**Study Design**: Descriptive cross-sectional study.

**Place and Duration of Study:** The study was conducted in the Department of Microbiology at AFIP (Armed Forces Institute of Pathology), CMH Rawalpindi and Army Medical College Rawalpindi in collaboration with Departments of Pathology (Microbiology) at Pakistan Railways Teaching Hospital (PRH), Islamic International Medical College Rawalpindi from 01th April 2017 to 30th September 2017.

**Materials and Methods:** Collectively 100 isolates of *candida* yielded from HVS clinical samples. Phenotypic tests including growth on CHROMagar *Candida* and API 20C AUX were used for reporting different *Candida* species. Clinical *Candida* isolates along with reference institutional control strains of *Candida* species were used in the study. Data was analyzed using simple descriptive statistics (frequencies, percentages) for each categorical variable.

**Results:** Among 100 *candida* isolates 92 (92%) isolates of *Candida* were identified correctly to level of species by CHROMagar *Candida*, in comparison to 100% identification of *candida* species using API 20C AUX. Results of present study revealed that CHROMagar *Candida* can be used to report three species of *Candida* considering the morphology and colour of colonies of these particular species, and to distinguish them as *C. albicans, C. tropicalis, and C. glabrata*.

**Conclusion:** Both phenotypic tests CHROMagar plates and API 20 C AUX are effective in the documentation of *Candida species*. However API 20 C Aux is found to be more accurate than CHROMagar because less commonly isolated *Candida species* cannot be documented using CHROMagar. Moreover being less costly; use of CHROMagar *Candida* is helpful in rapid identification and constructing suitable therapeutic plan for patient's management in laboratories with limited resources.

**Key Words:** *Candida Species, HVS, Identification Methods.*

## Introduction

Fungal infections have worldwide spread. Since 1980s, a steady rise in incidence as well as prevalence of these infections has been found contributing significantly to morbidity and mortality. Mycotic infections are frequently found in patients with depressed immunity [1,2,3] like cancer patients receiving chemotherapy, transplant patients and AIDS patients who are more prone to develop infections caused by *candida*.[4] Infections due to *Candida albicans* remains the most frequent etiology of human diseases due to genus *candida*, but the incidence of infections due to *non albicans candida* is also increasing.[5,6] There is a variety of methods for identifying *Candida species* from clinical samples, among them CHROMagar *Candida* differential medium is commonly used to isolate presumptive *C. albicans, C. dubliniensis, C. tropicalis* and *C. krusei*. Its sensitivity and specificity is considered satisfactory

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*Funding Source: NIL; Conflict of Interest: NIL*
*Received: Feb 29, 2018; Revised: May 10, 2018*
*Accepted: May 15, 2018*

for these species.[7,8] The biochemical characterization is done using the API® 20C AUX (BioMerieux, France), which relies on variations in the assimilation of carbohydrates.[9] However, it presents limitations related to cost and to distinguish between some species.[7] Presently the emergence of *non albicans Candida* species is a major problem to be addressed in several institutions.[10,11] In 70% to 90% vulvovaginal candidiasis cases, *C. albicans* is found to be main causative agent to be followed by *C. glabrata* causing 10% - 20% of vaginal candidiasis.[5,6,12] Most species of *Candida* are involved in causing vulvovaginitis but *C. krusei, C. parapsilosis* ,and *C. tropicalis* are infrequent causative agents.[13]

Conventional methods used for *candida species* identification like assimilation and fermentation reactions are described as clumsy and beyond the range of expertise in local laboratories. Evaluation of identification methods in resource-limited settings for *candida* species such as microscopy, colonial morphology and biochemical studies require unique research studies for effective management and prompt diagnosis of fungal infections.[14]

*Candida* vaginitis is usually diagnosed without proper diagnostic procedures but there is possibility that women may be uninfected or may be suffering from another illness. Culture on Sabouraud's dextrose agar (SDA) for diagnosis of fungal infections is considered as gold standard, while isolation and identification using different phenotypic assays can take up to 2 – 4 days.[15,16] Rapid identification of mycotic infections is possible with use of different brands of chromogenic media. These chromogenic agars, reduces the time required for the identification of yeast by distinguishing common *Candida* species on the basis of specific color that generated because of reaction of substrate with enzymes secreted by microorganisms after incubation for 48 hours at 37 $^{0}$C.[17,18]

Use of API 20C AUX kit for identification of *candida* species including *C.albicans* and *non albicans candida* is easier and has greatly reduced the laboratory time involved in the speciation of *Candida* isolates.[19,20]

Owing to limited knowledge and practice of these phenotypic methods, this study was aimed to compare and evaluate the efficacy of CHROMagar and API 20C AUX in identification of *Candida* species.

## Materials and Methods

A descriptive cross-sectional study was conducted from 01$^{st}$ April 2017 to 30$^{th}$ September 2017 after approval from Research and Ethical Review Committee. Non-probability convenient sampling was done and non-parametric data were collected. The data of *Candida species* were analyzed by using frequencies distribution test on SPSS (version 20) software. A total of 100 *candida* isolates were collected from Microbiology labs of Armed Forces Institute of Pathology (AFIP), Army Medical College (AMC) and Islamic International Medical College (IIMC) in collaboration with Combined Military Hospital (CMH), Military Hospital (MH) and Railway Hospital Rawalpindi respectively. Study included HVS specimens of pregnant, nonpregnant and postmenopausal women that revealed growth of *candida* species on Sabouraud' s culture plate. Direct Gram-stained smear examination was done for all collected candida isolates after culture on Sabouraud's dextrose agar (SDA) (Oxoid, UK) incubating at 37 °C for 24 – 48 hours.

Institutional control strains were used to compare the results of present study. Conventional methods, such as germ tube test, macroscopic appearance and structural description of colonies i.e. colour, size and texture on Sabouraud's dextrose agar (SDA), and CHROMagar *Candida* were used to confirm the growth of control strains.

CHROMagar *Candida* medium in each liter contained peptone (10 g), glucose (20 g), agar (15 g), and chloramphenicol (0.5 g) and Chromogenic mixture. (2 g), while pH of the medium was maintained at 6.1 according to instructions of manufacturer. *Candida* colonies of study samples from SDA agar were inoculated onto CHROMagar. Specimens were streaked for isolation onto the surface of the medium. The plates were kept for incubation at 30°C for 48-72 hrs in an inverted position. Forty two hours incubation time is obligatory for complete color development of *Candida* colonies. The diverse species of *Candida* species revealed dissimilar colours of colonies i.e. *C. albicans* colony appeared light to medium green, *C. tropicalis* colonies gave dark blue to metallic-blue colour and *C.glabrata* colonies looked light mauve to mauve. Moreover, these colonies were flat with a whitish border. Other candida species like *C. krusei* can give rise to light to

dark mauve colour. Rest of non albicans candida species produced light creamy to light pink colour that were later on identified with API kit.

