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# Teaching Professional Ethics in Undergraduate Medical Education: An Islamic Perspective

Khalid Farooq Danish[1], Usman Mahboob[2], Rahila Yasmeen[3]

## Teaching Ethics to Undergraduate Medical Students

Pakistan is an Islamic country where overwhelming majority of undergraduate medical students are Muslims.[1] In our settings thus moral ethical values take their origin from religion and get reinforced by religious knowledge.[2] This fact is of great advantage to the educators in this country as they do not have to advocate too strongly the practice of ethical values. They only need to reinforce the faith of the students in order to ensure observance of ethics by the students. Similarly the concept of accountability is built-in to religious beliefs and does not need any different emphasis.[3] It appears that it will require strong role modeling for this purpose.[4,5] However, for those who have faith in Islam, the Holy Quran sets the Holy Prophet as the role model in these words:

لَقَدْ كَانَ لَكُمْ فِي رَسُولِ اللَّهِ أُسْوَةٌ حَسَنَةٌ لِمَنْ كَانَ يَرْجُو
اللَّهَ وَالْيَوْمَ الْآخِرَ وَذَكَرَ اللَّهَ كَثِيرًا ﴿٢١﴾

**033.21 Ye have indeed in the Apostle of God a beautiful pattern (of conduct) for anyone whose hope is in God and the Final Day, and who engages much in the Praise of God. [6]**

It is further reinforced by these words of the Holy Quran:

مَنْ يُطِعِ الرَّسُولَ فَقَدْ أَطَاعَ اللَّهَ

**004.080 He who obeys the Apostle, obeys God.[7]**

Whereas this fact does not lessen the requirement of an appropriate faculty development program for preparing role models, the very program has to focus on the Holy Prophet as the role model.

Here, it is pertinent to refer to the concept of ethics in Islam and its practice by the Muslim doctors. The basis of ethical concepts in Islam is not client satisfaction or professional responsibilities, but it is the divine teachings conveyed to the mankind by the Prophet Muhammad (PBUH).[8] This is in contrast to the usual perception that the Islamic ethics is based on Islamic law.[9] The origin of these concepts is not any philosophical thought but the code created by the Creator, Almighty Allah. A Muslim doctor is bound to abide by the ethical principles of Islam not by the reason of client satisfaction or merely good professional behavior but for the sake of his own eternal salvation.

In the words of the Holy Quran:

فَمَنْ يَعْمَلْ مِثْقَالَ ذَرَّةٍ خَيْرًا يَرَهُ ﴿٧﴾

**099.007 Then shall anyone who has done an atom's weight of good, see it![10]**

إِنَّ الَّذِينَ يَخْشَوْنَ رَبَّهُمْ بِالْغَيْبِ لَهُمْ مَغْفِرَةٌ وَأَجْرٌ كَبِيرٌ ﴿١٢﴾

بَلِ الْإِنْسَانُ عَلَىٰ نَفْسِهِ بَصِيرَةٌ ﴿١٤﴾
وَلَوْ أَلْقَىٰ مَعَاذِيرَهُ ﴿١٥﴾

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إِنَّمَا الْأَعْمَالُ بِالنِّيَّات

**Actions are dependent on intentions**[13]

These explanatory notes are intended to put the Islamic ethics in the right perspective before any detailed deliberations are added on the topic. Islamic medical ethics was documented formally by the chief Muslim physician of 970 A.D. Al-Tabari.[14,15] Since then many texts on Islamic bioethics have been documented.[16] Many of them interpret Islamic ethics in the perspective of philosophical ethical foundations.[2]

Consistent with aforementioned facts, the curriculum for medical ethics should be strongly need-based and should be supported by religious teachings in an Islamic state. Discrepancies in practice and principles need to be specifically recognized and addressed in the ethics curriculum.[17] The principle attributes of professionalism; subordination of one's self interests, adherence to high ethical and moral standards, responsiveness to societal needs, and demonstration of humanistic values can be best fulfilled in this manner.[18]

A proposed outline of a 5-year MBBS ethics course may look like this:

The table shows many teaching and learning strategies to teach Ethics however we have mentioned two teaching learning strategies that will be explained in detail. They are the 'one minute preceptor' (OMP), and critical incident meetings.

**Table I: A proposed outline of a 5-year MBBS ethics course**

| Years | Curricular content | Teaching strategies | Assessment tools |
|---|---|---|---|
| 1 | Basic concepts, religious principles, situational ethics related to ethical issues, dilemmas and conflicting situations. | Interactive lectures, small group discussions. | Written examinations: MCQs, EMQs, SAQs, SEQs. |
| 2 | | | |
| 3 | Application of ethical principles in patient encounters, demonstration of ethical behaviors, feedback on ethical issues. | Task-based learning (TBL), PBL, role plays, Case-based learning (CBL), critical incident meetings.[19] | OSCEs, peer assessment, patient assessment, multisource assessment. |
| 4 | Behavioral aspects of ethics, professionalism, application of ethics in patient encounters, ethical judgments in conflicting situations, ethics related communication skills | TBLs, OMPs, role plays, community based learning, reflection sessions on clinical encounters and critical incidents. | OSCEs, peer assessment, patient assessment, multisource assessment, professionalism mini-evaluation exercise (P-MEX).[20,21] |
| 5 | | | |

**One Minute Preceptor**

The OMP is a convenient learning strategy in which the teacher provides feedback to the student regarding a patient encounter that has taken place in the presence of the teacher and the teacher has an opportunity to watch the entire encounter.[22] Five simple steps are followed by the teacher in the OMP:[22]

1. Get a commitment
2. Ask for evidence
3. Teach a general principle
4. Appreciate good performance
5. Correct errors

The step 3 provides opportunity to a teacher to recognize and address an ethical challenge in the encounter and provide essential corrective or narrative feedback.

**Critical Incident Meetings**

The earliest studies related to critical incident technique arose from failure of the American pilots to learn flying.[23] Further studies continued in the same context of aviation psychology.[24] The critical incident technique in its developed form was described by Flanagan in 1954.[19] He defined an incident as “any observable human activity that is sufficiently complete in itself to permit inferences and predictions to be made about the person performing the act.”[19] In order to be critical the intent behind the act should be fairly obvious by the situation in which it occurs. In its practical form, the activity is carried out in a small group format in a safe educational environment. An ethically challenging situation is described as a critical incident and reflections are invited by the group participants.[25] There is no absolutely right or wrong answers to the situation and only opinions and points of view are

presented, discussed and reflected upon.[25] Development of a safe educational environment appears to be a problem.[26] It provides the participants discuss the issues of beneficence, non-maleficence, autonomy, and justice.[25]

**Assessing Professional Ethics**

Assessment instruments for professional ethics with good validity and reliability are scarce.[27,28] Some of these instruments for such assessments have been mentioned in the table I.

The appropriate assessment instrument for OMPs is OSCE. A short patient encounter may be thoroughly simulated in a tactfully prepared OSCE with a judicious use of standardized patients (SP). Standardized patients (SP) training may pose a problem particularly if specified behavior patterns are to be simulated, such as breaking bad news, obtaining informed consent for surgery, or conflict resolution.

Assessment of critical incident meetings may be multisource assessment, and peer assessment. Such an assessment may be useful for formative purposes but its use as summative evaluation appears to be difficult.[29]

**Conclusion**

This thesis on different aspects of ethics and professionalism explains the contextuality of teaching and assessing professional ethics from an Islamic perspective in a Muslim country. It appears that apart from religious teachings, human conscience has provided a great driving force behind the ethical thought. However, in an attempt to remain secular, most scholars have underrated the impact of divine teaching on human ethical values and behaviors including Muslim scholars. We perceive that the ultimate source of ethical values and human conscience is the divine teachings either in the form of religious texts or the so called intuitions. While discussing ethics and professionalism, they need to be given due regard. Moreover, this editorial may open new avenues in adding a moral aspect to the readers' identity.

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ORIGINAL ARTICLE

# Prevalence and Susceptibility Pattern of Methicillin Resistant Staphylococcus aureus (MRSA)

Abdul Bari Khan, Uzma Mussarat


## ABSTRACT

**Objective:** To determine the prevalence and susceptibility pattern of MRSA isolated at a single tertiary care hospital at Rawalpindi and to compare MRSA susceptibility pattern with MSSA (Methicillin sensitive staphylococcus aureus).

**Study Design:** Descriptive cross sectional data based study.

**Place and Duration of Study:** The study was conducted at department of microbiology, Pakistan Railways Hospital Rawalpindi, from January 2012 to March 2014.

**Materials and Methods:** Culture reports data were retrospectively collected from microbiology laboratory. The antibiotic susceptibility patterns of all staphylococcal strains were determined by modified Kirby Bauer antibiotic sensitivity method. The data was analyzed on the basis of antimicrobial susceptibility pattern, location of the patient (OPD, ward patients) and specimen type (wound swab, pus, HVS & effusions).

**Results:** A total of 167 isolates were used in the study. Among these isolates 55 (33%) were MRSA and 112 (67%) were MSSA. The majority of S.aureus isolates were obtained from patients with skin and soft tissue infections. All (100%) strains of MRSA isolated during study period were found to be sensitive to Vancomycin, and linezolid and 95% to Teicoplanin, as well as they showed higher susceptibility against chloramphenicol (88%),Fusidic acid (70%) and Rifampin (48%)while MSSA showed higher susceptibility to Gentamicin (92%),Erythromycin (86%) and Ciprofloxacin (71%) as compared to MRSA.

**Conclusion:** This study showed a high prevalence of MRSA in this tertiary care hospital of Rawalpindi. Present study conclusively shows that Vancomycin, Linezolid and Teicoplanin remain the first choice of treatment for MRSA infections. Still alternative antibiotics like chloramphenicol, Fusidic acid, and Rifampin are available to maintain and reserve the efficacy of Vancomycin, Teicoplanin and Linezolid in treating life threatening illnesses.


**Keywords:** *Antimicrobial Susceptibility, MRSA, MSSA, Prevalence, Staphylococcus aureus.*

## Introduction

Staphylococcus aureus remains a compelling human pathogen as it is one of the most significant pathogen known for nosocomial as well as community acquired infections.[1,2] MRSA infections have been associated with increase in morbidity and mortality of patients in hospitals. Knowledge of susceptibility pattern of the antimicrobials commonly recommended for such patients has gained significance for appropriate management of patients with staphylococcal infections in general and post-operative surgical site infections in particular.

Methicillin resistance in S.aureus was reported in October 1960. Resistance to multiple antibiotics among the staphylococci isolates in hospitals has been recognized as one of the major challenges in hospital infection control.[3] Staphylococcus aureus is a major pathogen that causes a wide range of diseases, including wound infections, carbuncles and boils, nosocomial pneumonia, endocarditis, osteomyelitis, toxic-shock syndrome, food poisoning and septicemia.[4]

During the past 15 years, the appearance and world-wide spread of many MRSA clones have caused major therapeutic problems in different hospitals all over the world leading to drainage of considerable resources to control their spread. MRSA now accounts for more than 60% of S.aureus isolates in United States hospital ICUs.[5]

It is considered that over use and misuse of antibiotics has accelerated the progression of MRSA that has led to the emergence of strains which have systematically acquired multiple resistance genes.[6] In the early 1940s almost all S.aureus strains were susceptible to penicillin. In 1944 the first report of penicillin-resistant S.aureus appeared, and today virtually all strains of S.aureus are resistant to natural penicillins, aminopenicillins and antipseudomonal penicillins. Keeping all this in view, this study was aimed to determine the prevalence and sensitivity

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pattern of staphylococcal isolates both MRSA and MSSA at Pakistan Railways Hospital, Rawalpindi. Moreover the efficacy of low priced antimicrobials has been assessed to prevent drug resistance against most commonly used antimicrobials against S.aureus infections. It is expected to help the clinicians to understand emerging trends of drug resistance among clinical isolates of S.aureus and provide a platform to initiate epidemiological studies for staphylococcal infections.

## Materials and Methods

A descriptive cross sectional data based study was carried out from January 2012 to March 2014. No statistical test applied for data analysis. Clinical specimens received in microbiology laboratory of Pakistan Railways Hospital were processed and all S.aureus isolates were included in the study. The strategy for specimen collection included patient's location in hospitals (outdoor patients/ hospitalized patients), source (wound swab/pus, HVS or urine) of the isolate and the antibiotic susceptibility profiles. One hundred and sixty seven (167) isolates of S.aureus were obtained from samples received for culture and sensitivity from different departments of the hospital during the study period. The inclusion criterion of study data was to take sample from a patient once, no duplicate result from the same patient was considered.

In Microbiology Laboratory, the samples were cultured on Blood agar, MacConkey agar and Mannitol salt agar for 24-48 hours. The antibiotic susceptibility testing was performed by the Kirby Bauer's disc diffusion technique using Clinical and Laboratory Standards Institute (CLSI) recommendations. The characterization of MRSA was done by using Cefoxitin (30 µg) disc and following the interpretation criteria of Clinical and Laboratory Standards Institute (CLSI).[7] Antimicrobial sensitivity testing discs of Oxoid diagnostics were used by the participating laboratory of Pakistan Railways hospital that include Erythromycin (15 µg), Cefoxitin (30 µg), Minocycline (30µg), Ampicillin (10 µg), Chloramphenicol (30 µg), Linezolid (30 µg), Vancomycin (30 µg), Teicoplanin (30 µg), Fusidic acid (5 µg) and Rifampin (5 µg) to assess the sensitivity pattern of these isolates.

The other antibiotics tested include Gentamicin (10 µg), Co-trimoxazole i.e combination of Sulphamethoxazole and Trimethoprim (300 µg), Ciprofloxacin (5 µg), and Cephradin (30 µg). Inoculum was prepared by making a direct saline suspension of isolated colonies selected from an 18 to 24 hours old blood agar plate. Turbidity of the suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard and six to eight discs were applied on a 100mmMueller Hinton agar plate as per CLSI guidelines. S.aureus ATCC 25923 was used as the quality control strain for disc diffusion.

## Results

Out of the total 167 staphylococcus aureus, 55 (33%) were found to be MRSA and 112 (67%) were MSSA. Among these 55 MRSA isolates, the source from OPD patients were 48% in 2012, 20% in 2013 and 23% in 2014, whereas from indoor patients the values were 52%, 80% and 77% in 2012, 2013 and 2014 respectively. The distribution of these MRSA isolates among outdoor and indoor patients is shown in table I.

**Table I: Year wise Prevalence rate of MRSA in OPD Patients and Indoor Patients**

| | 2012 | 2013 | 2014 (Jan-March) | Grand Total |
|---|---|---|---|---|
| Category of patients | No. of MRSA isolates n=27 | No. of MRSA isolates n= 15 | No. of MRSA isolates n=13 | No. of MRSA isolates n=55 |
| OPD patients | 13 (48%) | 3 (20%) | 3 (23%) | 19(35%) |
| Indoor patients | 14 (52%) | 12 (80%) | 10 (77%) | 36(65%) |

It was found that 45(81%) of these isolates were from pus swabs/pus, followed by HVS 6 (11%) and from catheter tip/urine samples that were 4(8%). The prevalence rate of MRSA in the study period on yearly basis &in different categories of samples is shown in table II.

The antimicrobial susceptibility & resistance pattern of MRSA & MSSA isolated during January 2012 to March 2014 is shown in Table III and IV. Out of the total of MRSA strains isolated during study period 81% were found to be resistant to Co-trimoxazole (combination of Sulphamethoxazole & Trimethoprim), 62% to Gentamicin, 81% to Minocycline, 54% to Erythromycin and 52 % to Rifampin. However, all (100%) MRSA strains were

**Table II: Year wise distribution of MRSA from different sources**

| | 2012 | 2013 | 2014 (Jan-March) | Grand Total |
|---|---|---|---|---|
| Specimen | No. of MRSA isolates n=27 | No. of MRSA isolates n=15 | No. of MRSA isolates n =13 | No. of MRSA isolates n=55 |
| Wound/ pus | 22 (82%) | 13 (87%) | 10 (77%) | 45 (81%) |
| Urine | 3 (11%) | - | 1 (8%) | 4 (8%) |
| HVS | 2 (7%) | 2 (13%) | 2 (15%) | 6 (11%) |

found sensitive to Vancomycin, and Linezolid, 95% to Teicoplanin and 88% to Chloramphenicol.