API 20 C AUX (Biomerieux, France) was used to perform Carbohydrate fermentation tests. Dehydrated substrates were added in 20 different cupules which allowed the performance of 19 assimilation tests. Semi solid minimal medium was used to inoculate the cupules and the growth of yeast was seen when utilized the added substrate as the soul carbon source. The reactions were read by linking them to growth controls and documentation was done by referring to the Analytical Profile Index.

**Fig 1: API 20C AUX**

Results of API were finally compared with the culture results and speciation of *Candida* was also done.

## Results

Results of germ tube test revealed 68 out of 100 isolates were positive for germ tube and remaining 32 were negative as shown in Fig 2.

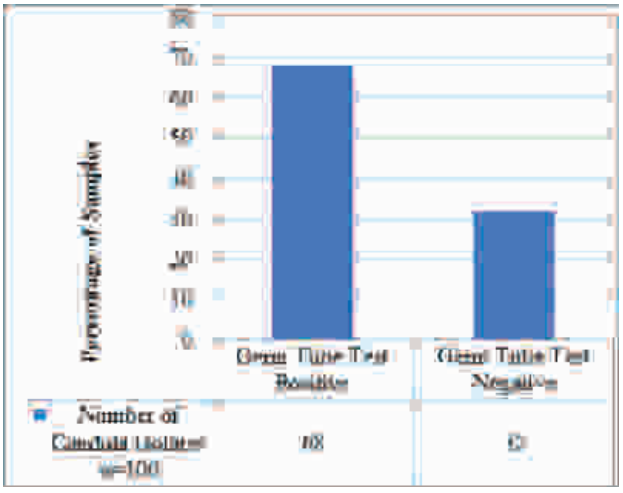

**Fig 2:Frequency of Germ tube positive isolates**

Among 100 samples grown on CHROMagar plates, 16 (16%) showed pinkish purple growth and were labeled as *C. glabrata*, 8 (8%) revealed blue coloured colonies and were termed as *C. tropicalis*, while 68 (68%) samples showed green coloured colonies of *candida albicans*. Among rest of 8 samples, 4 samples showed light pink colonies and 4 samples revealed blue to mauve coloured colonies which after API testing identified as *C.famata* 4(4%), *C. guilliermondii* 2(2%), *Saccharomyces cerevisiae* 1(1%) and *C. lusitaniae* 1(1%). Moreover, *Candida albicans* was found to be dominant over the *non albicans species*. The comparative identification results are shown in Table I.

**Table I: Identification of Samples using CHROMagar and API 20C Aux**

| S.No | Candida Species | No. of Isolates (total:100) | Colony characteristics on CHROMagar Candida | Identification by API 20C AUX |
|---|---|---|---|---|
| 1 | *C. albicans* | 68 | Apple green colonies; consistent | *C. albicans* |
| 2 | *C. glabrata* | 16 | White large glossy pale pink to violet colonies | *C. glabrata* |
| 3 | *C. tropicalis* | 8 | Steel blue, purple diffusion into surrounding agar | *C. tropicalis* |
| 4 | *C. famata* | 4 | White to light pink colonies | *C. famata* |
| 5 | *C. guilliermondii* | 2 | Small pink to purple colonies | *C. guilliermondii* |
| 6 | *C. lusitaniae* | 1 | Pink gray purple | *C. lusitaniae* |
| 7 | *Saccharomyces cerevisiae* | 1 | Pink to purple | *Saccharomyces cerevisiae* |

The API 20 C AUX tests recognized the *Candida* species on the basis of fermentation and utilization of different sugars. The confirmatory test by API yielded seven species of *Candida* in total i.e. *C. albicans, C. glabrata, C. tropicalis, C. guilliermondii, C. famata, Saccharomyces cerevisiae* and *C. lusitaniae*. API test results were matched with CHROMagar results, germ tube test results and microscopy of these selected samples. Overall CHROMagar identified strains correctly with more than 90% discrimination while comparing with API 20 C AUX results. The results of API are tabulated in Table 1. The API 20 C AUX system correctly identified about 100% of the isolates compared to 92% by CHROMagar culture technique. The CHROMagar culture plates correctly identified all organisms except 8 isolates. These 8 isolates revealed pink to

purple coloured colonies that were identified differently using API 20C AUX. The reason for this deviation is because CHROMagar has been known to identify frequently found species of *Candida i.e. C. albicans, C. glabrata, C. tropicalis*.

## Discussion

In developing countries especially where resources are limited, deficiency of training skills and non-availability of proper reagents that are contributory factors in making final diagnosis of mycotic infections, identification of fungal infections to species level become quite difficult. Moreover to minimize the monetary burden on the underprivileged patients, laboratories only perform germ tube test and limit their report only to identification of *C. albicans*.[21]

In present study, all 100 candida isolates gave distinct colours on CHROMagar thus helped in the recognition of *candida species* causing vulvovaginitis in study population. This data conforms to finding of a study conducted by Horvath et al.[22] There is nonconformance of our data with a study conducted by Grace L et al who reported 78% identification of *candida species* using CHROMagar.[23] This finding might be due to direct subculture of specimen on CHROMagar plates. LYnn L et al reported that CHROMagar readily identify *C. albicans, C.glabrata, C.krusei, and C. tropicalis*.[22] Diagnosis of vaginal candidiasis is usually based on clinical symptoms and direct microscopic examination as stated by Nyirjesy et al and Faraji et al [24,25]; Although microscopic examination of clinical samples is quick, easy method and may recognize the probable causative agent, but CDC recommend that vaginal culture is mandatory to confirm the diagnosis.[26] In this study, different laboratory methods were used for prompt diagnosis of *candida species*, among these Gram staining and germ tube test were found easy and trustworthy techniques for the documentation of *Candida spp*. CHROMagar is found to be a novel medium enabling isolation and identification of different *Candida species*. This media correctly identified 92% of *Candida* strains which is in accordance with the prior study done by Ozcan et al.in 2010.[27]

Based on different colors and morphology of colonies "CHROMagar *Candida*", provided a fast and convincing recognition of frequently found yeasts species, which would ordinarily be missed during conventional plating on solid medium. According to Nejad et al the major advantage of "CHROMagar *Candida*" was its ability to detect the presence of mixed species,[28] and results of this study prove that use of CHROMagar was helpful in correct identification of *C.albicans, C.glabrata* and *C.tropicalis* depending on the colour and morphology of colonies. Chromogenic culture media are very helpful in identification of *C. albicans* [29] but its main limitation is less power of discrimination among *non albicans Candida* species.[30] In present study, 92 % of *Candida* isolates were correctly identified after growth on CHROMagar , in contrast to the study conducted by Dalia et al they stated that this medium correctly identified *C. albicans* with excellent sensitivity and specificity, but revealed lower sensitivity for *non albicans candida*.[31] The data suggested that species which were identified by CHROMagar were almost the same as were confirmed by API 20C Aux. It means that in the presence of CHROMagar, germ tube test to confirm *C.albicans* can be excluded.