Antibiotics like Tetracycline (88% resistance) and

**Table III: susceptibility pattern of MRSA against different antibiotics**

| | 2012 | 2013 | 2014 (Jan-March) | Grand total |
|---|---|---|---|---|
| **Antibiotics** | **Sensitive (%)** | **Sensitive (%)** | **Sensitive (%)** | **Sensitive (%)** |
| Vancomycin | 100 | 100 | 100 | 100 |
| Linezolid | 100 | 100 | 100 | 100 |
| Teicoplanin | 85 | 100 | 100 | 95 |
| Chloramphenicol | 93 | 100 | 71 | 88 |
| Fusidic Acid | 67 | 57 | 85 | 70 |
| Rifampin | 60 | 50 | 33 | 48 |
| Erythromycin | 58 | 43 | 36 | 46 |
| Gentamycin | 38 | 25 | 50 | 38 |
| Cotrimoxazole | 13 | 44 | 0 | 19 |
| Minocycline | 38 | 20 | 0 | 19 |

**Table IV: Comparison of Susceptibility pattern of all MRSA& MSSA in study period (Jan 2012 – March 2014)**

| Antibiotics | MRSA Sensitive (%) n=55 | MRSA Resistant (%) n=55 | MSSA Sensitive (%) n=112 | MSSA Resistant (%) n=112 |
|---|---|---|---|---|
| Linezolid | 100 | 0% | N/A* | - |
| Vancomycin | 100 | 0% | 100% | 0% |
| Teicoplanin | 95 | 5% | N/A* | - |
| Chloramphenicol | 88 | 12% | N/A* | - |
| Fusidic Acid | 70 | 30% | N/A* | - |
| Rifampin | 48 | 52% | N/A* | - |
| Erythromycin | 46 | 54% | 86% | 14% |
| Gentamycin | 38 | 62% | 92% | 8% |
| Ciprofloxacin | 22% | 78% | 71% | 29% |
| Minocycline | 19 | 81% | N/A* | - |
| Cotrimoxazole | 19 | 81% | 31% | 69% |
| Cephradin | N/A* | - | 100% | 0% |
| Ampicillin | 0% | 100 | 28% | 72% |

*N/A: Not applicable

Ampicillin (72% resistance) were found to be ineffective against MSSA too. Rest of the antibiotic showed less than 30% resistance towards the isolated MSSA.

## Discussion

Increasing emergence of MRSA is a global problem and its prevalence is ever increasing with time. Finding of a prevalence rate of MRSA amounting to 33% in the present study is in close proximity to findings of 31.9% of MRSA in an Australian study involving 32 laboratories from all states and territories of Australia.[8] While a study done in India also revealed prevalence of MRSA varying between20- 25 per cent in western part of India[9] to 50 per cent in South India[10] with prevalence rate as high as 31.1% in clinical samples. Moreover the present data is also close to a study conducted in Nepal where Pandey et.al found at Kathmandu Medical College, Teaching Hospital that 29% of S. aureus isolates were resistant to methicillin.[11]

In the present study another significant observation was the increased emergence of MRSA isolates during first three months of 2014. This increase in MRSA prevalence could be attributed to many explanations like infection control measures, antibiotic prophylaxis and treatments used in each ward/hospital and, not less importantly, the clonal and often epidemic nature of these microorganisms.[12] The limitation of our study is that details of patients presenting in OPD and having MRSA infection are not known whether they were referred from other hospitals/ clinics or they got MRSA infection due to selective pressure of post-operative antibiotics, when they were discharged from our hospital. In such a situation one cannot comment that these patients were infected with MRSA at home or during their stay at hospital.

This study also showed that all MRSA isolates were significantly less sensitive against routinely used anti staphylococcal antibiotics as compared to MSSA isolates. However, significant difference was observed in case of Gentamycin, Erythromycin, and Ciprofloxacin. This antimicrobial susceptibility pattern of MRSA and MSSA isolates against antimicrobial agents has been summarized in Table IV. More than 50% of MRSA isolates were resistant to Gentamycin, Rifampin, Minocycline, Co-trimoxazole (Sulphamethoxazole + trimethoprim), Ciprofloxacin and erythromycin. Least amount of resistance was observed in Vancomycin (0%), Linezolid (0%) and Teicoplanin (5%) and last but not the least Chloramphenicol (12%) & Fusidic acid (30%). β

lactam antibiotics like penicillin (100% resistance) and co-trimoxazole were found to be ineffective against MSSA too. However Erythromycin and Gentamycin showed less than 20% resistance towards the isolated MSSA. This sensitivity pattern exposes the options of using antibiotics like Teicoplanin, Chloramphenicol, Fusidic acid and to some extent Rifampin as well for treating MRSA cases. This susceptibility pattern of drugs matches with a study conducted by Faiza et al in Agha Khan University which showed overall variable susceptibility pattern with high resistance rates to Cotrimoxazole (59%) and Rifampicin (50%) were observed. Resistance to Chloramphenicol (10%) and Fusidic acid (9%) was low.[13]

A US based study has revealed an increase during the ten years spanning between 1999-2008.They have also found a relatively increasing trend in community acquired cases as well.[14] While another nationwide study of US hospitals revealed about 369000 infections by MRSA in US hospitals in 2005.[15] However different countries may have different statistics at various hospitals in different regions. This depends upon many factors like characteristics, size of hospital and antibiotic use policy etc.

Mubbisher et al reported 44%MRSA isolates in two hospitals of District Kohat, Khyber Pakhtunkhwa province, Pakistan.[16] This higher percentage could be attributed to increased use of antimicrobials and higher rate of surgical procedures in that particular hospitals of Kohat. Mehta et.al,[17] in their study on control of MRSA in a tertiary care center in India, had reported an isolation rate of 33% from pus and wound swabs in 1998. Whereas, in 2009; it goes up to 40% as shown by Sangeeta Joshi et.al.[18] In the present study,81% of the total MRSAs were isolated from pus (as shown in Table II), this finding is again very close to a study conducted at Karachi by Fayyaz et.al who reported this figure to be 85.6% MRSA from pus in their study.[19]

Rifampicin is an oral antimicrobial agent with good tissue penetration. This agent could be used to treat MRSA infections in our setting but the problem is that Pakistan is a country where infections with Mycobacterium tuberculosis (TB) is common, so increased usage of Rifampicin is not advisable as a routine to treat MRSA infections because of potential development of resistance in TB. However, its use is justified in less complicated cases, where it can be used in combination of other antibiotics.

The side effect with Chloramphenicol treatment may occur such as bone marrow suppression or idiosyncratic aplastic anemia. This complication is manifested by high dose (4 g/day), prolonged therapy, and markedly elevated levels of Chloramphenicol in serum (20 mg/ml) and is reversible. Keeping in view the low cost and oral preparation of Chloramphenicol coupled with very high rate of in vitro efficacy makes this antimicrobial an ideal choice to treat wide variety of infections caused by MRSA. It is also imperious that since this compound has shown very promising results against MRSA isolates, the availability of this antibiotic must be ensured in the market for the benefit of patients. This study has revealed a reasonable susceptibility of MRSA against Rifampin and Fusidic acid as well. Rifampin has excellent oral bioavailability and tissue penetration and activity in bio films. Rifampin has potent intrinsic anti staphylococcal activity and is not used alone due to rapid emergence of resistance. Clinical studies have suggested benefits of addition of Rifampin to Fluoroquinolone regimens for treatment of S. aureus and MRSA bone and joint infections, especially device-associated infections and chronic osteomyelitis.[20]

Vancomycin and Linezolid remains the first choice of treatment for MRSA infection worldwide. Still there is possibility of developing toxicities of Linezolid after more than 2 weeks that include anemia and thrombocytopenia, thus hematologic parameters must be monitored. Other serious toxicities reported with prolonged Linezolid therapy include lactic acidosis syndromes, optic neuritis, and peripheral neuropathy.[21] In one study, 80% of 66 patients with chronic S.aureus osteomyelitis were cured after prolonged courses of Linezolid (mean 13 weeks), but treatment-limiting toxicities occurred in one third of patients.[22] Thus, Linezolid is not an ideal agent for very prolonged treatment courses or chronic suppressive therapy.

To preserve its value, use of Vancomycin should be limited to those cases where it is clearly needed. Our study proves the sensitivity of Linezolid, Teicoplanin, Chloramphenicol, Fusidic acid and Rifampin against MRSA that would be beneficial to control emerging resistance with Vancomycin.

## Conclusion

In this study, Vancomycin, Linezolid and Teicoplanin are the antibiotics found to give almost uniform sensitivity that is 100%. Therefore, it is construed that these antibiotics remain the mainstay for treatment of MRSA infections. However, still there is choice for clinicians to select Chloramphenicol, Fusidic acid and Rifampicin to treat less serious MRSA infections as these drugs are effective not only in combination but also economical. The use of these antibiotics will preserve the efficacy of Vancomycin, Linezolid and Teicoplanin against serious infections with MRSA.

This study provides a guideline to epidemiologists to understand the nature of MRSA isolates in this tertiary care hospital. It will be helpful to make policies by infection control committee to revise their strategies to combat the emerging infections in their hospitals i.e. cost effectiveness of antibiotics. It is recommended that there is need of clinical studies involving use of Chloramphenicol under medical and laboratory supervision. In addition clinical trials of using combinations of Rifampicin and Fusidic acid with other anti MRSA antibiotics are also recommended.

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ORIGINAL ARTICLE

# Cardiac Autonomic Modulation in Psychologically Stressed Subjects as reflected by Heart Rate Variability

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## ABSTRACT

**Objective:** To compare the frequency domain parameters of heart rate variability in stressed and non stressed subjects.

**Study Design:** It was a cross sectional study.

**Place and Duration of Study:** The study was conducted at Islamic International Medical College from June 2014 to December 2014.

**Materials and Methods:** Eighty subjects between 20-40 years of age were inducted in the study after fulfilling DASS questionnaire and were divided into stress and control group. Ten minutes ECG of the subject was taken using power lab and analyzed for heart rate variability following the guidelines of Task Force of European Society of Cardiology and the North American Society of Pacing Electrophysiology. Frequency domain indices of heart rate variability were compared among stressed and control group using fast fourier transform.

**Results:** Psychologically stressed subjects have significantly decreased high frequency in absolute unit and normalized unit ($p \leqslant 0.05$) and increased low frequency in normalized unit and absolute unit ($p \leqslant 0.05$ and .001 respectively) and low to high frequency ratio when compared with controls ($p \leqslant 0.001$). There was significant negative correlation among LF ms2 and HFms2($p < 0.001, r = -.423$), LF ms2 and HFnu ($p \leqslant 0.001, r = -.386$),HF ms2 and LFnu ( $p \leqslant 0.05, r = -.361$ ) and HFms2 and LF/HF ($p \leqslant 0.05, r = -.553$),LF/HF and HFnu ( $p \leqslant .001$ $r = -.553$), LFnu and HFnu ($p \leqslant 0.05, r = -.237$). There was also statistically significant positive correlation of LF/HF and LFnu ($p < .001. r = .824$).

**Conclusion:** Assessment of Heart rate variability is an important measure of autonomic nervous system and effect of psychological stress on autonomic nervous system can be indexed by determining heart rate variability.


**Keywords:** *Stress, Heart Rate Variability, Frequency Domain Methods, Low Frequency, High Frequency, Low Frequency to High Frequency Ratio.*

## Introduction

"Stress is defined as a state of physiological / or psychological imbalance resulting from disparity between situational demand and individual's ability /or motivation to meet these demands".[1] Psychological stress is becoming a serious health problem worldwide and is identified as a big health hazard which reduces productivity and satisfaction.[2] Stress causes activation of sympathetic branch of autonomic nervous system which comprises of sympathetic and parasympathetic nervous system. Persistent sympathetic activation occurring in stress leads to disturbances in blood pressure, heart rate and heart rate variability.[3] High resting vagal tone is a sign of autonomic flexibility and shows that autonomic nervous system is capable of generating adequate response to external challenge by adjusting heart rate, respiration and arousal.[4] Decrease vagal tone as in stress predicts mismatch between environmental demands and cardiac reactivity making heart more prone to arrhythmias.[5] Heart rate variability (HRV) is the most important noninvasive quantitative tool to access cardiac autonomic function.[6] It is the most important predictor of mortality and morbidity in healthy and diseased population and measures the interaction of all the physiological factors that adjust the heart rate, reflecting continuous interaction between neuronal modulatory function and sinoatrial node function. Low heart rate variability is associated with different medical and psychological health problems.[3] HRV is analyzed by time and frequency domain methods.[7] For short term recording of HRV, frequency domain method is frequently used because of easy setting and are based on spectral analysis of HRV.[8] Three frequency components are usually identified in spectral band, the high frequency (HF) component of HRV, spans the 0.15-0.4 Hz and is due to heart rate variation induced by respiration and predominantly mediated by vagal outflow, lower-frequency (LF) component of HRV, defined as 0.05-0.15 Hz, is postulated to be mediated by sympathetic and

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parasympathetic system and very low frequency (VLF) <0.04 Hz which is thought to be mediated by sympathetic system but exact physiological interpretation of this is not clear. LF and HF are reported in normalized units to avoid skewness of distribution. Low to high frequency (LF/HF) ratio reflects balance between sympathetic and parasympathetic nervous sytem.[9] Decrease HRV is associated with stress.[3] Decrease vagal control on heart as shown by reduced HF component is known to be a leading cause for the development of cardiovascular disease and arrhythmias.[5]

The field of education is highly demanding and challenging and renders students as well as the educationists to deal with complex learning environment.[10] Mental stress interferes with an individual's ability to accomplish normal tasks, leading to various psychological problems and low self confidence. There is no study in Pakistan which has described the effect of stress on autonomic nervous system in terms of heart rate variability which is an important predictor of mortality and morbidity. Autonomic imbalance is a key mechanism for the development of cardiovascular diseases and diabetes mellitus. This study was conducted with an aim to access the affect of stress on the autonomic nervous system through frequency domain parameters of heart rate variability by comparing stressed and non stressed subjects.

## Materials and Methods

This cross sectional study was conducted in physiology lab at Islamic International Medical College, Riphah University from June 2014 to December 2014 after approval from research ethical committee Islamic international medical college. A total of eighty healthy subjects from both genders, ranging in age of 20-40 years were included in study. All the subjects were healthy and free of any disease. They were randomly grouped as stressed and control after filling DASS questionnaire proforma (Depression anxiety stress scale).[11] Those who scored between 19-25 on DASS were labeled as having moderate stress and those who scored between 0-14 were labeled as control. Subjects having any chronic disease like asthma, diabetes or hypertension were excluded from the study. After taking written informed consent, the subjects were asked to report to physiology lab in morning between 8.00 to 9.00 am. Weight of the subjects was recorded, subjects were made to relax for 5 minutes and their blood pressure was measured using sphygmomanometer. Their recording of HRV was undertaken from ten minutes ECG in sitting position using ADInstrument power lab model Yam 4/25T.

Ten minutes ECG was taken to analyze HRV, according to the standard guidelines, published by Task Force of European Society of Cardiology and the North American Society of Pacing Electrophysiology.[12] HRV was recorded in quiet environment at ambient temperature. ECG of the subjects was recorded in a sitting position by connecting MLA 250 shielded lead wires to Bio AMP cable which was plugged in power lab. Positive electrode was connected with left wrist and negative to right wrist and ground to right leg. HRV recorded by analyzing ECG. Data in power lab was analyzed using software Lab chart 7 Pro. Frequency domain was accessed using Fast Fourier transform to determine low frequency, high frequency and low to high frequency ratio.

Statistical software SPSS 21(Statistical packages for social sciences) was used for the analysis of the data. Mean ± SD of the variables was calculated. The normality of each quantitative variable was checked separately through Shapiro Wilk test. To avoid the skewness of distribution all the HRV indices were log transformed and normality checked again. Independent sample t- test was used to check difference among two groups. A p value of $\leqslant$ 0.05 was taken significant. Association among different heart rate variability indices was checked by Pearson correlation.

## Results

The study included eighty subjects divided into 2 groups, stressed and control. The differences in frequency domain parameters of heart rate variability among stressed and control groups were compared. These indices were LF, HF and LF/HF ratio. Mean age of the stressed subjects was 25 ± 6 and for control was 27 ± 8 years. Descriptive statistics of stressed and controlled groups are given in the table I.

Table II shows frequency domain indices' of heart rate variability. HF in absolute unit and in normalized units was markedly decreased in stressed group in comparison to controls (p≤.05). LFnu and LF/HF ratio

**Table I: Descriptive statistics of stressed and control groups**

| Characteristics | Stressed n=40 Mean ±SD | Controls n= 40 Mean ± SD |
|---|---|---|
| Age (years) | 26 ±6 | 27 ± 8 |
| Weight (kg) | 66.43 ± 15.68 | 64.05 ± 11.46 |
| BMI (kg/$m^2$) | 26.45 ± 0.823 | 25.46± 0.46 |
| Systolic blood pressure (mmHg) | 118 ± 6 | 120 ± 5 |
| Diastolic blood pressure (mmHg) | 77 ± 7 | 78 ± 7 |
| Heart rate (HR)/min | 83.70 ± 9.30 | 82.41 ± 10.423 |

was significantly increased in stressed subjects, compared to control (P ≤ 0.001), Low frequency in absolute units was significantly higher in stressed group when compared to controls (p ≤.05).