On completion of study it is acknowledged that CHROMagar medium is appropriate and affordable diagnostics medium in a resource-limit setting because approximate cost per culture for complete identification of *Candida* using SDA, Corn meal agar, and API 20C Aux in Pakistan is around Rs. 1,200 while CHROMagar *Candida* is around Rs. 250 per specimen culture. Additionally, 4 - 5 samples can be inoculated on one CHROMagar plate without compromising its effectiveness that makes it more cost efficient.

Present study supports the statement that the most suitable and popular procedure for *candida* species identification is the use of commercially available kits for carbohydrate assimilation and / or enzyme detection.[31] In the present work, all *Candida* isolates were correctly identified to the species level by API 20C AUX. Results of present study about effectiveness of API 20C are in accordance with other studies.[32,33,34,35] API tests are evaluated as best methods for the final documentation of all *Candida species* but owing to the fact that in our present hospital settings where limited resources are available this technique is difficult to practice on routine basis. Comparison of CHROMagar and API 20C AUX (Biomerieux, France) reveals that use of CHROMagar is less costly while API identification

method is costly and laborious but API 20 C has an advantage that it can identify more species of *non albicans Candida* as compared to CHROMagar, which can identify only three to four commonly involved *Candida species*.

## Conclusion

This study reveals CHROMagar *Candida* and API 20C AUX having good potential to rapidly identify *candida species*. In resource limit settings, CHROMagar can be used as useful adjunctive medium in the clinical laboratory but for identification of yeasts at species level, the use of API 20C Aux with a wider database is preferable. API 20C is better for the diagnosis of candidiasis especially due to less frequently found *candida* species and should be adopted as routine diagnostic procedure in the clinical microbiological laboratories.

This study has involved evaluation of two phenotypic methods in identification of *candida* species in small number of cases with vaginal *candidiasis* because of cost and availability issues. Future studies including large sample size and comparison of these two methods with other diagnostic methods used for identification of *candida* species are needed. Moreover comparison and evaluation of these two methods in identifying *candida species* in cases other than vulvovaginal *candidiasis* are also recommended.

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ORIGINAL ARTICLE

# Determination of Mean Distance of Obturation from Radiographic Apex by Using Electronic Method of Working Length Measurement

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**ABSTRACT**

**Objective:** The aim of the study was to determine the mean distance of obturation from the radiographic apex, in maxillary and mandibular teeth, using electronic apex locator for working length measurement.

**Study Design:** Cross sectional study.

**Place and Duration of Study:** The study was conducted in the Department of Operative Dentistry, at Islamic International Dental Hospital, Riphah International University from September 1st, 2016 to March 15th, 2017.

**Materials and Methods:** The mean distance of obturation from radiographic apex was evaluated in 97 canals of 43 patients, between 14 to 55 years of age. Working length was determined by using Dentaport ZX apex locator. Canals were prepared by using step back technique and obturated by cold lateral condensation technique. Postoperative radiographs were taken by using paralleling technique and the results were evaluated in SPSS version 24.

**Results:** The mean distance of obturation from radiographic apex was found to be -0.52mm with standard deviation (SD) of + 0.57.No statistically significant difference was found between the apical limit of canal filling on the basis of tooth vitality and tooth type. (P-value > 0.05).

**Conclusion:** This study suggested that appropriate use of apex locator can decrease the required number of radiographs during endodontic treatment and can be used reliably with no statistically significant difference in mean distance of obturation from radiographic apex when used in maxillary and mandibular teeth.

**Key Words:** *Electronic Apex Locator, Obturation, Working Length Determination.*

## Introduction

An accurate working length determination is one of the critical steps in the endodontic "Triad of success" thorough microbial disinfection, ideal canal preparation and hermetic seal. It determines how far the canal preparation and later on obturation should be extended.[1,2] Inaccurate working length determination can lead to iatrogenic errors, patient discomfort and possible infection. It is generally agreed upon that canal preparation and root canal filling should end at or short of apical constriction.[3] Moreover, optimal healing occurs when instrumentation and filling is contained within the region of apical constriction. Conventional methods used for working length determination are use of anatomical averages and knowledge of anatomy, tactile sensation, moisture on paper point and radiography.[4]

Radiographic method is the most common method for working length determination, however, this method has limitations.[5] Radiographic method, provides an estimation of the apical constriction which is histological landmark. Although clinically beneficial, averages utilized to define the radiographic apex from apical constriction could result in over filling or under filling of canal.[6] Additionally, radiation hazard both to patients and dental personnel is one of limitations of radiography.[2] This led to the development of electronic apex locators (EALs), which has helped in making the assessment of working length more accurate and predictable.[7] Most electronic apex locators are based on the theory of Sunada.[8]

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*Funding Source: NIL; Conflict of Interest: NIL*
*Received: Jan 26, 2018; Revised: Jun 02, 2018*
*Accepted: Jun 04, 2018*

Dentaport ZX (J.Morita Corp, Tokyo, Japan) is an impedance type locator[7,9] and has proved effective in working length determination even in the presence of different electrolytes, blood and irrigant solutions in the canals[10,11] Moreover, electronic apex locators have demonstrated efficiency in teeth with difficult canal morphology[12], intracanal exudate[13], large apical foramen[14] or periapical lesion.[13]

Current literature suggests that apex locators should be considered as useful adjunct to the radiographic method, not its replacement, and it improves the accuracy of working length determination.[7,15] Additionally, use of EALs could possibly decrease the quantity of radiographs taken for the determination of working length.[2] Even though new generations of apex locators give accurate reading, current practice is to confirm electronic reading, radiographically.[2] On the other hand, completing endodontic treatment without the need for a working length radiograph would not only save the time and cost of root canal treatment but will also significantly reduce the radiation exposure of the patients. The current study helped to ascertain, how the use of EALs alone for working length determination affect the extent of root canal filling. The objective of the study was to determine the mean distance of obturation from the radiographic apex, in maxillary and mandibular teeth, using electronic apex locator (Dentaport ZX) for working length measurement.

## Materials and Methods

The cross-sectional study was conducted in the Department of Operative Dentistry, at Islamic International Dental Hospital (IIDH), Riphah International University from 1st September, 2016 to 15th March, 2017. Sample size was calculated using World Health Organization sample size calculator with confidence interval=95% .Estimated population mean was 0.5, estimated standard deviation was $0.5^2$ and absolute precision required was 0.1. Sample (n) was comprised of 97 canals of 43 teeth. Non-probability consecutive sampling technique was used. The study was approved by ethical review board of IIDH. All teeth requiring endodontic treatment with completely formed roots were included in the study. Teeth with resorbed roots/open apices, previously endodontically treated, calcified canals and patients with heart pacemaker were excluded. In the present in vivo, ex vivo study 97 canals of 43 patients, between the age group of 14 to 55 years were studied. Both male and female requiring root canal treatment of single and multi-rooted teeth were treated by following the procedure as mentioned below. An informed written consent of the patient was obtained. A standardized data collection proforma was used for recording the required details. A preoperative periapical radiograph placed in a film holder (Hawe X-Ray Film Holder System) was taken by paralleling technique.[16] After administration of local anesthesia standard access cavity was prepared.[17] Tooth was isolated by rubber dam and vitality was assessed on the basis of bleeding on initial instrumentation of canal. The irrigation of the pulp chamber was carried out by 2.5% sodium hypochlorite solution and chamber was dried by performing aspiration.

After that Dentaport ZX, third generation apex locator was used on EMR Mode (electronic measurement of root canal mode). The size 15 k file (Mani) was advanced into the canal and apical line indicator on the LED of the apex locator was adjusted at 0.5 position. The rapid tone was noted with the illumination of LED green light at 0.5 position. After that silicone stopper was adjusted against reference point. The distance from file tip and silicone stopper was then measured and registered as working length. Cleaning and shaping was done using k files size 15-40 (Mani) using a step back technique. During cleaning and shaping, sodium hypochlorite was used as an irrigant in 2.5% concentration. Subsequently, paper points were used to dry the canal prior to obturation. Obturation was performed by utilizing gutta percha and endomethasone sealer with cold lateral compaction technique. A postoperative periapical radiograph was taken by paralleling technique. Endodontic treatment was completed within 1 to 3 visits depending on pathological status, patient's cooperation, time available and difficulty of case. Post obturation periapical radiographs were evaluated on radiograph illuminator by using 2.5x magnifying loupes (keeler SuperVu Galilean loupe, 18 inches working distance). Distances were measured in millimeters from the end of obturation to radiographic apex of tooth to an accuracy of 0.5 mm twice, at two separate occasions. The mean of two readings was calculated and recorded. A negative sign (-ve) was used if obturation was short

of apex and positive (+ve) sign was used if obturation extended beyond the apex. All the collected data was analyzed using Statistical Package for Social Sciences (SPSS version 24). Descriptive statistics were used. Mean and standard deviation was calculated for obturation length measurements (in millimeters). Frequency and percentages were calculated for gender, age, status and type of tooth. Independent sample t-test was utilized to determine the difference between mean distance from tip of root canal filling to radiographic apex in vital and non-vital teeth and for calculating the p-value. ANOVA test was used to determine the difference between mean distance from tip of root canal filling to radiographic apex in anterior, premolar and molar teeth.

## Results

The total number of patients who were assessed for eligibility was 70, out of which 25 patients were excluded as they did not meet the inclusion criteria. Of the 45 patients enrolled for the study 2 patients were excluded from the study one because of instrument breakage during canal preparation and other because of inability of an instrument to reach the apical foramen, resulting in 43 teeth with 97 canals for the outcome analysis. The age of the patients ranged between 14 years and 55 years as shown in fig 1.

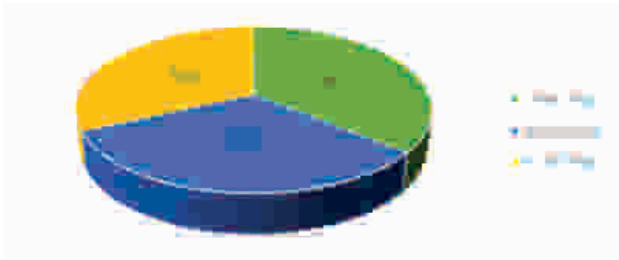

**Fig 1: Age Distribution**

The mean age was found to be 33.83 years with the standard deviation of 13.93. Out of 97 patients, 61 were males and 36 were females. Canals of molar teeth were comparatively more, 74 in number, followed by anteriors and premolars which were found to be 12 and 11 in numbers, respectively. The distance of obturation from radiographic apex was calculated at two different occasions and their means were calculated for all 97 canals. The mean of two readings was entered in SPSS version 24 and the mean of all 97 readings was found to be -0.52mm and standard deviation was found to be $\pm$ 0.57mm.

**Table I: Comparison between Mean Distances of Obturation from Radiographic Apex in Vital and Non-Vital Teeth**

| Distance from radiographic apex | Tooth Status | N (%) | Mean | SD | P-value |
|---|---|---|---|---|---|
| | Vital | 47 (48.5%) | -0.43 | ± 0.47 | 0.135 |
| | Non vital | 50 (51.5%) | -0.60 | ±0.64 | |

Using independent t-test it was found that there was no statistically significant difference between mean distance from tip of root canal filling to radiographic apex in vital and non-vital teeth (p value > 0.05) , as shown in the table I.

**Table II: Comparison between Mean Distances of Obturation from Radiographic Apex in Different Teeth Type**

| Tooth type | n (%) | Minimum | Maximum | Mean | SD | P-value |
|---|---|---|---|---|---|---|
| Anteriors | 12 (12.4%) | 0.00 | 1.5 | -0.52 | ±0.43 | 0.202 |
| Premolars | 11 (11.3%) | 0.00 | 0.60 | -0.23 | ±0.21 | |
| Molars | 74 (76.3%) | 0.00 | 3.00 | -0.56 | ±0.62 | |
| Total | 97 (100%) | 0.00 | 3.00 | -0.52 | ±0.57 | |

The mean distance from the radiographic apex to the tip of root canal filling was found to be -0.52 mm, -0.23 mm and -0.56 mm for anteriors, premolars and molars, respectively. Using ANOVA test, it was found that there was no statistically significant difference between mean distance from tip of root canal filling to radiographic apex in anterior, premolar and molar teeth (p-value=0.202), as shown in the table II. The mean distance from tip of root canal filling to radiographic apex was found to be - 0.49 mm for maxillary teeth and -0.55 mm for mandibular teeth with standard deviation of ±0.56 and ±0.59, respectively. Using independent t-test it was found that there was no statistically significant difference between mean distance from tip of root canal filling to radiographic apex in maxillary and mandibular teeth (p value > 0.05) , as shown in the table III.