**Table II: Comparison of Frequency domain parameters of heart rate variability in stressed and control group**

| Parameters | Stressed n=40 Mean ±SD | Controls n= 40 Mean± SD | t value | p value | Normality test |
|---|---|---|---|---|---|
| LFms$^2$ (Reference value 1175 ms$^2$) | 678.07 ±475.37 | 505.99 ± 496.16 | -2.174 | 0.03* | .002† |
| HF ms$^2$ (Reference value 975ms$^2$) | 297.43± 186.76 | 415.83 ± 224.33 | 2.640 | .010* | .006† |
| LF/HF Reference value (1.5-2) | 2.96± 2.49 | 1.277±0.83 | -5.726 | 0.000** | .000†† |
| LF nu Reference value :54 | 61.52 ± 14.29 | 44.8488 ± 16.75 | -3.766 | 0.000** | .09† |
| HF nu Reference value 29 | 31.99 ± 29.85 | 39.72 ± 12.02 | 3.602 | 0.001* | .000†† |

*p value < 0.05 is significant and **p value < 0.001 is highly significant

†p value < 0.05; p ††< 0.0001 shows that particular variable is non normal (Shapiro Wilik's test)

Table III shows partial Pearson correlation controlling for heart rate, carried out between various heart rate variability indices. There was significant negative correlation among LF ms2 and HFms2(p < 0.001,r = -.423), LF ms2 and HFnu (p⩽ 0.001,r = -.386),HF ms2 and LFnu ( p ⩽ 0.05, r = -.361 ) and HFms2 and LF/HF (p ⩽ 0.05, r = -.553),LF/HF and HFnu ( p ⩽ .001, r = -.553), LFnu and HFnu (p ⩽ 0.05, r = -.237). There was also statistically significant positive correlation of LF/HF and LFnu (p < .001, r = .824).

**Table III: Partial Pearson correlation controlling for HR between various HRV indices**

| | LFms$^2$ | HFms$^2$ | HF nu | LFnu |
|---|---|---|---|---|
| HFms$^2$ | .000** (-.423) | | | |
| HF nu | 0.000** (-.386) | 0.409 (.094) | | |
| LFnu | 0.000** (.458) | 0.001* (-.361) | 0.034* (-.237) | |
| LF/HF | 0.003* | 0.050* | 0.004* | 0.000** |

* Correlation significant at p value < 0.05

** Correlation highly significant at p value < .001

r values are shown in brackets

## Discussion

The current study examined the different frequency domain parameters of HRV indices in stressed and control subjects. These indices were LF, HF, LF/ HF ratio. Compared to controls, stressed subjects exhibited decrease HF, increased LF/HF ratio and LF. Mean figure of HF in controls was higher, compared to stressed, which is showing that they have good vagal control as compared to stressed.

In a model of HRV analysis, Montano et al., (2009) showed that HRV was analyzed as HF component which is regarded to be the marker of cardiac vagal control, the LF component is a measure of sympathetic outflow to heart and LF/HF ratio which reflects the balance between sympathetic and parasympathetic system controlling the heart rate.[13] The present study validates that stress is associated with vagal withdrawal as shown by reduced HF and was confirmed through this study. Literature shows that psychological stress is linked with decrease in vagal control reflected as decrease in HF component. Hernandez- Gaytan et al., (2012) confirmed low HF in doctors complaining of psychological stress at work.[14] A study conducted by Eller et al., (2011) reported decrease in HF component of HRV in teachers and engineers also which confirm the result of our study.[15] Hintsanen et al., (2007) conducted a study showed that HF was decreased in office workers with high effort reward imbalance (ERI) along with increase in LF/HF ratio.[16] Takada et al.,(2010) conducted a study in Japanese worker suffering from psychological stress and reported that HF is considerably lower in stressed group and

on receiving treatment ,their HF was increased.[17] A study conducted by Minakuchi et al., (2013) reports similar findings.[18] Kemp et al., (2012) also showed decrease in HF in depressed subjects.[19]

Findings based on sympathetic assessment were LF and LF/HF ratio. LF/HF ratio was significantly raised among stressed group as compared to control showing sympathetic over activation in stressed group. LF is regarded as a marker of sympathetic control of heart and is increased in response to stress. LF expressed normalized units showed significant difference in stressed and controls. This finding was consistent with the study conducted by Collins et al., (2005) which reported high LF/HF ratio in high strain group in working hours.[20] A study conducted by Takada et al., (2009) showed higher LF/HF ratio and reduced HF in Japanese stressed workers which again proved our hypothesis. Takada showed that depressed workers who took medication for depression had improvement in HF and LF/HF ratio.[17] This finding further supports our study. Minakuch et al., (2013) conducted a study in which he showed that in response to mental stress LF/HF ratio increases significantly.[18] However a study by Hynynen et al., ( 2011) reported no significant association of any HRV parameter with stress.[21] Petrowski K et al., in his study also proved that in stress LF/HF ratio increased significantly.[22] Strong positive association between LF and LF/HF ratio controlling for heart rate was seen, a finding consistent with the finding of a study conducted by Ramakers (1998).[23] He also reported significant negative correlation between high frequency and low frequency and LF/HF ratio and high frequency, the findings also reported by present study. This show that heart rate variability parameters are affected by stress and decrease in high frequency; an index of sympathetic activity is associated with increase in low frequency which is an index of sympathetic activity.

## Conclusion

Stressed subjects exhibit reduced HRV as compared to non stressed subjects. HRV provides important information for evaluation of cardiac autonomic control. Reduced HRV is a predictor of cardiovascular diseases. Additional research is needed for the evaluation of HRV in frequency and time domain indices.

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ORIGINAL ARTICLE

# Gastroprotective Effect of Sagu Pearls on Diclofenac Sodium Induced Gastric Ulcer

Shazia Muazam[1], Rehana Rana[2], Saira Jawed[3], Shabana Ali[4]


## ABSTRACT

**Objective:** To see the possible gastro protective effect of Sagu pearls on Diclofenac Sodium (NSAID) induced gastric ulcer by enhancing mucosal barrier.

**Study Design:** Randomized control trial.

**Place and Duration of Study:** The study was carried out in the department of Anatomy, Islamic International Medical College, Rawalpindi, in collaboration with National Institute of Health, Islamabad. It was conducted for a period of six months, from 15th September 2014 till 30th March, 2015.

**Materials and Methods:** Fifty adult rats of both sexes of Sprague Drawly strain were divided into three groups: Group I (control); Group II (ulcer group) given Diclofenac sodium orally at the dose of 50mg/kg body weight, daily for 2 weeks and Group III given Sagu pearls daily at the dose of 200mg/kg body weight for 2 weeks along with Diclofenac Sodium. Animals of all the groups were sacrificed on day 16 and their stomachs were studied macro and microscopically. Statistical analysis was done to see any significant difference between the groups. Anti-ulcer effects were assessed on the qualitative and quantitative parameters like ulcer size and index, histological determination of depth of the mucosal lesion and mucus thickness.

**Results:** Results highlighted the probable protective effect of Sagu pearls by exhibiting almost 90% decrease in ulcer index in group III accompanied by a continuous thick mucus layer on the surface of mucosal cells confirmed by Periodic Acid Schiff (PAS) stain.

**Conclusion:** Sagudana can provide protection against NSAID induced gastric ulcer by strengthening mucus barrier and thus can be used as an adjunct along with NSAIDs.


**Keywords:** *Gastric Ulcer, Starch (Sagu), Non-Steroidal Anti-Inflammatory Drugs, Anti-Ulcer Agents.*

## Introduction

Peptic ulcer, an interruption in the continuity of gastrointestinal mucosal lining occurs most commonly in patients aged 30 to 50 years but above 60 years account only for 15% of cases.[1] Aggressive factors leading to imbalance in gastric mucosal offensive and defensive factors are generally accepted as the cause of gastric ulcer.[2] Untreated ulcers can increase morbidity by anemia, haematemesis or perforations.[3] Such ulcers are a cause of economic burden as their treatment imposes at least 10% of the total cost of treatment of digestive disorders.[4]

NSAIDs, commonly prescribed medicine for general to chronic ailments in Pakistan[5], are one of the aggressive factors for ulcerogenesis.[6] Those who receive high dosage of NSAIDs even for shorter span of time or those using moderate dosage but for a long period have shown large number of ulcerative lesions.[7] Statistical data collected from randomized control trials conducted in hospitals infers that about 1 in 10 NSAID induced ulcer bleeds.[8] Numbers of adjunct therapies are prescribed along with the usage of NSAIDs to reduce their ulcerative effects but some are not cost effective or have side effects of their own along with noncompliance of patient.[7] There is a need to find some natural, cost effective prophylaxis along with NSAIDs to minimize patient's agony. Sagu starch, an inexpensive natural polysaccharide, marketed in the form of small globules known as sagu pearl or Sagudana, has been used traditionally for 70 years during sickness.[9] Both homeopaths and allopaths consider it as best food during fever as it is a promising natural tonic and easily digested, non-irritating food in inflammatory cases.[10] It is a very rich source of carbohydrates.[11] Number of ulcer protective natural herbs, has been found by different researchers, with different parts of plant being used, after removing their unwanted chemicals.[12] Sagu starch being a pure polysaccharide

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is totally inert and highly viscous which can be used easily.

Effect on quality of life, time lost from work due to ulceratic pain, expense of hospitalization and expensive adjuvant therapy along with NSAIDs, having their own side effects, made us find cost effective, nontoxic and easily available prophylaxis. The idea that the surface-active agents would be preferable to acid inhibitors as they do not alter the bactericidal activity of the stomach along with low cost and high yield as compared to other sources of starches and traditional belief of people made sagu pearls as the choice of prophylactic substance.

## Materials and Methods

It was a randomized control trial conducted in Anatomy Department IIMC in collaboration with NIH after the approval from Institutional Review Committee, for a period of six months, from 15th September 2014 till 30th March, 2015. Fifty adult Sprague Drawly rats of both sexes, more than 3 months old, weighing approximately 180-250g, were selected by balloting method and purchased from animal house of NIH, Islamabad. Rats with any obvious physical pathology were excluded. Twenty five male and twenty five female rats were divided in 3 groups and kept in separate cages, under standard laboratory conditions in NIH. They were acclimatized for one week at a room temperature of 23-25°C with a 12 hour dark/light cycle and were allowed to feed and drink ad libitum on standard pellet diet and tap water.

Sagu pearls, 200gms/pack, of local brand were purchased from market. Diclofenac sodium (50 mg) (Voltral) of Novartis Pharma (Pakistan) Limited was purchased from local pharmacy.

Group I (control) where n =10 was divided into sub groups: IA having 5 male rats and IB having 5 female rats. They had free access to feed and water. Group II (ulcer group) where n =20 were divided into subgroups: II A having 10 male rats and II B having 10 female rats. They were given diclofenac sodium at a dose of 50 mg/kg body weight[13], once daily for 15 days, orally mixed in water so as to reduce the bias of stress as ulcerative factor. Group III (Test group) where n = 20; was divided into sub groups III A having 10 male rats and III B having 10 female rats. They were given Sagu pearls at the dose of 200 mg/kg body weight[14], once daily along with diclofenac sodium as above, for 15 days in the form of gruel. Gruel was made by boiling 100 granules in 700ml of water to form thin paste. At the end of 2 weeks the animals of all groups were anesthetized and sacrificed. The stomachs were isolated, opened along the greater curvature, washed gently with saline and were examined by hand lens for change of color, hemorrhagic area or presence of crater. Presence of ulcer was confirmed by measuring ulcer size[15] and ulcer index.[16] The samples were then placed in10% neutral-buffered formalin for 24 hours. 2 mm wide parallel strips from glandular portion were dissected and embedded in Paraffin. The prepared serial sections were then stained with Haematoxylin and Eosin (H&E) for detecting the depth of lesion and Periodic Acid Schiff (PAS) stain for detection of neutral mucins on the mucosal surface. The depth of lesion was graded as type 1, 2 and 3 according to criteria laid by Natalie.[17] Ulcer index was calculated according to the formula notified by Sharma[16]:

UI=UN +US +UP × 10-1

Where UI= ulcer index, UN= average number of ulcers per animal in each group

US= ulcer score per group and UP= percentage of animal with ulcers in each group.

The mucus thickness was measured as the vertical distance between cell surface and luminal mucus surface with linear eyepiece micrometer at 40 magnification of objective. The mean value of 4 different measurements was taken.[18] The data was entered and analyzed using SPSS 20.0. One Way Analysis of Variance (ANOVA) was applied to compare the mean differences among groups. A p–value of <0.05 was considered as statistically significant.

## Results

Macroscopic examination of opened stomach in control group exhibited pink colored glandular part with prominent rugae. Mucosal damage of varying severity ranging from pin point erosions to dark brown lesions were seen in both Groups II and III .

Ulcer index was higher in group II as compared to group III.

H & E stained slides of gastric mucosa of Group I (control) revealed gastric glands having columnar surface mucous secreting cells with basal oval nuclei followed by predominant mucous neck cells and

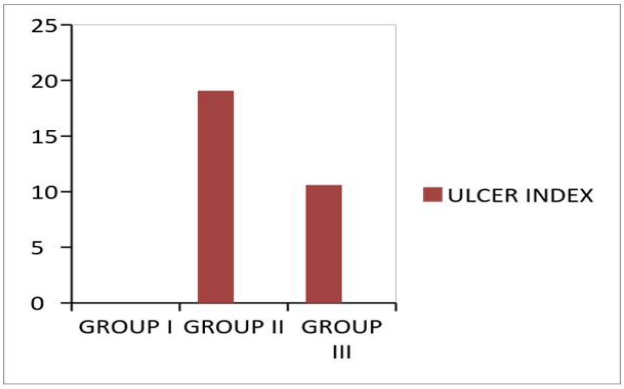


**Fig 1: Comparison of Ulcer Index in stomachs between the three groups**

parietal cells. The parietal cells appeared as deeply eosinophilic rounded to ovoid cells with central rounded nuclei. At the base of the gland abundant chief cells with few parietal cells were observed. The glands fully occupied the thickness of lamina propria along with blood vessels and few dispersed lymphocytes. Smooth muscle layer, muscularis mucosae, limited the mucosal layer from submucosa. The submucosa was evident as loose connective tissue with blood vessels. The depth of lesion in Group II and III was determined on the basis of extent of destruction of cells which was graded as type 1, 2 and 3 according to the involvement of upper or lower part of lamina propria respectively.17 In Group II the damaged cells appeared as shrunken with pyknotic nuclei extending from neck till base of gland. Submucosal edema with congested blood vessels was prominent (Fig 3). Group III had predominance of proliferating mucous cells extending along the neck of the gland. Glands with dilated lumen were prominent. No submucosal odema was detectable as compare to Group II.

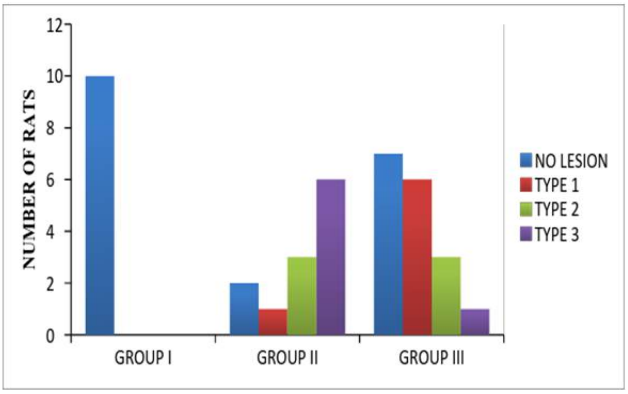


**Fig 2: Comparison of depth of lesion between the three groups**

**Table I: Comparison of depth of ulcerative lesions between the three groups**

| Type of Lesion | Group I (n = 10) | Group II (n = 12) | Group II (n = 17) | P-value |
|---|---|---|---|---|
| No Lesion | 10 (100%) | 2 (16.7%) | 7 (41.2%) | 1* |
| Type 1 Lesions | 0 (0%) | 1 (8.3%) | 6 (35.3%) | |
| Type 2 Lesions | 0 (0%) | 3 (25%) | 3 (17.6%) | |
| Type 3 Lesions | 0 (0%) | 6 (50%) | 1 (5.9%) | |

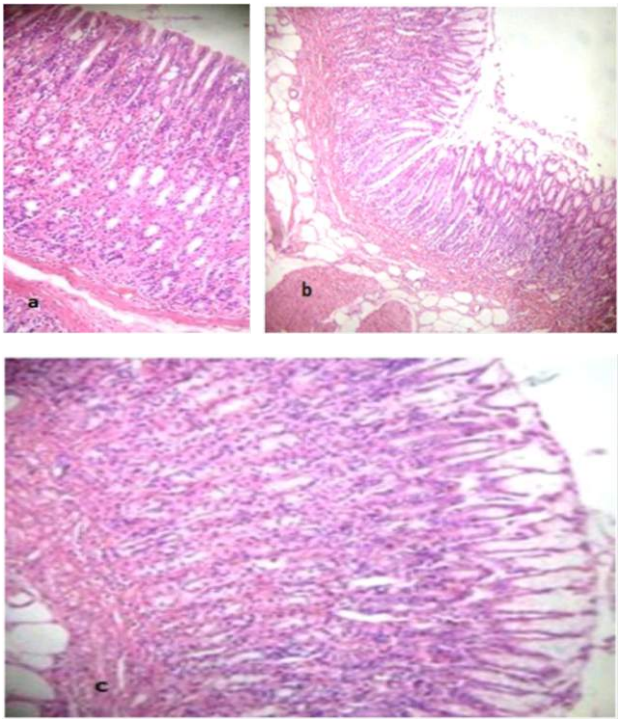


**Fig 3: Photomicrograph of glandular mucosa of (a) control Group showing normal glandular architecture with intact surface mucous cells and normal submucosal thickness(b) Group II showing sloughed cells with ulcer and sub mucosaloedema (c) Group III with intact mucus layer and normal architecture H&E 100x**

In Group I a continuous magenta colored layer and a positive reaction was observed in surface and mucous neck cells while interrupted mucosal layer and weak PAS reaction was observed in mucous neck cells in Group II. Group III exhibited a continuous, thick magenta color at the surface and strong PAS positive reaction extending to the pits of the glands. (Fig 4).