**Table III: Comparison between Mean Distances of Obturation from Radiographic Apex in Maxillary and Mandibular Teeth**

| Distance from radiographic apex | Arch | N(%) | mean | Std Dev | p-value |
|---|---|---|---|---|---|
| | Maxilla | 47 (48.5%) | -0.49 | ±0.56 | 0.618 |
| | Mandible | 50 (51.5%) | -0.55 | ±0.59 | |

## Discussion

The utilization of electronic apex locators for determination of working length has increased substantially in the current years[2] and they have been increasingly incorporated into the modern practice of endodontics.[18] The purpose of the present study was to determine the mean distance of canal filling from the radiographic apex, using electronic apex locator (Dentaport ZX) for working length measurement.

Several researchers in the past have worked to evaluate the accuracy of apex locators. Researchers utilized teeth which were to be extracted for comparing the electronically determined length to various reference points, such as radiographic apex, apical foramen and apical constriction in different in vivo studies[19,20,21] On the other hand, electro conductive material like saline was used for simulating the clinical conditions in the in vitro studies.[22] Contrary to the aforementioned studies, the present clinical study is true representative of what occurs during typical endodontic treatment, thereby, incorporating errors that might occur in the oral cavity.[2]

In the present study the mean distance of obturation from radiographic apex was found to be -0.52 mm. The findings were in line with the results of the study conducted by L Smadi et al[2] who found the mean distance of obturation from radiographic apex as -0.5mm when only EAL was used for working length determination. Obturation length found in the present study was within acceptable clinical limit as concluded by Fouad and Reid.[15] Fouad and Reid[15] deemed root canal filling as acceptable when it was found to be 0 to 2mm short of radiographic apex. Likewise, Ravanshad et al[23] in their research concluded that there was no difference in the radiographic length measurement of root canal filling of two groups, when working length was determined by EAL only and Radiograph only.

Overfilling of canals was not noted in the current study. The results are clinically acceptable as they are supported by the meta-analysis by Schaeffer et al[24] in which it was found that most successful endodontic treatments are those in which obturation is within 1mm of radiographic apex followed by those which ends within 1 to 3 mm and both were found superior to obturation beyond the apex in terms of success. Likewise, Swartz and colleagues[25] in their study concluded that the chances of failure of root canal treatment increases to four times when canal is overfilled as compared to under filled canal. However, Halse et al[26] studied canals, which were slightly overfilled radiographically, 10 to 17 years after obturation and gave the conclusion that obturation with filling material in slight excess generally results in successful endodontic treatment. Contrary to this, Kojima et al[27] and Wu et al[28] found higher success rates when obturation was close to or at the radiographic apex.

In the current study, the preoperative pulpal status did not seem to influence the functionality of the Root Zx apex locator. Therefore, the difference in the apical limit of canal filling in the teeth with vital and non-vital pulp was found to be statistically insignificant. Similar findings were reported in previous studies.[2,19,21] Conversely, Pommer and colleagues[29] noted higher accuracy of apex locators in the teeth canals with vital pulp tissue. However, recent meta-analysis[30] concluded that vitality of the pulp have no influence on the accurate functioning of electronic apex locators.

The results of the present study suggest that apex locator can reliably be used as a method of working length determination and in future can replace radiographic method as a mean of working length determination. This suggestion would adhere to As Low As Reasonably Achievable principle[31] (ALARA) in relation to radiographic exposure at one end and greater accuracy of modern apex locators as concluded in other studies[19,23] at the other end. This suggestion, however, is not in keeping with the suggestion of Hoer and Attin[32] who suggested that apex locators should always be used in combination with radiographs. Alternatively, Saad and al-Nazhan[33] argued that it is possible to perform successful endodontic treatment by utilizing only apex locator for working length determination.

The small sample size of 97 canals is one of the limitations of this study. This sample size did not allow detailed analyses of results. Increasing the number of teeth will give more valid and reliable statistical data. The other limitation is that this is single arm study and comparison is not made by using the gold standard method .i.e. radiographic method as a method of working length

determination to evaluate the difference between two methods. Lastly, only one brand of apex locator (Root Zx) was used in the current study; therefore, the results cannot be generalized to all the apex locators. Further studies, with larger sample size and multiple brands of electronic apex locators under varying clinical conditions, are suggested to verify the results of current research

## Conclusion

The routine practice of utilizing apex locator for working length determination is reliable and appropriate with no statistically significant difference of the apical extent of root canal filling in the teeth with vital or non-vital pulp. Within the clinical setting of the current study, it is proposed that accurate use of electronic apex locator alone can limit the need of taking diagnostic radiographs for measurement of working length. Subsequently, it will also reduce the radiation exposure of patients.

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ORIGINAL ARTICLE

# Factors Influencing the Student's Learning and Motivation in a Blended Learning Approach Used in MHPE Program: A Qualitative Analysis

Ayesha Naveed[1], Raheela Yasmeen[2], Amer Siddiq[3], Barik Hassan Ahmed[4]

**ABSTRACT**

**Objective:** To explore the factors that influence the postgraduate students' learning and motivation in a blended learning approach used in MHPE program.

**Study Design:** It was a Qualitative, Phenomenology study.

**Place and Duration of Study:** This study was conducted at three different institutions of Pakistan who are running their Masters in Health Professions Education Program (MHPE) and duration was from March 2017 to August, 2017.

**Study Population:** Sampling technique was purposive homogenous sampling, and the interviews were taken from 10 postgraduate (MHPE) students from three institutions of Pakistan.

**Materials and Methods**: Qualitative data was collected through one on one semi structured interviews. Interview questionnaire was formulated after going through extensive literature research and expert validation. All interviews were audio recorded, transcribed, coded, analyzed manually as well as by importing it into NVivo software version 11 and thematic analysis was done.

**Results:** Thematic analysis revealed many factors which influenced students' learning in a meaningful way. These factor were clustered into six major areas which are; 'Active learning environment', 'Role of course design and educational psychology', 'Role of teachers', 'Role of assessments', 'Learners satisfaction and motivation for learning' and 'learning hampering factors' on the basis of similarities with each other.

**Conclusion:** Students hold a positive perception of Blended Learning Approach being offered in the MHPE program. New learning skills were acquired by the students and motivation was maintained throughout the course. Students were also able to transfer these learning skills to their undergraduate students. Research findings suggests that a Blended learning approach course design can be successfully used in postgraduate education. This study provides guidance for faculty members, educators and curriculum planners to effectively plan their modules while incorporating a Blended learning approach in medical education.