## Discussion

In our study a variety of macroscopic mucosal gastric lesions ranging from mere color change to hyperemia to gross lesions were noticed in group II. This is in accordance with the changes reported by all studies conducted on gastric ulcers.[19,20] There was significant increase in the ulcer index in group II as

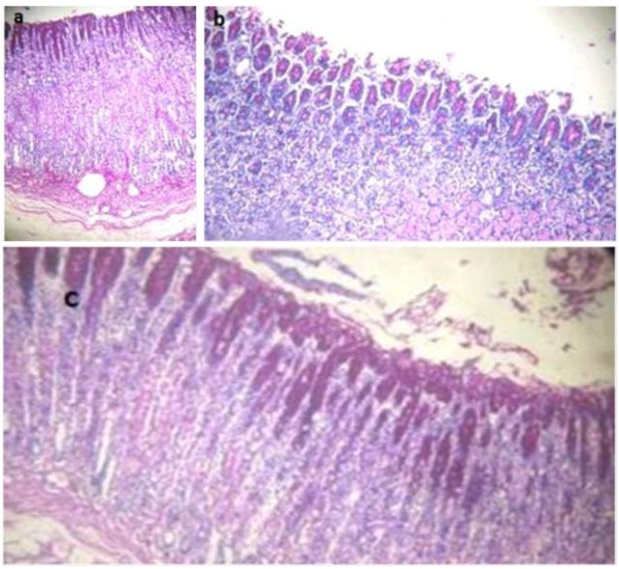


**Fig 4: Photomicrograph (a) showing continuous mucus layer in Control (b) showing interrupted mucus layer and weak PAS reaction in Group II (c) showing thick continuous mucus layer and strong PAS reaction extending into pits in Group III. PAS stain 100x**

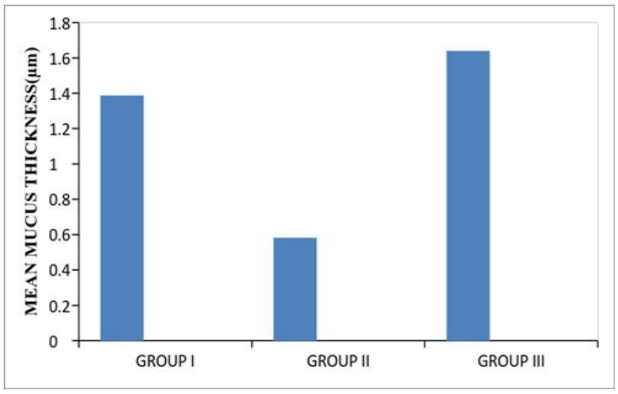


**Fig 5: Comparison of mean mucus thickness (µm) on the luminal surface of mucosal cells of stomach, between the three groups.**

**Table II: Post-hoc comparison of Mean Mucus thickness (µm) between the three groups**

| Group Comparisons | Mean mucus thickness | |
|---|---|---|
| | Mean difference | p-value |
| Group I vs. Group II | 2.009 | 0.003 * |
| Group I vs. Group III | - 0.652 | 0.425 |
| Group II vs.Group III | - 2.661 | 0.001 * |

* p< 0.05 = Significant

compared to Group I. Moreover there was a statistically significant decrease in the ulcer index in group III compared to group II. Regarding the depth of lesion, group II showed all three types of lesions. Microscopically sloughed off surface mucous cells and mucus neck cells were evident forming erosions. Cells with highly eosinophilic cytoplasm and pyknotic nuclei extended from the neck to the base of group II whereas they were restricted to basal parts only in group III. In agreement with above results it's seen that diclofenac sodium produced reactive gastropathy. This damage may be due to excessive hydrogen ion movement from the lumen to inside when diclofenac is given in therapeutic doses for a longer period of time as is the case with aspirin.[20]

Regarding group III examination of H&E stained slides showed an intact mucus layer with hyperplastic mucous cells extending deep down the length of gland, depth of lesion was significantly reduced as compared to the other group.

In this study significant difference was observed in mucus thickness between group II and group III. PAS stained slides revealed attenuation of mucus layer in group II given only diclofenac as has been observed by Singh et al[21] in an aspirin induced gastic ulcer. Thick mucus layer and intense reaction (magenta color) extending into gastric pits, the lumen of the gland and neck region was seen in group III. This is in agreement with Mohammad[22]who also measured the mean optical density of magenta color. Flemstorm and Isenberg[23] have highlighted that gastric mucosal barrier plays a vital role in the protection of gastric wall from aggressive factors responsible for damage. Silva[24] while explaining mucus gel layer emphasized that it provides a diffusion barrier against aggressive factors, entraps microorganisms and holds bicarbonate ions thus controlling intraluminal pH. Jainu[20] has proved that depletion of sulphated mucin glycoprotein leads to small erosions in stomach.

Available literature has shown multiple mechanisms of action of NSAIDs in inducing gastropathy.[2,25,26] Most commonly accepted mechanism is interruption of mucosal layer which in turn is due to depletion of prostaglandin. Cyclooxygenases are the key enzymes in prostaglandin biosynthesis and the target enzymes for the widely used NSAIDs.[27]

PAS stain highlighted deep magenta color on the surface as well as lower down the pits. It can be due to mucilaginous polysaccharides in sagu starch. Same effects are seen by Maria et al[28] who worked on rhamnogalacturonan, a polysaccharide. Prabha et al

has seen the effect of plaintain[29] which stimulates the growth of gastric mucosa because of its water soluble polysaccharides. Wei, Hui and Mao[30] work on finding safe herbal medicines in treating gastric ulcer notified the work of Bhattacharya and Banerjee who had proved local mucus enhancement by plants like Piper Betel extract.

The prophylactic gastro protective mechanism is based on the ability to strengthen defensive factors like prostaglandin synthesis in addition to other factors. Prostaglandins can provide gastric cytoprotection without reducing gastric acid secretion[31] but by enhancing mucosal barrier and its blood flow. Sagu pearls cytoprotective role may be attributed to the polysaccharides which stimulated prostaglandin synthesis leading to mucosal regeneration.

Non availability of electron microscope has limited the study to know the exact histological changes occuring in the gastric mucosal cells after administration of sagu pearls.

## Conclusion

This study has shown the prophylactic effect of sagu pearls, as has been confirmed by morphological and histological parameters. Lesser depths of lesions along with thick mucus layer generation on the luminal surface of mucosal cells, in rats given sagu pearls, are significant enough to prove our alternative hypothesis. Effect of sagu pearls on peptic ulcers induced by physical and chemical agents should also be seen to find its effect on acid secretion.

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ORIGINAL ARTICLE

# The Relationship of Health Literacy, Perceived Health Information Need and Preventive Health Related Behavior in Urban Karachi

Muhammad Yahya Noori[1], Mehak Kazi[2], Amna Shahid[3], Asima Faisal[4]


## ABSTRACT

**Objective:** To determine the relationship of health literacy with perceived need of health information and health related behaviors in urban population of Karachi.

**Study Design:** Cross sectional survey.

**Place and Duration of Study:** Community based study carried out on the students of Institute of Business Management and Ojha Institute of Chest Diseases, Dow University of Health Sciences for four months during February to May 2015.

**Materials and Methods:** A cross-sectional survey was performed in Karachi using a 33 item validated questionnaire on 100 randomly selected subjects using simple random sampling. Descriptive and analytical data analyses were carried out using SPSS version 20. Linear regression was used to find the association between health literacy and perceived health information needs as well as preventive actions. The information regarding demographics was obtained using dichotomous and multichotomous items, health literacy, perceived health information needs and preventive actions was collected using Likert type scales.

**Results:** The participants had a mean age of 27.8± 8.3 years with at least intermediate level education. The mean health literacy score of respondents was calculated to be11.14 ±2.84 on 19 point scale (Range 4-16). On perceived health information needs, the mean score turned out to be 28.27 ± 8.52 on a 51 point scale (Range 13-47). As for preventive health actions on a 48 point scale, the mean score was 21.47 ± 8.61 (Range 8-44). Linear regression analysis showed a weak positive ($r=0.383$) relationship between health literacy and preventive health actions ($\beta=9.25$ $R2=0.147$, $p< 0.05$), however, a weak negative ($r= -0.306$) relationship between health literacy and perceived information needs ($\beta$ 38.58 $R2=-0.094$, $p< 0.05$).

**Conclusion:** Health literacy has an association with preventive actions against different diseases, however it also decreases their feeling of need for more health education and may result in a false sense of security. It is important that proper literacy programs must be initiated to increase preventive measures against common diseases in the society, so that the burden and cost of these diseases can be minimized.


**Key words:** *Health Literacy, Perceived Information Needs, Health Related Behaviors.*

## Introduction

In any society, health literacy plays an important role in maintaining well being as knowledge and practices allow it to stay healthy.[1,2] It is unfortunate that individuals with low health literacy are generally unaware of potential threats and unable to manage and maintain proper health.[3] Therefore, in any community the health prospects of its population depend upon the cognitive awareness about health information.[4,5] Inadequate health literacy puts the person at a higher risk of getting a disease regardless of the absence of illness.[6]

Health literacy ranges from basic functional level, where a person is capable of reading and understanding health related material to conceptual level, where he can make informed health choices to improve his quality of life after evaluation of relevant options.[7-9] It is believed to be a stronger predictor of health outcomes than socioeconomic status, gender, age and education.[10,11] Health literacy can be considered a tool for empowerment as it allows one to understand his obligations and rights and helps to become an informed consumer of health services.[10] It has been reported that individuals in rural communities fail to follow physician's direction for maintenance of their health due to lack of functional health literacy.[6]

It is unfortunate that a significant population in Pakistan lives in poverty and is without basic needs and lacks access to basic health facilities.[12,13] It is evident that this population has very low health literacy despite the government led health literacy programs on vaccination, family planning and other epidemic diseases.[14,15] The level of health literacy in educated individuals also needs to be assessed.

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Generally, this population is considered an informed consumer of health care services, but the true level of knowledge is unknown.

Since health literacy a strong predictor of health outcomes,[6,16] this study was designed to identify level of acquired health literacy, perceived health education needs and preventive health measures that the general educated population takes about their health along with relationship between health literacy and preventive health measures to justify the need of health literacy with other factors of well being.

## Materials and Methods

A cross-sectional survey was performed in Karachi using a 33 item validated questionnaire for measuring health related attributes (Table I) after permission of investigators from primary study.[17] Sample size was calculated to be 97 using online sample size calculator[18] using 95% confidence level and 10% margin of error. The study subjects were randomly chosen from Institute of Business Management and Ojha Institute of Chest Diseases, Dow University of Health Sciences, by skipping every second subject with the aim of establishing role of health literacy and preventive actions.

Informed consent was taken before filling the questionnaire. Data analysis was carried out using SPSS version 20. Linear regression was used to find the association between health literacy and perceived health information needs as well as preventive actions.

Health literacy was measured on a 19 point scale. Score <9 points was taken as below average, 9 - 13 points reflect above average and scores > 13 was taken as good health literacy. Perceived health information needs were measured on a 44 point Likert type scale perceived health information needs were measured on a 47 point Likert type scale.

## Results

The questionnaire was filled by hundred participants (59 females and 41 males). The mean age of the respondents was 27.8± 8.3 (Range 16-78) and all of them were educated to at least intermediate level.

The mean health literacy score of respondents was 11.14 ±2.84 on 19 point scale (Range 4-16). The major source for health related information was reported to be friends and TV/ media.

The perception about health information needs was identified through a series of questions. The mean score turned out to be 28.27 ± 8.52 on a 51 point scale (Range 13-47). Some misconceptions about diet and its impact were noted. Majority of respondents could relate quality of life with the availability of health information. However, a large proportion of population reported to not having enough information about health services available in the country and not having enough information about water and sanitation issues (64% each).

As for preventive health actions on a 48 point scale, the mean score was 21.47 ± 8.61(Range8-44). Majority of respondents expressed need for proper guidance about common diseases such as diabetes (66%) and hypertension (76%). Most of them expressed dissatisfaction about provision of information from their health care providers about the disease (67%) and the medications (66%). The scores are summarized in table II.

Linear regression analysis was performed to explore the relationship between health literacy and preventive actions, which showed a weakly positive

**Table I: Measured attributes related to Health Literacy, Perceived information needs and preventive health actions**

| Variable | Attributes |
|---|---|
| Health Litracy | Language fluency and understanding, Cognitive awareness, Recognition of Symptoms knowledge about adverse health outcomes |
| Perceived information Needs | Information about maintenance of a health, Information availability self management of health |
| Preventive Health Actions | Dietary measures to remain healthy, Prevention of prevalent diseases hygiene practices |

**Table II: Scores related to Health Literacy, Perceived information needs and Preventive Health Actions**

| Variable | Maximum Point on Scale | Minimum Point on Scale | Mean Score | Standard Deviation |
|---|---|---|---|---|
| Health Literacy | 19 | 4 | 11.14 | 2.84 |
| Perceived information Needs | 47 | 13 | 28.27 | 8.52 |
| Preventive Health Actions | 44 | 8 | 21.47 | 8.61 |

relationship (r=0.383) between health literacy and preventive health actions (β=9.25 R2=0.147, p< 0.05) (Fig I) and a weakly negative (r= -0.306) relationship (β 38.58 R2=-0.094, p< 0.05) between health literacy and perceived information needs (Fig II).

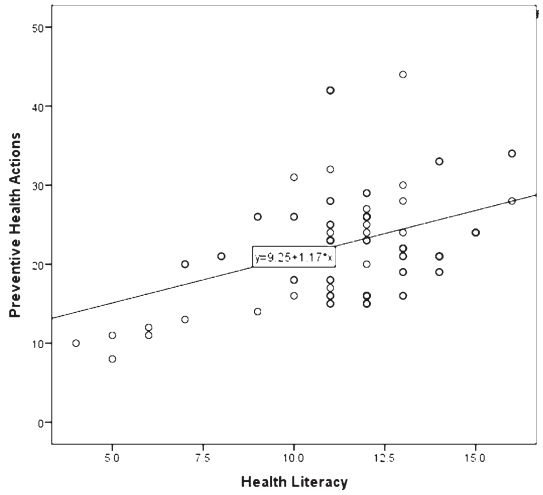


**Fig 1: Linear Regression analysis of Health Literacy and Preventive Health Actions.**

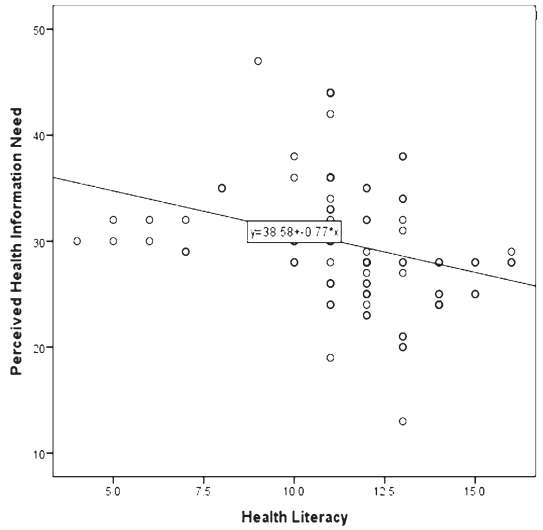


**Fig 2: Linear Regression analysis of Health Literacy and Perceived Health Information Needs**

## Discussion

The study was carried out to find the association between health literacy and perceived health information needs and health prevention activities in the population of Karachi. Our target population was mainly urban and educated. Our findings show health literacy to be an important factor for practicing prevention. This finding has an important implication on designing health literacy programs, as targeting the educated population with specific information can develop an effect like herd immunity and play an important role in improvement of health status of the society[19,20] since most of the participants appeared to obtain their health related knowledge from either friends or media, which is not a common phenomenon.[17]

Our results also showed that diet related information must be disseminated in a more robust manner as a quarter of the sampled respondents had misconceptions about fat in diet. There was a high proportion of respondents who expressed dissatisfaction about availability of information about relevant health services even from health care providers, which again shows that health promotion and information dissemination system must be improved in the community. It also signifies that care providers must be convinced that they are the most important source of health related information for the general public, and it is their duty to educate the people they treat.

Using Linear regression analysis, it was observed that there is a weak positive correlation between health literacy and preventive health actions, however, the relationship between health literacy and perceived information needs is weakly negative. This shows that health literacy does have a role in encouraging people in taking preventive actions against different diseases, which have been shown earlier.[5,10,16] However it also decreases their feeling of need for more health education and may result in a false sense of security. In the light of above findings and previous reports from Pakistan,[13-15,21] it is important that proper literacy programs must be initiated to increase preventive measures against common diseases in the society, so that the burden and cost of these diseases can be minimized.

One of the limitations of this study was limited sample size, which may raise questions regarding generalization of this study, however, randomization may adjust for this issue and it can be safely assumed that it provides a snapshot of the community at this point of time. Our findings strongly suggest that there is a significant information deficit in the educated population of one of the most advanced cities of the country. Therefore it is important to develop proper promotional programs so that the preventive attitude in the community can be

strengthened as this will allow the country to gradually travel towards the international goal of health for all.

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ORIGINAL ARTICLE

# Effect of Aqueous and Ethanolic Extract of Syzygium Aromaticum on Blood Glucose in Diabetic Rats

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## ABSTRACT

**Objective:** To compare the effect of 50% Aqueous and 50% Ethanolic extract of Syzygium aromaticum on blood glucose level in STZ induced diabetic rats in comparison with insulin.

**Study Design:** Randomized control trial.

**Place and Duration of Study:** This study was conducted at National Institute of Health Islamabad from July 2011 to December 2011.

**Materials and Methods:** Forty adult rats of Sprague dawaley specie were equally divided into 5 groups (I-V). Group- I control. Group (II-V) received a single intraperitoneal injection of STZ and rats having fasting blood glucose above 200mg/dl were selected. Group-II served as diabetic control , group III received 50% aqueous extract at a dose of 750 mg/kg body weight for sixty days and group 1Vrats received 50% ethanolic extract of Syzygium aromaticum at a dose of 750 mg/kg body weight for sixty days. Group V (standard) received the dose of 0.6 units/kg body weight of humulin insulin 70/30 subcutaneously bid for sixty days. After giving the injection of STZ fasting blood samples were taken at zero 15, 30 and 60 days and comparison is done between the glucose lowering effect of aqueous and ethanolic extract of Syzygium aromaticum.

**Results:** The 50% ethanolic extract of Syzygium aromaticum showed more reduction in blood glucose level than the 50% aqueous extract of Syzygium aromaticum. The levels of blood glucose markedly decreased in group-1V receiving 750 mg/kg body of ethanolic extract as compared to group III receiving the same dose of aqueous extract. Group V receiving insulin showed the level of this parameter almost closer to the blood glucose levels of group III rats.

**Conclusion:** The reduction in blood glucose with 750 mg/kg body weight of ethanolic extract of Syzygium aromaticum is more than with aqueous extract and insulin.


**Keywords:** *Syzygium Aromaticum Extract, Diabetes Mellitus, Glucose Lowering Effect.*

## Introduction

In diabetes mellitus hyperglycemia causes cellular lesions and enhances the non-enzymatic glycosylation of proteins and advanced glycosylation end-products are formed which injure cells by structural rearrangement of proteins.[1] Diabetes mellitus is a clinical syndrome with increased blood glucose level and depending on the need of insulin it is divided into absolute deficiency of insulin (type 1) or relative deficiency of insulin (type 2).[2] It has become a common disorder affecting approximately 180 million people all over the globe.[2] Streptozotocin (STZ) is used to induce diabetes in rats and causes hyperglycaemia.[3] STZ is effective after intraperitoneal administration of single injection.3 The doses range between 40 and 60 mg/kg body weight but higher doses can also be used.[3] It is a nitric oxide donor which leads to DNA damage.[3] after entering the cell it brings about changes in the DNA of pancreatic beta cells leading to its fragmentation.[3] Many undesirable effects of insulin and other oral hypoglycemic agents necessitated the search for more safer and effective anti diabetic agents.[4] In the last few decades many herbal plants have shown antidiabetic potential.[5] Syzygium aromaticum (clove) a herbal plant belongs to the family myrtacea.[6] genus Syzygium species aromaticum in urdu also called "laung" Scientific name of clove is Syzygium aromaticum (Linn) Merrill and Perry syn. Eugenia caryophyllata.[6] Clove has many uses and is used as a topical antiseptic.[7] local anaesthetic in dentistry.[7] In the treatment of gastrointestinal symptoms.[7] also as ant-inflammatory, insecticidal, antiplatelet, antioxidant, insulin-mimetic, and antihypertensive agent.[7] Clove oil is used as a painkiller for dental emergencies.8 Clove buds are used in food products as a flavoring agent condiment.[8] The compounds in clove are eugenol, isoeugenol, caryophyllene and triterpenes including oleanolic acid (OA).[9] This

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compound Oleanolic acid causes attenuation of the activities of glycogenic enzymes with concomitant increases of hepatic and muscle glycogen concentrations of STZ-induced diabetic rats.[10] Syzugium aromaticum has anti oxidant and antimutagenic potential.[11]

The purpose of this experimental study is to compare glucose lowering effect of ethanolic and aqueous extract of Syzygium aromaticum on STZ induced diabetic rats in comparison with the standard drug insulin, a drug commonly used in diabetic patients.

## Materials and Methods

This randomized control comparative study was conducted in the department of plant and scienceat National Institute of Health Islamabad from July to December 2011. A total of Forty adult healthy male Sprague dawley rats were selected and randomly divided in 5 groups with eight rats in each group. Female rats and male rats with weight less than 200 gm and more than 250 gm were not included in the study. The animals were kept in the animal house NIH for one week to get acclimatized under standard laboratory environment, with the room temperature at 260 C, humidity at 70%, 12 hours dark and light cycle was maintained.[12] Free access to rodent pellet and water ad labium was available throughout the study. A total of 250 grams of dried Syzygium aromaticum buds were purchased from the herbal dealer in the local market. The sample was submitted to the Department of Plant Sciences National University of Science and Technology Islamabad for identification of plant sample (NUST/NCVI /MQH/ZRC/ 001). Dried Syzygium aromaticum clove buds were didvided into two groups 125g in each group and were crushed and soaked into 50% ethanol and 50% aqueous soluton, each was stirred in the flask with magnetic stirrer for 24 hours at room temperature. After 24 hours the filtrate were separated and kept in a separate flask. The process was repeated thrice, and filtrate was concentrated at 40o C under reduced pressure in a rotary evaporator. The extracts was stored at temp of -20°C till used for experimental purpose.[13]

Rats with fasting blood glucose level between 70-135 mg/dl were selected after one week of acclimatization, a single intraperitoneal injection of freshly dissolved streptozotocin (60 mg/ kg body weight) in 0.1m citrate buffer (pH 4.5). was used for inducing diabetes, 5% dextrose, solution was given over night to counter the hypoglycemic shock.[14] After 48 h of STZ injection blood samples were taken from the tail vein. Animals with fasting blood glucose level above 200 mg/ 100 ml were selected for further experiments. The animals were randomly divided into five groups (n= 8). All the groups received standard diet and tap water for sixty days. Group-I. (control group) received 10 ml/kg of 0.9% saline solution.Group-II till group V received STZ (60 mg/kg body weight) as a single intraperitoneal injection. Group III received an aquoeus extract of strength 750mg/kg body wt by gavage, Group-IV received ethanolic extract of the strength 750mg/kg body wt by gavage.[13,14]Group-V. (Standard) received insulin (humulin 70/30) with dose of 0.6 units/kg body wt subcutaneously twice daily, for sixty days.[15] One drop of blood was with-drawn from the tail vein of the animals, at zero, 15, 30 and 60 day. quantitative estimation of blood glucose was done by using glucometer (Easy glucometer) and glucose oxidase based test strips. (Easy gluco auto coding test strips).[16] Twenty four hour after the last dose of the extract, the animals were anesthetized with ether and blood was with-drawn by cardiac puncture. The blood was allowed to clot for 5 minutes. Serum was separated by centrifuge at 3000 rpm for 10 minutes and stored at -20°C. Quantitative estimation of blood glucose was carried out by enzymatic method using a commercially available Kit (Randox, UK) based on glucose oxidase method.[17]Data was entered into SPSS version 20. Mean and standard deviation of the parameters were calculated and results of different study groups were compared. Changes in glucose level between all the groups were compared using One-Way ANOVA followed by Post-hoc Tukey test. A p-value of $< 0.05$ was considered significant.

## RESULTS

The readings of blood glucose showed that injection of STZ caused a significant ($p<0.001$) increase in the serum glucose level of the rats of group II, III, IV, and V as compared to the control group. On the other hand the administration of the dose of 750mg/kg body weight of aqueous and ethanolic extract of Syzygium aromaticum caused a significant ($p<0.001$) reduction in the blood glucose level of group III, and IV, as compared to group 11 (diabetic control). The reduction in the blood glucose level of group 1V

receiving 750 mg/kg body weight of ethanol extract of Syzygium aromaticum was significantly higher ($p<0.001$) as compared to the other experimental groups. It was also seen that simultaneous administration of insulin (humulin) resulted in significant ($p<0.001$) decrease in the serum glucose level of group V as compared to the group II (diabetic control) but the difference between group-III and group-V was insignificant ($p=0.996$)

**Table I: Serum glucose levels (mg/dl) in all the study groups**

| Groups | Glucose level mg/dl On day zero | Glucose level mg/dl On day 15 | Glucose level mg/dl On day 30 | Glucose level mg/dl On day 60 |
|---|---|---|---|---|
| Group-I (n = 8) | 121.87 ± 5.86 | 123.50 ± 5.58¶ | 125.00 ± 5.73¶¥€π | 127.75 ± 5.17¶¥€π |
| Group-II (n = 8) | 232.00± 7.42* | 234.00 ± 6.80* | 236.00 ± 7.09*¥€π | 237.87 ± 6.55*¥€π |
| Group-III(n = 8) | 229.25 ± 5.72* | 222.12 ± 5.38*¶ | 189.87 ± 3.75*¶€ | 167.12 ± 2.64*¶€ |
| Group-IV (n = 8) | 231.62 ± 6.92* | 205.75 ± 8.06*¶¥ | 180.37 ± 6.78*¶¥ π | 154.62 ± 5.97*¶¥π |
| Group-V (n = 8) | 230.25 ± 7.42* | 213.25 ± 7.38*¶ | 191.25 ± 6.71*¶€ | 166.12 ± 5.81*¶€ |
| p-value | < 0.001** | < 0.001** | < 0.001** | < 0.001** |

All values have been expressed as mean±SD

** = Highly Significant * = Significant from group-I

¶ = Significant from group-II ¥ = Significant from group-III

€ = Significant from group-IV π = Significant from group-V

## Discussion

In our study a comparison of aquoeous and ethanolic extract of Syzygium aromaticum on blood glucose of streptozotocin induced diabetic rats is made and the results are compared with the standard drug insulin at a dose of 0.6 units/kg body weight. We used 50% aqueous extract and 50% ethanol extract with dose of 750mg/kg body weight respectively. In our study group II showed significant elevation in the blood glucose as compared to group I (control) group. The administration of aqueous extract of Syzygium aromaticum to group III, ethanol extract to group IV, and humulin insulin to group V brought the level of this diagnostic parameter in all the experimental groups to almost normal as compared to group II (diabetic control group) rats. When we compare the mean values of glucose between group III, IV with group V although the reduction in the serum glucose level of all the three groups is seen but reduction is more in group 1V as compared to the other two groups. It is seen that the group V receiving insulin also reduced the blood glucose but the level of this parameter is almost close to the reduction in blood glucose level brought by group III. It is observed that Syzygium aromaticum ethanol extract causes 35% reduction in the serum glucose level and the Syzygium aromaticum aqueous extract causes 30% reduction in blood glucose level which is close to the reduction in the blood glucose brought about by insulin. In our study we used 50% ethanol extract of plant because the constituents in the Syzygium aromaticum are more soluble in ethanol.[18] Similar concentration of extract was used by Tajuddin A, et.al who used 50% ethanol extract of clove in rats.[19] The dose 750 mg /kg body weight was selected because zunnera et al in one of her study on the diabetic rats suggested that maximum glucose lowering effect of syzygium aromaticum was seen with the extract having the strength of 750 mg/kg body weight.[20] We used aqueous extract in our study because aqueous extract of Syzygium aromaticum also has the potential of lowering blood glucose. Rao BK et.al conducted a study on the genus Syzygium and concluded that the aqueous extract of the Syzygium also possess the potential of lowering blood glucose.[21] Our results indicate that group IV receiving 50% ethanolic extract at a dose of 750 mg/kg body weight causes more reduction in blood glusoe as compared to group III and group V, similar results are seen with Abubakar Gidadoet et al who studied the effect of aqueous and ethanolic extract of plant on blood glucose and concluded that ethanolic extract caused more reduction in blood glucose.[22] The main constituents in the Syzygium aromaticum are Olaenic acid and Eugenol.[23] Musabayane et al. critically reviewed the analytical chemistry of Eugenol, and Olaenic acid and found that both posses antioxidant activity and are the major scavenger of free radicals.[24] Segas et al, proposed that glucose lowering effect of Syzygium aromaticum can be through anti oxidant means.[25]It has been reported in many studies that extract of herbal plants when used in the treatment of diabetes mellitus resulted in the activation of pancreatic beta cells and improved granulation showing insulinogenic effect.[26] Khan A (2006) in one of his study said that Syzygium aromaticum has the

potential to cause regeneration of pancreatic beta cells and stimulate the functioning cells of islet of

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ORIGINAL ARTICLE

# Oral Cytomorphometry of Smokers and Non Smokers

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## ABSTRACT

**Objective:** The objective of the study was to observe and compare the changes in buccal exfoliated cells between smokers and Nonsmokers.

**Study Design:** Cross sectional comparative study.

**Place and Duration of Study:** Study was carried out at Islamic International Dental College, Islamabad and Post Graduate Medical Institute, Lahore. The duration of study was six months i.e 1st September 2013-1st March 2014.

**Materials and Methods:** Convinient, non-probability sampling technique was used. Quantitative data was obtained. The study groups consisted of 66 subjects divided into two equal groups of smokers S and non- smokers M, of ages between 15yrs-60yrs. Cellular diameter CD, nuclear diameter ND and nuclear to cytoplasmic ratio N/C ratio was assessed in buccal mucosal smears taken from clinically normal mucosa of smokers and normal subjects using exfoliative cytology. SPSS version 17.0 was used for data entry and statistical analysis. ANOVA and post-hoc tuckey were used for statistical analysis.

**Results:** The mean cellular diameter of smokers and non-smokers was 54.41± 3.30µm and 43.81±2.01µm respectively. The mean nuclear diameter of smokers and non-smokers was 12.68± 0.90µm and 9.97± 0.80µm respectively. And the mean N/C ratio of group smokers and non-smokers was 1: 4.43± 0.38 and 1: 4.42± 0.41 respectively. The ONE WAY ANOVA test showed significant results (p=0.000) for cellular diameter CD, nuclear diameter ND and N/C ratio both, while post hoc tukey test gave highly significant results for CD and N/C ratio i.e p=0.000.

**Conclusion:** Exfoliative cytology and cytomorphometry can help in the early detection of cellular changes as these techniques are easy, non-invasive and reproducible. Moreover, there is significant cause effect relationship between smoking and variables as nuclear diameter ND and N/C ratio.


**Keywords:** *Smokers, Oral Exfoliative Cytology, Cytomorphometry.*

## Introduction

Oral squamous cell carcinoma comprises of 90-95% of all oral cancers.[1] In Pakistan, oral cancer is the second most common cause of cancer in women and third most common in men.[2] The five years survival rate for oral squamous cell carcinoma has remained at approximately 50% for the past several decades.[3] Prognosis of oral squamous cell carcinoma lacks improvement because most of the lesions are diagnosed or treated at advanced stages. The prognosis for patients with squamous cell carcinoma that is treated early is much better, with 5 years survival rate as high as 80%.[4]

Tobacco is an important causative factor for oral cancer. Different forms of tobacco like smokeless tobacco, naswar, cigarettes, cigars, pipes are proved etiological factors for oral cancers.3Many cellular alterations are caused by use of tobacco in the buccal cells. Studies have been carried out to find out role of techniques that can assess the cellular changes as a result of tobacco use.[5,6] Moreover, to evaluate their possibility to serve as a screening tool for early diagnosis of oral dysplastic lesions or that may lead to malignancy. Relating this prospect, exfoliative cytological techniques are being used to detect the influence of tobacco on the oral mucosa.[7]

Exfoliative cytology is a simple non-invasive diagnostic technique that is useful in early assessment of cellular changes in oral lesions.[8] But still this technique is not widely accepted as a screening tool because the results of various studies are quite variable and thus cannot be used as a standardized early diagnostic tool. Moreover, majority of studies carried out internationally assessed the cytomorphological and not the cytomorphometric cellular changes affecting the oral epithelium in tobacco chewers but not the smokers. Thus, objective of the present study was to evaluate the role of exfoliative cytology in differentiating the cytomorphometric parameters between smokers and non-smokers. Furthermore,

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quantitative techniques basedon the assessment of variables as nuclear diameter ND, cellular diameter CD and nuclear to cytoplasmic ratio N/C ratio may increase the sensitivity of exfoliative cytology for the early diagnosis of oral cancers as these techniques are accurate, objective and reproducible.[9]

## Materials and Methods

The objective of the study was to:

1. Observe the cytomporphometric changes in buccal mucosal smears of smokers and non-smokers.
2. To compare these changes in buccal exfoliated cells between smokers and Non-smokers.