**Key Words**: *Blended Learning Approach, Postgraduate Programs, Student Learning and Motivation.*

## Introduction

In recent years, medical education has undergone an exceptional change with more focus on interactive and student-centered learning. As students became more computer literate, and the demand for technology-based learning at a time convenient to the learner increased, it led to technological modernization and the use of e-learning tools which has become a key component of medical education.[1] While these virtual learning opportunities have enhanced the prospects, they have also posed challenges to education. Key limitations included limited interactions with peer and teachers, communication problems, and insufficient sense of bonding between the teacher and student. This has led to the adoption of “blended learning (BL)”, by combining the strengths of computer-mediated instruction and face-to-face class, blended-learning environments can produce improved student outcomes and facilitate acquisition of competencies

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*Funding Source: NIL; Conflict of Interest: NIL*
*Received: Nov 07, 2017; Revised: April 18, 2018*
*Accepted: April 28, 2018*

that may not otherwise be achieved. This approach increases student engagement, enhance critical-thinking development, and improve learning outcomes.[2] Blended learning approach supports learners' needs for cognitive and social presence and rely on a strong teaching presence by instructor.[3] The main idea behind this approach is that the design should be focused on learner directed activities.[4]

Faculty make use of "blended" virtual and physical resources to welcome different learning styles and speed of cognitive learning. Learning material can be presented in a variety of formats, each reinforcing another and can utilize subject experts for delivery of each unit. This variety in delivery methods, ranging from experiential to instructional and structured to unstructured, can revitalize subjects that have lost their appeal or are emerging in our country, like Health Professions Education.[5] This nontraditional format allows professionals especially from out of state, to pursue their degrees while keeping full time jobs. Blended learning is effective in the field of medical education as it can fill the gap between theory and practice so encourage the learner to solve problems and exchange experiences.[6]

However, attention should be paid to the andragogy used, and not just the technology because the expectations of students and the methods they use to benefit from can differ.[5,7]

This study gives an idea of the extent to which students are developing in a professionally meaningful way, how their learning journey in this blended environment was, and how they acquired self-directed, and lifelong learning skills.

## Materials and Methods

The qualitative phenomenology study was conducted in three universities of Pakistan from March 2017 to August 2017. It comprises of 10 Masters' students from Health Professions Education Program who were selected after purposive homogenous sampling. They underwent one to one interviews for which semi structured, open ended questionnaire was used, which was formulated after going through literature search. Primary areas of interest related to our research question were identified and questions were formulated around those areas to explore them into more details, to find out the answer to our research question. This questionnaire was sent to 5 different subject specialists in medical education for validation to improve the validity and credibility.

A pilot interview was conducted with one of the student, using validated questionnaire comprising of 9 open ended questions before actual interviews, to improve the quality and sequence of questions. This interview lasted for 20-25 mins.

The research question and the tool were aligned. Data was transcribed and deductive coding was done[8,9] followed by thematic analysis[10,11] of the data and common categories were identified. They were grouped into six major areas based upon similarities which highlighted the factors that influenced students' learning and motivation in a Blended Learning Approach Master's Program, and answered our research question.

Triangulation was done by taking Field notes, Member checking, and Inter interviewee comparisons.

**Ethical Considerations**

Informed consent was obtained from all participants. They were informed about the voluntary nature of participation. The participants were assured of anonymity, confidentiality and security of information. They were also assured of information about the results of the study if so desired by anyone. No reward or payment was assured to any of the participants.

**Table I: Interview Questions**

| |
|---|
| **Student expectations and satisfaction with the course** |
| Why have you joined this course, what were your expectations with this course? |
| **Educational psychology and principles of adult learning** |
| How do you find this course design relevant to your need as a learner? If no why? |
| **Students' Learning** |
| How this program (f2f or online) enhanced your learning and in what way? |
| PROBE: What type of learning is promoted and how? Deep, superficial, strategic |
| **Student Motivation** |
| How the blended learning approach in this course influenced your motivation to learn the subject matter? |
| **Influence of Educational Environment on Students Motivation to Learn** |
| How educational environment created in this course |

influenced your motivation to learn?
PROBE: Is the environment conducive to your learning?
Does it promote collaborative learning? Does this promote peer to peer learning?
Which type of learning does this educational environment promotes? (deep, superficial, strategic)

**Role of Teachers**

How teachers played their roles in students learning?
PROBE: What is the student teacher relationship in this blended learning environment?

**Role of Assessments**

How assessment methods used in this BLA postgraduate program influenced your learning?
PROBE: Which assessment methodology enhanced your learning more and in what way?

**Strength and Weaknesses of the Blended Learning Environment**

What was the overall strength of the blended learning environment?
How this blended learning environment hampered your learning?

## Results

Themes/factors identified were grouped under 6 major areas, each area comprising the factors which influenced students' learning in blended postgraduate program, as shown in figure below:

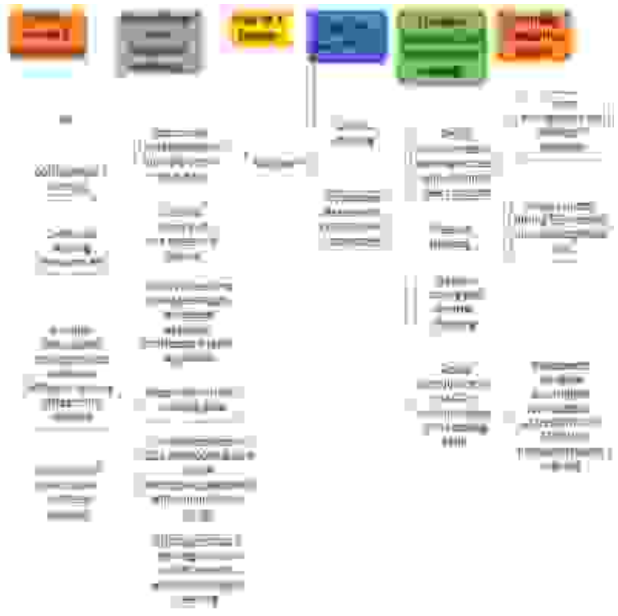

**Fig 1: Major Areas and the Factors Influencing Students' Learning and Motivation in a Blended Learning Approach Master's Program**

Given below in table are the themes along with quotes from the participants:

**Table II: Factors Influencing Students' Learning and Motivation in a Blended Learning Approach Post Graduate (MHPE) Program**

| Major area | Active learning environment | Participants Quotes |
|---|---|---|
| **Themes** | 1. P2P learning and experiential learning enhanced students learning | "This course promoted peer to peer learning a lot, I think it is the only beauty of this course and it makes us easy to learn from each other's experiences" |
| | 2.Collaborative learning and social interactions enhanced interest and motivation | "Our group was very good we used to discuss things, there was a good collaborative learning environment each one of us has his own strength and learning style in this way we learned lot and this kept us motivated throughout" |
| | 3. Conducive and social constructivist learning environment kept them motivated | "Learning environment was very conducive all physical, teachers and group dynamics were very good, this educational environment has a major role in keeping us motivated" |
| | 4.Blend of instructional strategies addressed different learning styles of students and kept them engaged | "there was diversity of teaching style, and blend of instructional strategies, which addressed different learning style of the students, this change of flavor in the teaching sessions kept us engaged and maintained our interest in the sessions throughout" |
| | 5. Constructivist and student centered approach in this blended program addressed learners' needs | "Most of the adult learning principles are followed in this course. Constructivist approach, relevance, participation, student centered learning, students autonomy all were there and I find them as very much relevant to my need as learner, we were trained how to apply that knowledge in practical scenario and Because of this course the traits of lifelong learner has been acquired by me" |

| Major area | Course design and educational psychology in BLA | Participant Quotes |
|---|---|---|
| Themes | 6.instructuctional strategies helps in applying theory into practice at workplace | "I liked this blended course design because all the tools used for the instructional strategies were applied on us and we had fair enough idea how to apply them on our student and how they will be perceived by the students. It has improved my teaching a lot and I really enjoyed the course a lot" |
| | 7.Course relevancy to the needs of a learner as self-directed learner, and learning through collaboration and social interaction | "this courses design is relevant to my need as learner because it give me a chance to learn both in campus activities from teacher and peer as well as to involve self-directed learning while we are off campus" |
| | 8. variety of learning strategies taught in F2F and off campus increased academic confidence and self-regulation | "It promotes all sort of learning, deep, superficial and strategic. We learned which strategy is to be applied when according to the type of learning relevant to us, we learned how to self-regulate our learning and this variety in the teaching sessions increased our academic confidence" |
| | 9. Acquisition of new learning skills helps in achieving the trait of | In this blended course we learned metacognitive skills interactive teaching, critical |
| | lifelong and active learner. | thinking so we were able to deliver a more interactive lecture rather than a simple lecture and we developed the traits of life long learner" |
| | 10. Social interaction in small group activities developed skills of active participation, leader ship and communication | "In task based group learning we used to learn from each other by participating, we developed leadership qualities and improved communication skills. small group learning is the main strength of this course" |
| | 11. Principals of adult learning and theory of social constructivism followed in this blended course facilitated students' learning | "Feasibility, time management cost effectiveness, and Promotion of adult learning in both aspect of this blended program are the main strengths of this program" |

| Major areas | Role of a teacher | Participants Quotes |
|---|---|---|
| Themes | 12. Teachers as facilitators helped to develop the traits of independent and lifelong learners | "Role of teachers is like a facilitators, we had a very cordial relationship with our teachers, scaffolding is always there They guide us whenever we need them, it is an adult learning environment and we have to move on with our own journey as a lifelong learner" |
| **Major areas** | **Role of assessments** | **Participant Quotes** |
| Themes | 13. Assessment drives learning through incorporation of formative assessments and constructive feedback by the teachers | "Assessments drives our learning and especially the formative assessment and feedback given by the teacher improved our learning" |
| **Major areas** | **Learners satisfaction and motivation for learning** | **Participant Quotes** |
| Themes | 14. Learners' satisfaction achieved through active involvement in the learning process, | "I am very much satisfied with this program because I gained more than what I expected from this program as it is student centered, |
| | exploration of the concepts, creative thinking, active communication in SGs, and improvement in teaching skills | active learning process, and more exploratory. Creative thinking, communication skills, teaching skills improved, we get a chance to learn from our facilitators along with a chance to be more self-directed and active learner" |
| **Major areas** | **Learning hampering factors in BLA** | **Participant Quotes** |
| Themes | 15. Time management, meeting of deadlines, and Heavy projects increased the cognitive load, due to detachment of teacher in distant sessions. | "limitation is the time management , we fought through out with the time, deadlines do not let you learn freely as there was a cognitive overload too much information in short period of time, This was disturbing for us" |
| | 16. Assessments designed according to principle of ASSESSMENT OF LEARNING hampers the students' learning | "summative assessment need a revision whether there should be summative exams or not, and new assessment methodologies should be employed rather than those traditional ones" |

## Discussion

Various factors influencing the students' learning identified through this study are presented in a form of a model in which various theories of teaching and learning are also imbedded. This schema can be used at level of curriculum planning, and individual teaching and learning. The role of the institution is to ensure that the time and resources are available for effective learning to happen. (See below fig 2).


**Fig 2: Conceptual Model of the Study**

Participants of this study were satisfied and thought that this blended learning strategy helped them to learn new skills like problem solving strategies, metacognition, critical thinking, reflection, creative thinking, and deep as well as strategic learning, which improved their learning and helped them as an active and lifelong learner. The feedback from the study was mainly positive and the factors which enhanced students learning were the blended course design, and students' autonomy. Student in our studies were professionals and they considered this blended course relevant to their needs as a leaner as it allowed them to learn with more freedom and autonomy, as supported by Knowles theory of adult learning.[12] Other factors include variety of teaching and learning strategies, acquisition of new learning skills like metacognition and reflection, role of a teacher as a facilitators, constructivist, student centered, collaborative, and conducive learning environment. Continuous scaffolding was provided by teachers, and student learnt by active participation. Peer to peer learning, and learning from experience of others. Social interaction with teachers and other learners plays a fundamental role in the development of understanding[13] which is supported by Vygotsky theory of social constructivism (1978)[14] Social constructivists believes that the process of sharing individual perspectives called collaborative elaboration[15] results in learners understanding and this construction cannot be achieved by individual learning. Feedback from peers and teachers followed by reflection helped to develop the skills of critical thinking and problem solving, consequently becoming a reflective learner. Hands on activities and learning by active participation in face to face sessions, small group activities and blended course design were considered as the main strength of this blended learning environment. Thus, all of the principles of adult learning were well incorporated in this learning approach. The blended learning format allowed participants to combine work, studies, and private life. Improved possibilities for anytime and anywhere freedom was considered as essential. Pahinis et al recorded a similar positive response among postgraduate dental students at the end of a blended learning course taught to different groups of learners in a dental school.[16]

Similarly, when the other studies were analyzed the great majority of the students were happy and wanted the blended approach to be more widespread.[17]

This study suggests that students prefer face-to-face interactions over online communication mechanisms. Students understood the course concepts better in face to face sessions than in virtual or eLearning environment. This is because they learn by interacting with facilitators and by experience, this finding is supported by Experiential learning theory (Kolb, 1974)[18] It describes how learners learn from experience. Whereas, Contrary to this, in previous studies there were different perspectives of the students. In some of the studies it is found that the online components of blended cardiovascular pharmacotherapy course were well received by students and enhanced long term knowledge.[19] Whereas, on the other hand Boje et al found that although students were enthusiastic about use of a virtual biotechnology laboratory in a blended pharmacy course, they preferred face to face

discussion over asynchronous discussion boards.[20, 13] Although online discussion forums are effective at promoting reflective activities, face to face discussions are better suited to situations where students are learning directly from each other or from an expert instructors.[21]

At last the limitations of this blended learning approach; Students' concerns about blended learning increases at times due to concerns of time management, meeting deadlines and pressures of completing online course components/assessments. Another factor is to get most out of campus course tutorials, and getting prepared for 'assessment of learning'. Moreover they showed criticism towards detached course during off campus sessions from facilitators which may be the main reason of cognitive overload, as teachers played a major role in keeping students motivated and facilitated them in their learning journey. They suggested that there is a need to divide information into chunks and deliver them in pieces or intervals with comfort breaks. A final issue worth mentioning was the student's access to online learning resource, articles and journals. They should be given full access to online data bases in order to improve their learning via best evidence based literature available on line.