A cross- sectional comparative study was carried out at Histopathology Department, Post Graduate Medical Institute Lahore and Oral pathology department, Islamic International Dental College, Riphah International University, Islamabad from 1st September 2013 to1st March 2014.

The study group consisted of 66 adult males divided into two groups: smokers and non-smokers. The age group was 15 years and above. Smokers included were smoking cigarettes only for 3 years or more; 3-5 times daily and without any visible lesion in the oral cavity, respectively. While those included in control group were normal healthy individuals without any habit of using tobacco, pan or gutka. Also the subjects included in both groups were not having any chronic debilitating diseases.

Informed consent was obtained from all the subjects to obtain the cytological smears. Data was collected through convenient, non- probability sampling technique. Data that was collected was quantitative. Scrapings were obtained using a moistened wooden spatula. Using a gentle scraping motion cells were scraped from clinically normal looking buccal mucosa from the both groups. Three smears were taken from each individual to prepare three slides per case. The scrapings were smeared onto the centre of the previously marked glass slides and were immediately fixed in 95% Alcohol. All cytological smears were stained with hematoxylin and eosin, Giemsa and pap stains.[10] Each case has three slides and these three were individually stained with H&E, Giemsa and Pap stain. Two types of micrometers are used to measure an object under a microscope i.e stage micrometer and ocular micrometer. Ocular micrometer is precalibrated using a stage micrometer on required optical combination before making accurate measurements.[11] The ocular micrometer was precalibrated with the help of stage micrometer according to which one division of ocular micrometer was equal to 3µm using the following equation:

100 div on ocular micrometer = 30 divisions on stage micrometer (one div =10µm)

= 30×10

100 div on ocular micrometer = 300 um

1 div on ocular micrometer = x

x =3 µm

After calibration, variables like cellular diameter(CD) and nuclear diameter(ND) of the 50 cells in each smear were measured by using calibrated ocular micrometer fixed in eye piece of microscope on 40 x (Fig 1). The average of the values give the size of cell and nucleus in each subject, followed by calculating the N/C ratio (NCR). Data was entered in SPSS version 17.0 and all the mentioned variables were analysed. ONE WAY ANOVA and post hoc tuckey test were applied for two groups to compare the mean of CD, ND and their ratios.

## Results

Subjects included in the study were all adult males with age range between 15yrs-60yrs; with peak age range in the 4th decade of life. After cytomorphometry following results were calculated in smokers and non-smokers: i.e cellular diameter, nuclear diameter, and N/C ratio table I. The smears in this study were analysed quantitatively and the mentined parameters were measured. Fifty clearly defined cells were measured in each slide with precalibrated ocular micrometer. The cellular diameter and nuclear diameter were recorded on both axis and mean was taken to calculate the values.

## Discussion

On the whole, cytomorphometric results show that on all three stains i.e; H&E, pap and giemsa, the measurements were almost the same.

When variance analysis was conducted to analyse any difference in the cellular diameter between the two groups, a statistically significant difference was found ($p < 0.005$). The intergroup Post-hoc tukey analysis revealed that the difference in the cellular diameter between the smokers group (54.38 + 3.31µm) and the control group was significant.

Carcinomas in the oral cavity are caused by use of

**Table I: Mean of CD, ND and NCR in smokers and non-smokers**

| Stain | Control | | | Smokers | | |
|---|---|---|---|---|---|---|
| | CDµm | NDµm | N/C | CDµm | NDµm | N/C |
| H & E | 43.81 ±2.01 | 9.97 ±0.80 | 1:4.4 ±0.41 | 54.38 ±3.31 | 12.66 ±0.92 | 1:4.3 ±0.39 |
| GIEMSA | 43.81 ±2.01 | 9.97 ±0.80 | 1:4.4 ±0.41 | 54.41 ±3.29 | 12.68 ±0.91 | 1:4.3 ±0.39 |
| PAP | 43.81 ±2.01 | 9.97±0 .80 | 1:4.4 ±0.41 | 54.36 ±3.32 | 12.63± 0.91 | 1:4.3 ±0.38 |

One way ANOVA and post hoc tuckey test showed significant results i.e p value 0.000 for ND and NCR.

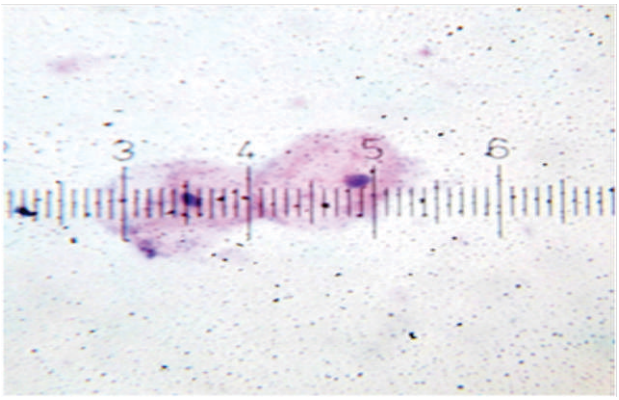

**Fig 1: Image of Individual buccal mucosal cell of smoker superimposed with focused precalibrated ocular micrometer.**

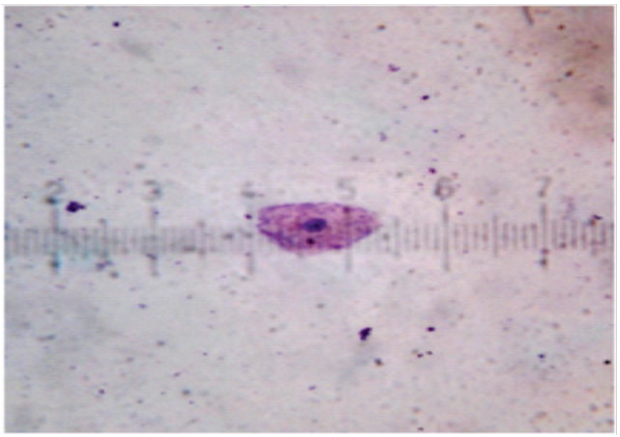

**Fig 2: Image of Individual buccal mucosal cell of control group superimposed with focused precalibrated ocular micrometer**

different forms of tobacco, thus making it possible to view the damage with naked eye.[12] Different forms of tobacco like smokeless tobacco, naswar, cigarettes, cigars, pipes are proven to be as prominent risk factors for oral cancers.[13]

The hostile effects of cigarrete smoking and smokeless tobacco use have been studied and documented by various studies.[14,15,16,17] Tobacco induced buccal changes at celluar level are also studied and documented in several articles.[18,19,20,21,22,23]

Oral exfoliative cytology has been proven to detect early changes in the cells even before the onset of the clinical lesion, and also this technique is inexpensive and easy with high sensitivity rates and diagnostic values.[24]

Hande and Chaudhary in 2010 conducted a study using cytomorphometry and showed that systemic and external factors affect the cytomorphometric variables such as ND, CD and N/C ratio.2 As the CD is increased in smokers in the present study, it may be due to any factor which is caused by smoking cigarettes. The results of the present study, i.e, increase in the CD of smokers as compared to the control group, contrasts with the other studies carried out like in case of Sumit babuta (2014) and Goregen (2011).[25,26] Whereby in a study conducted by Ramesh et al. (1999) CD was decreased in cigarette smokers.[27] Similarly, a study conducted by Ogden et al. (1997) also showed a decrease in CD of tobacco users.[7]

When variance analysis was conducted to analyse any difference in the nuclear diameter between the two groups, a statistically significant difference was found (p = 0.000). The intergroup Post-hoc tukey analysis revealed that the difference in the nuclear diameter between the smokers group (12.68 µm± 0.91) and the control group (9.97 µm± 0.80) was significant.

In the present study, smoker group showed an increase in ND in comparison with the control group. This may be due to various reasons including use of tobacco or increase in DNA content as stated by Hande and Chaudhary in 2010.[2] Einstein and Sivapathasundharam conducted a study in 2005 which showed that CD decreased while ND increased in the buccal mucosal cells of tobacco users in south of India.[28] Other studies which have been conducted in the past on the same subject showed the similar results which are consistent with the findings of the present study i.e smokers or tobacco users.[24,25] Ogden et al observed in 1989 5 % average increase in nuclear diameter of smokers when compared with those of the non-smokers.[8] While a study conducted

by Goregen in 2011 showed an increase of 16.5 % increase in ND of smokers as compared to non-smokers which was attributed to smoking.[26]

The analysis of variance test reported a significant difference in the N/C ratio between the two groups ($p<0.005$). The intergroup Post-hoc tukey analysis revealed that the difference in the N/C ratio between the control group (1:4.4 ± 0.37) and the smokers group (1:4.3 ± 0.38) was not significant ($p> 0.005$).

Franklin and Smith in 1980 carried out a study which showed that N/C ratio helps us to show the precise relationship in the altered cellular and nuclear diameter.[29] N/C ratio in the smokers was also higher when compared with the control group which could be because of increased CD and ND in the respective group. Increase in the N/C ratio can be indicative of an early dysplastic change because in squamous cell carcinoma the N/C ratio is increased to 1:1 from 1:4.[30]

The limitations of the study were that cytomorphometry can be computer assisted with the help of softwares that were not available for the present study. Computer assisted cytomorphometry can give more accurate and quick results as compared to manual cytomorphometric technique used in the present study. Cytomorphometry can measure the early changes in buccal smears of tobacco users which can help in the early detection of malignant changes to improve the prognosis of oral squamous cell carcinomas.

## Conclusion

This study suggests that cytomorphometric analysis showed significant results in terms of changes in CD, ND and N/C ratio between the control and study group. However, it is important here to highlight the fact that these changes depict cause effect relationship only and association of these changes with dysplasia or pre-malignancy needs further verification with the help of specific immune-markers.

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ORIGINAL ARTICLE

# Effect of Aqueous Extract of Walnut Leaves on Lipid Profile and Atherogenic Ratio in Hypercholesterolemic Rats

Rabia Azhar, Arif Siddiqui, Shazia Ali


## ABSTRACT

**Objective:** To determine the effect of aqueous extract of walnut leaves on lipid profile i.e. serum total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol triglycerides, LDL/HDL and atherogenic ratio (Total Cholesterol/HDL) of hypercholesterolemic rats.

**Study Design:** An experimental randomized control study.

**Place and Duration of Study:** The study was conducted at Islamic International Medical College, Riphah International University, with assistance from National Institute of Health, Riphah Institute of Pharmaceutical Sciences and Citilab, Islamabad, Pakistan. The duration of study was one year from April 2014 to March 2015.

**Materials and Methods:** A total of 30 male Sprague Dawley rats were included in the study. They were divided into 3 groups i.e. ten rats in each group. Group 1(Control group), Group 2 (Hyper-cholesterolemic control), Group 3 (Aqueous group) treated with aqueous extract of walnut leaves after induction of hypercholesterolemia in a dose of (200mg/kg) through gavage needle once daily, for four weeks. Blood sampling was done at the beginning (baseline), end of week 8, and end of week 12 to perform lipid profile, LDL/HDL and atherogenic ratios TC/HDL-cholesterol. Statistical analysis was applied by using SPSS version 17. All data was shown as mean ±SD and Student t test was applied between groups. p value of $< 0.05$ was considered as statistically significant.

**Results:** Hyper-cholesterolemic rats after treatment with aqueous extract (Group 3) had significantly lower levels ($p<0.001$) of serum cholesterol, low density lipoprotein and triglycerides while significantly high ($p<0.001$) levels of HDL-Cholesterol with significantly reduced ($p<0.001$) TC/HDL-Cholesterol and LDL/HDL ratios.

**Conclusion:** Aqueous extract of walnut leaves has hypo-lipidemic effect on serum total cholesterol, LDL-Cholesterol and triglycerides. It reduces TC/HDL, LDL/HDL ratios whereas it significantly increases the level of HDL-Cholesterol.


**Keywords:** *Juglans Regia, Atherogenic Ratio, Hypercholesterolemia, Lipoproteins.*

## Introduction

Hypercholesterolemia is a condition characterized by elevated serum total cholesterol, triglycerides (Tgs), low-density lipoprotein (LDL-Cholesterol), very low density lipoprotein (VLDL-Cholesterol) and decreased high-density lipoprotein (HDL-Cholesterol) levels.[1] It can develop primarily due to genetic cause or secondary to chronic diseases like hypothyroidism, diabetes mellitus and renal insufficiency.[2] Cholesterol is one of the essential component present in all foods of animal origin and is necessary for synthesis of cell membrane which plays a significant role in the maintenance of cell homeostasis and trans-membrane communication.[3] TGs are mainly synthesized in the liver or present in dietary fat and are carried in the form of chylomicrons and VLDL in capillaries where they are ultimately hydrolyzed by lipoprotein lipase into free fatty acids.[4] HDL plays a significant role in reversing cholesterol transport which is an important step in the eradication of surplus cholesterol from the body.[5] So increased levels of serum HDL prevents development of hypercholesterolemia and cardiovascular disease (CVD).[6] The LDL-Cholesterol are termed as "bad cholesterol", as it carries maximum of cholesterol in circulation which in turn increases risk of CVDs.[7] Studies have shown that hypercholesterolemic diet increase cholesterol, LDL, TG levels and decreases LDL-receptor activities in liver.6 Increased LDL, cholesterol and TGs are considered dangerous and are strongly linked with poor cardiovascular outcomes.[8,9]

TC/HDL and LDL/HDL ratios are two important components and predictors of CVDs. When TC/HDL ratio is more than one to five, the risk of CVDs increases, substantially.[10] The initial step in management of hypercholesterolemia includes diet modification and use of lipid lowering agents.[5] Medicinal plants are being used for the treatment of various diseases as they are considered safer and cost effective as compared to pharmaceutical

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medicines.[11] Walnut (Juglans regia L) is a medicinal plant that belongs to the family juglandaceae and is extensively cultivated in China, Japan, South Asia, South Eastern Europe and United States.[12] Different parts of juglans regia such as kernel, shell, leaves, septum, bark, epicarp have been used in pharmaceutical and cosmetic products.[13]

Previous studies have documented that walnut leaves have been used in folk medicine for the treatment of hypoglycemia, diarrhea, venous insufficiency, hemorrhoids and fungal or microbial infections.[7] Whereas, walnuts leaves extract have antimicrobial, antihyperglycemic, anti-inflammatory and anti proliferative activity.[14-17] Whole walnut improves cholesterol and lipoprotein levels and walnut bark is used for cleaning teeth.[18]

Despite various approaches on walnut, the effect of aqueous extract of walnut leave on lipid profile and LDL/HDL, TC/HDL ratios in hypercholesterolemia have not been explored. So, the purpose of this study is to investigate the effect of aqueous extract of walnut leaves on lipid profile, LDL/HDL and atherogenic ratios (TC/HDL) of hypercholesterolemic rats.

## Materials and Methods

This randomized control study was conducted at Islamic International Medical College, Riphah International University, Islamabad with access to Animal Housing Facility for laboratory rats at National Institute of Health (NIH), Islamabad for over a period of 1 year (April, 2014 to March 2015), after taking approval from Ethical Review Committee of Islamic International Medical College. A total of 30 male Sprague Dawley rats, aged 3 months, weighing 250-300 grams were included in the study. Rats were kept for 3 months at NIH Animal Housing Facility in a well ventilated room with 12 hours light and dark cycle, 50-70 humidity % at 24±2 oC room temperature.[19,20] Rats were fed on standard rat diet and availability of water was made ad libitum for a period of one week in order to get rats acclimatized for acclimatization before starting the experiment.

Rats were divided into three groups i.e. 10 in each group. Group 1 (control group) was fed on regular diet till the end of study. Group 2 (hypercholesterolemic group) and 3 (aqueous group) were fed on high fatty diet prepared at NIH comprising 17 % of calories as carbohydrates, 25% as proteins and 58% calories as fat for 8 weeks for induction of hypercholesterolemia.[21] Group 2 was considered as hypercholesterolemic control and then given regular diet till the end of study. Group 3 (aqueous group) which after inducing hypercholesterolemia, was given aqueous extract of walnut leaves (juglans regia) in a dose of (200mg/kg) through gavage needle once daily for four weeks. Aqueous extract was prepared from walnut leaves (juglans regia) collected from Muzaffarabad, Azad Kashmir and were identified and authenticated by Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan. The leaves collected, were coded and kept under voucher number 57 at the Herbarium, at Quaid-i-Azam University, Islamabad, Pakistan. Walnut leaves were first dried under shade and grounded into a fine powder with the help of electrical grinder. The aqueous extract of walnut leaves was prepared by using one hundred gram of grounded walnut leaves soaked in distilled water for 24 hours. The solution obtained was later filtered using Whatmann Filter paper No.1 and dried in a rotary evaporator at 55°C at research laboratory of Riphah Institute of Pharmaceutical Sciences, Islamabad. The extract obtained was in the form of dark brown semi-solid sticky paste and was stored in air tight glass bottles, protected from light and kept in refrigerator at 2-8 o C to be used throughout the experiment.[22]

Blood samples were collected three times (baseline, at the end of week 8 and week 12). At baseline and at the end of week 8 1.5 ml of blood samples were drawn through tail vein sampling from rats of all three groups while Final 1.5 ml of blood sample was drawn through cardiac puncture at the end of week 12 from rats of all groups.