## Conclusion

Students showed both evidence of learning and enthusiasm for the blended programs as they learned in a cooperative way with their peers and thus outshined academically.

Study findings suggests that a blended learning format can be successfully integrated into postgraduate learning programs in Pakistan as it provides a favorable transition to an enhanced flexibility, location convenience, and time efficiency.

There were many factors which helped students learning and maintained rather enhanced their motivation in this program like blended course design, constructivist and student centered learning environment, student autonomy, collaboration and interactive learning with peers and teachers, scaffolding from facilitators to make them self-regulated, active and lifelong learners. Their learning was driven by the formative assessments and timely feedback provided by the teachers, in face to face sessions. They learned self-regulation, active participation, communication, and leadership skills both in distant and face to face sessions. Their learning was hands-on and based on experience. Variety of teaching tools used in this blended format and blend of this in campus and off campus learning activity kept them engaged, addressed various learning styles and theories, and satisfied their needs as a learner. In short, principles of adult learning were very well applied in this course both in off campus and in campus sessions and helped them not only to gather knowledge, but also to understand, apply, and transfer it to new settings. So acquisition, application and transfer of knowledge helped the future educators to develop academically in a more effective way.

**Recommendations from the Study**

Students' representations should be the policy of the program in curriculum renewal of postgraduate (MHPE) program.

**Future Study**

The opinions and satisfaction levels of the instructors using the blended learning environment could be also examined, so as to more fully optimize the potential benefits of new education technology.

**Limitations**

Population size may be increased in future studies. Very few participants could be approached as all of them were busy professionals and there were time limitations, and feasibility issues regarding approaching them.

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**DISSERTATION/THESIS BASED ARTICLE**

An article based on dissertation submitted as part of

the requirement for a Fellowship can be sent for publication after it has been approved by the Research and Training Monitoring Cell (RTMC). The main difference between an article and dissertation is the length of the manuscript. Dissertation based article should be re-written in accordance with the instructions to author

**TABLE AND ILLUSTRATIONS**

Tables and illustrations should be merged within the text of the paper, and legends to illustrations should be typed on the same sheet. Table should be simple, and should supplement rather than duplicate information in the text; tables repeating information will be omitted. Each table should have a title and be typed in double space without horizontal and vertical lines on an 8 ½" x 11' paper. Tables should be numbered consecutively with Roman numeral in the order they are mentioned in the text. Page number should be in the upper right corner. If abbreviations are used, they should be explained in foot notes and when they first appear in text. When graphs, scattergrams, or histogram are submitted, the numerical data on which they are based should be supplied. All graphs should be made with MS Excel and be sent as a separate Excel file even if merged in the manuscript. For scanned photographs highest resolution should be used.

**S I UNITS**

System International (SI) Unit measurements should be used. All drugs must be mentioned in their generic form. The commercial name may however be mentioned within brackets, if necessary.

**FIGURES AND PHOTOGRAPHS**

Figures and Photographs should only be sent when data cannot be expressed in any other form. They must be unmounted, glossy prints in sharp focus, 5"x7" in size. These may be in black & white or in colour. Negatives, transparencies and X Ray films should not be submitted. The number of figure, the name of the author(s) should be printed on the back of each figure/photograph. The top of the figure must be identified by the author. These figures and photographs must be cited in the text in consecutive order. Legends must be typed on the same paper. Legends for photomicrographs should indicate the magnifications, internal scale and method of staining. Photographs in published articles will not be returned.

**REFERENCES**

References should be numbered in the order in which they are cited in the text. At the end of the article, the full list of references should give the names and initials of all authors (unless there are more than six when only the first six should be given followed by et al). The author's names are followed by the title of the article; title of the journal abbreviated according to the style of the Index Medicus (see "List of Journals Indexed", printed yearly in the January issue of Index Medicus); year volum and page number; e.g. Hall, RR. The healing of tissues by C02 laser. Br J. Surg: 1970; 58:222-225. References to books should give the names of editors, place of publication, publisher and year. The author must verify the references against the original documents before the article.

**ABSTRACT**

Abstracts of original article should be in structured format with following sub-headings: i. Objective, ii. Design, iii. Place & Duration of study iv. Materials & Methods, v. Result, vi. Conclusion. Four elements should be addressed: why did you start, why did you do, what did you find and what does it mean. Why did you start in the objective. What did you do constitutes the methodology and could include design, setting, patients or other participants, interventions, and outcome measures. What did you find is the results, and what does it mean would constitute; our conclusions. Please label each section clearly with the appropriate sub-headings. Structured abstract for an original article, should not be more than 250 words. Review article, case report and other requires a short, unstructured abstract. Commentaries do not required abstract.

**INTRODUCTION**

This should include the purpose of the article. The rationale for the study or observation should be summarized; only strictly pertinent references should be cited; the subject should not be extensively reviewed. Data or conclusions from the work being reported should not be presented.

**MATERIALS AND METHODS**

Study design and sampling methods should be

mentioned. Obsolete terms such as retrospective studies should not be used. The selection of the observational or experimental subjects (patients or experimental animals, including controls) should be described clearly. The methods and the apparatus used should be identified (with the manufacturer's name and address in parentheses), and procedures described in sufficient detail to allow other workers to reproduce the results. References to established methods should be given, including statistical methods; references and brief descriptions for methods that have been published but are not well known should be provided; new or substantially modified methods should be described, giving reasons for using them, and evaluating their limitations all drugs and chemicals used should be identified precisely, including generic names(s), dose(s) and route(s) of administration.

**RESULTS**

These should be presented in logical sequence in the text, tables and illustrations. All the data in the tables or illustrations should not be repeated in the text; only important observations should be emphasized or summarized.

**DISCUSSION**

The author's comment on the results supported with contemporary references, including arguments and analysis of identical work done by other workers. A summary is not required Brief acknowledgement may be made at the end.

**CONCLUSION**

Conclusion should be provided under separate heading and highlight new aspects arising from the study. It should be in accordance with the objectives.

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