Blood was then centrifuged at 3000 rev/min for 15min and serum was separated for analysis of lipid profile.[23] Blood samples for serum total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were estimated using Merck (Germany) kits based by enzymatic calorimeter method on automated chemistry analyzer and ratios was calculated as TC/HDL and LDL/HDL.

The data was analyzed using SPSS version 17. All data are shown as mean ±SD and Student t test was applied between group 2 (hyper-cholesterolemic control) and group 3 (aqueous group). p value of <

0.05 was considered as statistically significant.

## Results

During the experiment blood was retrieved for lipid profile analysis at various intervals from Sprague Dawley rats i.e. in the beginning of experiment (pre-cholesterol), after 8 weeks (mid-cholesterol) and at the end of experiment at 12 weeks (post-cholesterol) in control, hypercholesterolemic control, and aqueous extract group which are presented in fig 1.

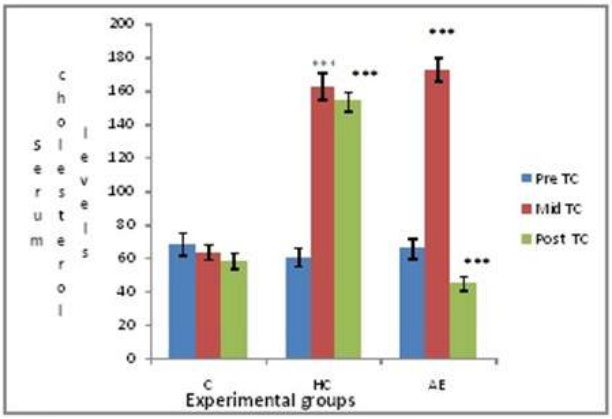


**Fig 1: Serum cholesterol levels (mg/dl) in C, HC and AE group at the start of experiment (Pre-Cholesterol), after 08 weeks (Mid-Cholesterol) & after 12 weeks (Post-Cholesterol) in Sprague Dawley rats. C=Control group, HC = Hypercholesterolemic control, AE = Aqueous extract. All Values are expressed as mean +- SD**
***** p<0.001 is considered significant on comparison with Hypercholesterolemic control group**

Group 2 and 3 were given hypercholesterolemic diet for 8 weeks, after which mid- cholesterol levels in group 2 (162.5±7.90 mg/dl) and group 3 (172.7±6.95 mg/dl) were significantly raised (p<0.001) as compared to the mid-cholesterol levels of control group 1 (63.5±4.56mg/dl), which confirmed the development of hypercholesterolemia. Group 3 hypercholesterolemic rats treated with aqueous extract for 4 weeks, had significantly reduced (p<0.001) post cholesterol levels (45.0±4.56 mg/dl) when compared with the post cholesterol levels of hypercholesterolemic control group 2 (153.7±5.92 mg/dl).

Analysis of serum TG levels at various intervals during the experiment which are shown in Figure 2. Mid-TG levels after giving hypercholesterolemic diet for 8 weeks in Group 2(153.4.6±3.78 mg/dl) and 3(159.7±2.24mg/dl) were significantly raised

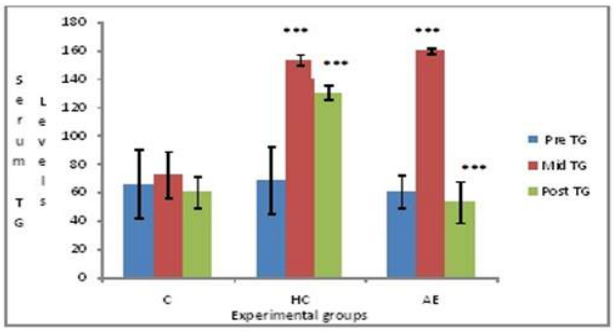


**Fig 2: Serum TG levels (mg/dl) in C, HC and AE group at the start of experiment (Pre-TG), after 08 weeks (Mid-TG) & after 12 weeks (Post-TG) in Sprague Dawleyrats C= Control Group, HC = Hypercholesterolemic control, AE = Aqueous extract. All values are expressed as mean +- SD**
***** p<0.001 is considered significant on comparison with Hypercholesterolemic control group**

(p<0.001) as compared to mid-TG levels of control group 1 (72.45±16.6mg/dl). Group 3 hypercholesterolemic rats treated with aqueous extract for 4 weeks after which post-TG levels 53.38±14.6 mg/dl were significantly reduced (p<0.001) as compared to post-TG levels of hypercholesterolemic control group 2 (130.5±4.95 mg/dl).

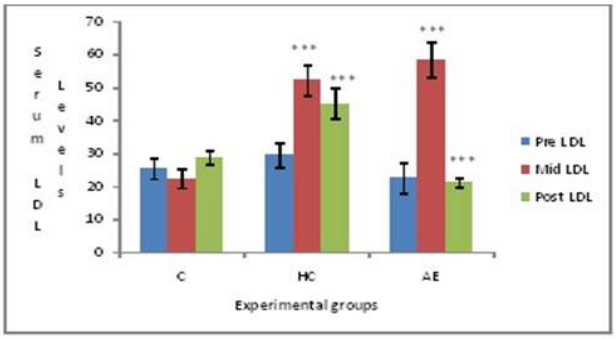


**Fig 3: Serum LDL levels (mg/dl) in C, HC and AE Group at the start of experiment (Pre-LDL), after 08 weeks (Mid-LDL) & after 12 weeks (Post-LDL) in Sprague Dawleyrats) C=Control group, HC = Hypercholesterolemic control, AE = Aqueous extract. All Values are expressed as mean +- SD**
***** p<0.001 is considered significant on comparison with Hypercholesterolemic control group**

Serum LDL levels analyzed at various intervals during the experiment which are presented in Figure 3.Group 2 and 3 were given hypercholesterolemic diet for 8 weeks after which mid-LDL levels in group 2 (52.25±4.68mg/dl) and group 3 (59.45±5.25mg/dl) were significantly raised (p<0.001) as compared to

mid- LDL levels of control group 1 (22.43±2.89mg/dl). Group 3 hypercholesterolemic

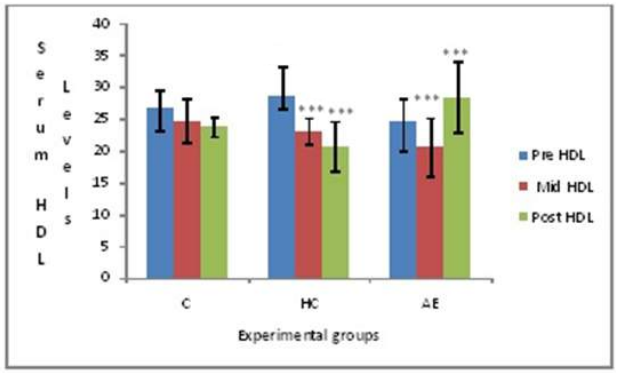


**Fig 4: Serum HDL levels (mg/dl) in C, HC and AE Group at the start of experiment (Pre-HDL), after 08 weeks (Mid -HDL) & after 12 weeks (Post-HDL) in Sprague Dawleyrats) C=Control group, HC = Hypercholesterolemic control, AE = Aqueous extract. All Values are expressed as mean +- SD**

***** p<0.001 is considered significant on comparison with Hypercholesterolemic control group**

to the mid- TC/HDL and LDL/HDL levels of control group 1. Group 3 hypercholesterolemic rats were treated with aqueous extract for 4 weeks, the post TC/HDL and LDL/HDL ratios were significantly reduced (p<0.05) on comparison with the post TC/HDL and LDL/HDL ratios of hypercholesterolemic control group 2.

## Discussion

Hypercholesterolemia is a major risk factor for cardiovascular diseases and diabetes mellitus.

The present study showed that aqueous extract of walnut leaves cause significant reduction in serum cholesterol, TGs, LDL, TC/HDL and LDL/HDL ratio and significant increased in HDL levels of hypercholesterolemic Sprague Dawley rats. This study was in agreement with the work done by Mahmoodi at al., (2011) who studied the hypolipidemic effects of walnut leaf powder on lipid profile in hypercholesterolemic rats. There was significant reduction (p<0.05) in serum total cholesterol, LDL and TGs and increased HDL as compared to hypercholesterolemic control.[6]

Gholamreza (2008) and Divband et al.,(2010)

**Table I: Comparison of TC/HDL and LDL/HDL ratio of Sprague Dawley rats at baseline and end of week 8 and week 12 of the experiment**

| Variables | Time | Group 1 (C) n=10 | Group 2 (HC) n=10 | Group 3 (AE) n=10 |
|---|---|---|---|---|
| **Atherogenic ratio TC/HDL** | baseline | 2.27 ±0.06 | 2.23 ±0.06 | 2.20 ±4.06 |
| | end of 8 week | 2.27 ±0.06 | 7.33 ±0.7** | 7.23 ±2.7** |
| | end of12 week | 2.49 ±0.2 | 6.6 ±0.4 | 1.84 ±0.1** |
| **LDL/HDL ratio** | baseline | 0.93 ±0.04 | 0.96 ±0.34 | 0.94 ±0.44 |
| | end of 8 week | 0.93 ±0.04 | 2.23 ±0.39** | 2.34 ±1.39** |
| | end of12 week | 1.04 ±0.13 | 2.01 ±0.3 | 0.85 ±0.06** |

TC: Total cholesterol, HDL: High density lipoproteins, LDL: Low density lipoproteins. C=Control group, HC= Hypercholesterolmic control, AE=Aqueous group. Data represents as mean ±SD

* p <0.05 with respect to corresponding control

** p<0.001 with respect to corresponding control

conducted a study in diabetic rats for a period of 4 weeks to study the effect of aqueous extract of walnut leaves on serum lipid profile and blood sugar. They reported that diabetic rats, given aqueous extract of walnut leaves caused a significant decrease in glucose (p=0.009), cholesterol (p=0.045), LDL (p=0.022), TGs (p=0.047) and a significant increase in HDL levels (p=0.045) as compared to diabetic control group. Also TC/HDL (p=0.006) and LDL/HDL ratio (p=0.035) in experimental group were significantly decreased when compared with the control diabetic group.[22,24] This study had showed similar results with the present study but they had used diabetic rats instead of hypercholesterolemic rats. Asgary et al., (2008), studied the effect of administration of ethanolic extract of walnut leaves on biochemical parameters in a dose of 200mg/kg for four weeks in alloxan-induced diabetic rats which showed similar results to our study.[25] Zavvarreza et al (2006), conducted a study and reported that the administration of Iranian walnut oil extract caused dose-dependent decrease in TGs, cholesterol and LDL level in rats that received

hypercholesterolemic diet.[22]

Banel (2009) who conducted a meta-analysis and literature review to investigate the effect of walnuts on blood lipids. When compared with control diets, supplemented diets with walnuts resulted in a significant decrease in total cholesterol, LDL levels and TGs while HDL were not significantly affected by walnut diets.[26] Finding of our study was not in accordance with their study as HDL levels were significantly raised in our study after treatment with aqueous extract.

In present study the HDL levels were significantly raised in aqueous group as compared to hypercholesteremic control group however these findings are not in consistance to work done by Iwamoto et al., (2000) who studied the effect of walnuts consumption on serum lipids in Japanese subjects for a period of 4 week. The study revealed a significant decrease ($p<0.01$) in total cholesterol, LDL levels and LDL/HDL ratio while HDL-Cholesterol were not significantly affected by walnut diets.[27]

The possible mechanism underlying the lipid lowering effect of juglans regia might be due to effects of compounds like phenolic acids and flavonoids which are the major antioxidant present in walnut leaf. Antioxidants like quercitin and cholorogenic acid reduce synthesis of cholesterol in liver through inhibition of HMG COA reductase enzyme and causes increased biliary excretion of cholesterol.[28] Studies have also shown that other components like fiber, micronutrients such as vitamin E and C, folic acid, copper, calcium, potassium, magnesium, plant protein (such as arginine), plant sterols are also present in walnut leaves they distribute lipid properly in physiologically manner to prevent lipid and cholesterol accumulation.[29,30]

## Conclusion

The present study concludes that aqueous extract of walnut leaves has remarkable lipid lowering effect in hypercholesterolemic rats that decreases serum cholesterol, triglycerides LDL, TC /HDL and LDL/HDL ratios with concomitant increase in HDL levels. Results suggest that administration of aqueous extract of walnut leave can be another option for treating people with hypercholesterolemia and may have beneficial role in the prevention of cardiovascular disease.

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ORIGINAL ARTICLE

# Review of Dental Operative Procedures and the Causes of Tooth Extraction at the Railway General Hospital Rawalpindi

Fatima Suhaib, Mir Rizwan Ahmad, Khalil ur Rehman

**ABSTRACT**

**Objective:** To study the frequency of various dental procedures and the causes of dental extractions at Railway General Hospital Rawalpindi and to suggest measures to improve the existing scenario.

**Study Design:** A descriptive cross-sectional study.

**Place and Duration of Study:** The study was conducted at IIMCT, Railway General Hospital from June 2014 to May 2015.

**Materials and Methods:** All consecutive patients undergoing dental operative procedures at IIMCT Railway General Hospital were studied. The patients were examined clinically and by dental X-Ray when required. The extent of caries was ascertained and Miller's Mobility Index was used to grade the extent of Periodontitis. Tooth extraction was done in patients having broken down roots, gross caries, tooth mobility grade >2 and those with impaction, trauma, cysts etc.

**Results:** A total of 3116 dental procedures were done that included 1902 (61.0%) tooth extractions, 930 (29.8%) fillings, 166 (5.3%) scalings and 118 (3.8%) root canal treatments. The number of scalings and root canal treatments significantly increased with addition of an extra dental surgeon ($p<0.001$). A total of 1902 teeth were extracted from 1560 patients. Caries was the leading cause of tooth extraction (60%). Most of the patients with advanced caries presented with broken down root (763/1902, 40%). Gross caries was the reason for extraction in 380/1902 (20%), periodontitis with mobile teeth (grade >2) in 569/1902 (30%) and other reasons like cysts, impaction and trauma etc. in 190/1902 (10%) of patients.

**Conclusion:** Caries and periodontal infections are the commonest dental problems and the reason for tooth extraction at the Railway General Hospital Rawalpindi where people mostly come from the lower socio-economic status. The quality of work in a public sector dental setting like Railway General Hospital can be improved by providing adequate number of dentists and other resources.

**Keywords:** *Dental Caries, Periodontitis, Tooth Extraction, Scaling, Root Canal Treatment.*

## Introduction

Tooth extraction is one of the oldest and the most frequently performed operative procedures in dentistry. The frequency of tooth extraction, at least in the developed centers of the world, is on the decline with a corresponding rise in the frequency of tooth restorative procedures like root canal treatment.[1-4] However, tooth extraction is still a preferred choice in dental centers with budget constraints, lack of expertise in using restorative procedures or where the dental surgeon is too busy to spare time for a complex solution.[5,6] When a tooth is removed without taking into account how that gap will be filled the patient may be left with a long term disability and morbidity. Carries ranks as the leading cause of tooth extraction followed by periodontitis.[7-11] Both of these conditions are related to poor oral hygiene and can be prevented by community based awareness programs.[12] The Railway General Hospital Rawalpindi provides free of cost services to the employees of Pakistan Railway. The hospital also takes care of the non-entitled resident population of the surrounding areas that mostly come from the low socioeconomic group. The hospital is affiliated with the Islamic International Medical College but its dental unit is without any such affiliation. Like most other public sector hospitals the dental unit of the Railway General Hospital Rawalpindi works within a tight budget. The cost restrictions and excessive workload with limited dental chairs often preclude dental restorative procedures like root canal treatment and preparation of dental implants and prostheses. This descriptive study aims to analyze the frequency of various dental procedures and the causes of dental extractions at this hospital and to suggest measures to improve the existing scenario.

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## Materials and Methods

This descriptive cross-sectional study was done at the Dental Department of Railway General Hospital Rawalpindi over a period of one year (Jun 2014 to May 2015). All consecutive patients undergoing dental operative procedures were studied. The age

and gender of the patients was recorded. During the first half of the year (Jun to Nov 2014) two dental surgeons were available whereas the third dental surgeon (FS) joined the department during the second half of the year (Dec 14 to May 15).Only one dental chair was available for the operative treatment. The patients were examined clinically and by dental X-Ray when required. The extent of caries was ascertained according to the destruction of the tooth structure. Miller's Mobility Index was used to grade the extent of Periodontitis.[13] The patients with mild to moderate caries were subjected to filling or root canal treatment. Tooth extraction was done in patients having broken down roots, gross caries, tooth mobility grade >2 and those with impaction, trauma, cysts etc. Statistical analysis was done by Chi square test with the computer software Epi Info version 5.0.

## Results

During the period of one year (Jun2014 to May 2015) a total of 3116dental procedures were done at the Dental Department of Railway General Hospital Rawalpindi. Month wise distribution of the dental procedures is shown in table I.

These included 1902 (61.0%) tooth extractions, 930 (29.8%) fillings, 166 (5.3%) scaling and 118 (3.8%) root canal treatments. With the addition of the third dental surgeon in the second half of the study year the numbers of scalings and root canal treatments significantly increased. The scaling increased from 48/1591 (3.0%) to 118/1525 (7.7%) (p<0.001) and root canal treatments increased from 33/1591 (2.1%) to 85/1525 (5.6%) (P<0.001).A total of 1902 teeth were extracted from 1560 patients. The ages of these patients ranged from 3 to 80 years (median age 41 years). Their male to female ratio was 1:1.36 and they included 149 (9.6%) children from 3-13 years of age. The main reasons for tooth extraction are summarized in Fig I.

**Table I: Month wise breakdown of the operative procedures done over one year from June 14 to May 15 at the Dental Department of Railway General Hospital, Rawalpindi**

| Procedure | Extractions | Fillings | Scalings | RCT | Total |
|---|---|---|---|---|---|
| Jun | 178 | 60 | 19 | 4 | 261 |
| Jul | 84 | 67 | 6 | 2 | 159 |
| Aug | 233 | 72 | 17 | 6 | 328 |
| Sep | 221 | 107 | 6 | 20 | 354 |
| Oct | 132. | 96 | - | 1 | 229 |
| Nov | 171 | 89 | - | - | 260 |
| Dec | 138 | 105 | 19 | 5 | 267 |
| Jan | 167 | 80 | 20 | 17 | 284 |
| Feb | 194 | 60 | 23 | 21 | 298 |
| Mar | 197 | 72 | 28 | 24 | 321 |
| Apr | 60 | 65 | 28 | 7 | 160 |
| May | 127 | 57 | - | 11 | 195 |
| Total | 1902(61.0%) | 930 (29.8%) | 166 (5.3%) | 118 (3.8%) | 3116 (100%) |

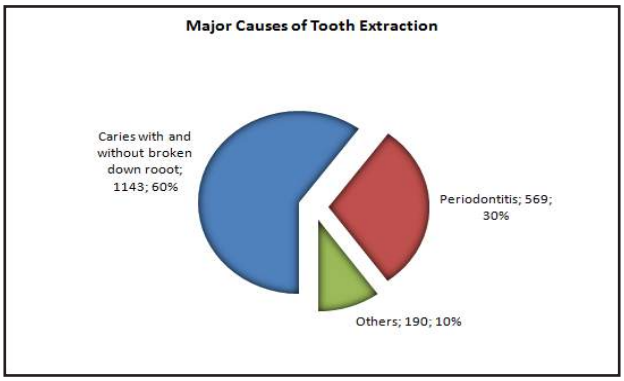


**Fig 1: Major causes of tooth extraction from Jun 14 to May 15 at the Dental Department of Railway General Hospital, Rawalpindi**

Caries was the leading cause of tooth extraction. Most of the patients with advanced caries presented with broken down root (763/1902, 40%). Gross caries was the reason for extraction in 380/1902 (20%), periodontitis with mobile teeth (grade >2) in 569/1902 (30%) and other reasons like cysts, impaction and trauma etc. in 190/1902 (10%) of patients.

## Discussion

Dental caries and periodontal infection due to poor oral hygiene are the two most common dental health problems in Pakistan.[5,12-15] This is clearly reflected in this study. Most of the patients with caries had advanced disease with the majority having completely lost their teeth and presented with broken down roots while the remaining had major loss of the tooth structure. None of such teeth could be restored by any dental procedure and extraction was the only choice available. Education on improving oral hygiene and timely visit to the dentist are the key components to prevent such unfortunate happenings.[12,15] Community based education and

awareness program through electronic and print media are highly recommended.

Dental Department in a hospital is often neglected and understaffed. This leaves the dentists with no time to undertake more complex restorative dental procedures. This is also highlighted in this study. When the number of dentists was increased from two to three the number of root canal treatments also increased significantly. In a busy dental clinic the dentists are often compelled to extract a tooth rather than spending extra time to salvage it through root canal treatment. The quality of work can also be improved by installing additional dental chairs. In a hospital setting the flow of work is often hindered due to trivial issues like lack of availability of local anesthetic, or X-Ray films etc. A couple of dips in the work load during the months of July 14 and April 15 in this study exemplify such problems.

The overall spectrum of workload and reasons for tooth extraction in this study is comparable to that in the other public sector dental setups in Pakistan.5 The results from a more developed centre from the private sector showed different results where 42.2% extractions were due to caries, 30.6% were due to periodontal infection and the remaining were for other reasons.[16] Data from many developed countries also suggest that caries is the commonest cause of tooth extraction but its incidence is much lower than in our study and that from the other under-developed areas of Pakistan.[10,11,14] The tooth loss due to caries is also reported to be higher in other developing countries.[6,9] The results from a Nigerian study show that almost 78% of the extractions were related to caries followed by trauma as the next most frequent cause of extraction.[6]

Our results and those from the other places clearly highlight that the incidence of caries and periodontitis is higher in the underdeveloped and the poor socio-economic class of people.[17,18] Though the level of poverty cannot be changed but the awareness can certainly be increased in such communities for reducing the incidence of caries and periodontal infections.

## Conclusion

Caries and periodontal infections are the commonest dental problems and cause of tooth extraction at the Railway General Hospital Rawalpindi where people mostly come from the lower socio-economic status. The quality of work in a public sector dental setting like Railway General Hospital can be improved by providing adequate number of dentists and other resources.

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ORIGINAL ARTICLE

# Out of Pocket Cost Born by Patients of Type 2 Diabetes Mellitus in Private Diabetic Clinics of Islamabad

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## ABSTRACT

**Objective:** To estimate the direct cost and its determinants in type2 diabetic patients visiting outpatient department of private tertiary care hospitals.

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

Place and Duration of Study: This research was carried out in the diabetic outpatient department of Shifa international hospital and Ali medical Centre from 15 November 2014 to 15 February 2014.

**Materials and Methods:** The descriptive cross sectional study was conducted on 108 diabetic patients (male 52%, female 54 %). By employing simple random sampling technique the data was collected from patients having diabetes from at least 5 years, with age limit between 30 to 80 years, with or without having complications through pretested interview administered questionnaire. The structured questionnaire was used for collecting data. SPSS 20.0 was used for data analysis. The percentages and frequencies were drawn in order to draw the results.

**Results:** The results showed that the average direct cost spent by a patient was 7704 PKR per month. More than half (66.7%) of the study subjects have suffered from diabetes since 5-10years. A larger group of respondents (50%) was treating diabetes with oral hypoglycemic.

Medication, consultation, and lab investigation charges were the main determinants of diabetic cost. Per month medication charges were 3997 PKR, followed by lab investigation charges of 2441PKR per visit and consultation cost was 1298 PKR. Most of the patients (86.1%) were having one complication due to diabetes. The cost of treatment increased with the increasing age and morbidities.

**Conclusion:** Diabetes is very expensive disease to manage. The affluent charges of managing diabetes and its day by day increased cases will put tremendous burden on the society.


**Keywords:** *Diabetes Mellitus, Direct Medical Cost, Determinants.*

## Introduction

The diabetic prevalence and its effects on health status have grown rapidly in south Asian region as compared to any other part of the world.[1] Diabetes shares a major chunk to the burden of disease that can be prevented and that leads to economic burden and loss of productive life years.[2] The resource deficit region like south Asia where most of the people having lack of access to even basic necessities of life with no health care insurance system or nationwide welfare system, the patients cannot tackle the burden of such expensive disease like diabetes that resulted in the form of different even more damaging complications.[3] DM is related to variety of micro and macro vascular complications with the greater risk of atherosclerosis manifestations.[4]

Diabetes Mellitus (DM) is requires a lot of monetary and other resources for its management due to its chronicity and severity of different types of complications.[5] Almost one third of the total cost of managing diabetes is attributed for the macro-vascular disease that resulted from the poor management of diabetes.[3] The Ramacchandran and colleagues reported in one of their study that the complications related to diabetes were more prevalent among low socio economic class as compared to higher socio economic class while the prevalence scenario of DM was vise verse among these two groups.[6] When the complications due to diabetes affect the breadwinner member of the family the whole family gets shock of poverty and bad health status.[7] High prevalence and rate of complications engender substantial negative implications on the economies of patient & their families.[8]

Internationally, the direct cost for patients suffering from diabetes among age group of 20 to 79 years was

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approximately $153 billion annually.[9] This is one of the most expensive disease to manage as it alone utilize approximately 8% of total health care budget in the developed countries like America, china and Canada.[10] The economic burden due to this disease is area of concern for many countries in the world including both developed and developing nations as the economic burden is two to five folds greater among diabetics as compared to non-diabetics.[11,12] The studies that calculated the estimated direct cost of diabetic are very few in the south Asian region especially in Pakistan. It is very important to have base line data regarding diabetes costing in order to make and implement prevention and treatment policies.

The objective of this study was to estimate the direct cost and its determinants in type 2 diabetic patients visiting outpatient department of private tertiary care hospitals.

## Materials and Methods

This was a descriptive cross sectional research that was conducted in the outpatient department of Shifa International Hospital and Ali Medical Center Islamabad. The data collection was completed within 4 months from 15 November 2014 to 15 February 2015. Study participants included in the study were; patients suffering from diabetes from at least 5 years, with age limit between 30 years to 80 years, with or without having complications. While, pregnant women who were having diabetes were excluded from the study as due to pregnancy the no of visits and cost can vary as compared to other participants. The total sample size that was taken for conducting this study was 130 inclusive of 20% non-response. The simple random sampling technique was adopted for selecting the sample. Sampling frame that included the patient's details who visited the diabetic OPD was obtained from hospital management. The sample was drawn randomly from the OPDs with the name list of the patients. All the patients' names were written and then randomly selected for the study purpose. A questionnaire was designed and pilot tested to collect the data from the participants.

The participants were interviewed by using the questionnaire that took about 10 to 15 minutes about direct medical and non-medical cost due to diabetes mellitus. Informed consent was taken from every study participant before taking the interview. Consent form in English with Urdu translation was used. The interviews were conducted by trained health professionals (pharmacist and nurses). They were given two days training prior the data collection process. Prior to the research initiation the study was approved by the Ethical Review Committe (ERC) of the hospitals.

Data was validated after double entry and then analysis was carried out using SPSS version 20 and Excel 2010. Cost of per dose of medicine was calculated for each patient through which per day cost and per month cost had been estimated. The frequencies and percentages were compiled for the demographic variables. While, mean and standard deviations were calculated for all the cost variables.

## Results

Out of total 108 Participants who participated in the study 52% were male while 48% were females. The mean age was 53.35 years. Almost half of the participants 43.5% were belonged to a group of age 41-50 years. The patients were having good educational background with 41% of the study subjects were graduate or having a high level of education. By occupation, 41% were employed in office, 19% were having their own business while others were unemployed. Most of the participant belonged 62.6% had a household income between 50,000 to 100,000 PKR one quarter of the subjects 24% had income between 100,000 to 200,000 PKR, 3.5% had greater than 200,000 PKR and only very small proportion (8%) had a household income less than 50,000 rupees. The details are given in the following table I.

Majority of the participants 66.7% were suffering from diabetes since 5-10 years while 30.6% had the disease from last 11-20 years and only 3.8% were found with disease age greater than 20 years.

A larger group of respondents (50%) was treating diabetes with oral hypoglycemic, 13% were using insulin, 26.9% were using a combination (insulin + oral hypoglycemic) and only 11% were treated diabetes with lifestyle modification (diet plan / exercise).

On average every patient visits to clinic after 10 weeks approximately. Median and standard deviation between visits were calculated as 3 and 1.35 respectively. Half of the patients 46.3% visited

**Table I: Socio-demographic characteristics of the Study Participants of Type2 Diabetes Mellitus (n= 108)**

| Characteristics | Number of patients | Percent (%) |
|---|---|---|
| **Age** | | |
| 30-40 years | 12 | 11.1 |
| 41-50 years | 31 | 28.7 |
| 51-60 years | 47 | 43.5 |
| 61-80 years | 18 | 16.5 |
| **Marital status** | | |
| Single | 4 | 3.7 |
| Married | 104 | 96.3 |
| **Education** | | |
| Un-Educated | 10 | 9 |
| Secondary | 20 | 18 |
| Intermediate | 35 | 33 |
| Graduation & above | 43 | 40 |
| **Occupation** | | |
| Office job | 44 | 41 |
| Businessman | 21 | 19 |
| Unemployed | | 40 |
| **Household Income (PKR)** | | |
| < 50,000 | 8 | 7.4 |
| 51000 – 100000 | 72 | 66 |
| 100000 – 200000 | 24 | 22.2 |
| > 200,000 | 4 | 3.7 |

doctor after 3 months. In taking medication approximately 99.1% of respondents follow doctor's advice followed by dietary intake 96.3% and consultation for treatment 95.4%.

More than two third, 86.1% of the study participants were suffering from Co-morbidities due to diabetes i.e. hypertension, liver problems, dyslipidemia, heart diseases, retinopathy, neuropathy, nephropathy etc. When respondents were asked about the per visit consultation cost with the physician, the mean cost was calculated as 1298.61 in Pakistani rupees. Minimum cost paid by a patient was 600 rupees and maximum cost was 1500. The average cost on lab investigation was 2441.40 rupees with standard deviation 1834.96. Overall cost paid by diabetic patients for medicines was 431780 rupees. The mean cost for medication was calculated as 3997.96 rupees ranging from 705 PKR to 15812 PKR. The deviation between medication costs was calculated as 3036.47.Fig 1.

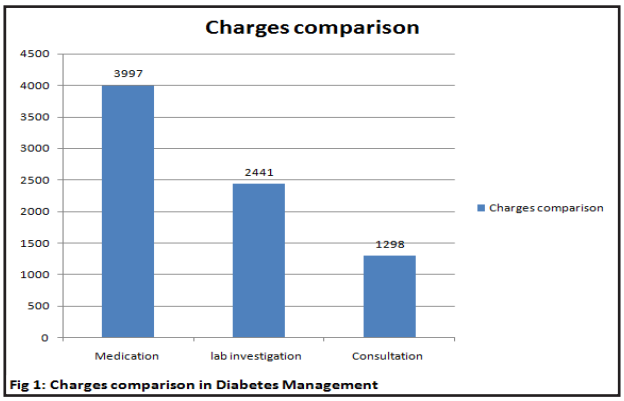


**Fig 1: Charges comparison in Diabetes Management**

The total direct medical costs (consultation cost+ lab investigation cost+ medication cost) borne by a diabetic patient were calculated as 832060 rupees and the average cost calculated was 7704.25 rupees. The three main determinants identified during the study were medication cost, lab investigation and consultation fee respectively that were contributing mainly in the diabetes care cost. Total direct non-medical cost for all study subjects was 1081.48 rupees.

## Discussion

Our research depicted three main reasons of diabetic care cost was medication cost, lab investigation and consultation fee. These results were comparable to the previous study that was held in Karachi, Pakistan. The study highlighted that patients on insulin were bearing 1.8 times more cost as compared to oral hypoglycemic. On average a patient was spending 7704 PKR per month on diabetic care and this cost was 7.9 times greater when compared to previous Pakistani study conducted by Liaqzat A. khawaja.

More than two third 93% of the patients were having at least one comorbidity that also increases the treatment cost. The overall treatment cost was greater in patient having more than two co-morbidities as compared to one or no co-morbidity which can be due to greater number of medications, lab tests, consultation, and hospitalization.[13] These results were in lined with other studies conducted din developing and developed countries.[14,15] The average health care cost was also increasing with

increased age; highest in age group of 61-80years followed by 51-60 years which might be due to increase in number of comorbidities. The treatment cost was also directly related with years of diabetes history.[14] Increased duration of diabetes among patients requires consultation not only from a medical specialist but also from other health care experts like cardiologist, urologist, and ophthalmologist and in worst cases from surgeons as well. Results in this study revealed that the largest component of cost was medication. Groover and colleagues reported that 95% of treatment cost is paid by patients in India.[16]

The limitation of this study was the smaller sample size and limited only to private patients, the studies like this should be conducted on larger samples on the patients visiting government facilities as well. This can give the share of health care cost bear by the government health facilities on every diabetic patient.

## Conclusion

It is concluded that diabetes is a very expensive disease to tackle. It requires lot of monetary resources for management. The major determinants of direct diabetes care cost are medication, lab, and consultation charges. The affluent charges of managing diabetes and its day by day increased cases will put tremendous burden on to health policy planners and society.

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