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Editorial

Ethical challenges in medical practice in the context of Coronavirus disease 2019 in Nepal

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The Coronavirus disease 2019 (COVID-19) pandemic has posed unexpected challenges to government, health care providers, policy makers and people globally. This disease is new, highly contagious and has no definite treatment, vaccine or pre-existing immunity. The governments of different nations globally are using several strategies to contain and treat the disease, but the situation has been a nightmare for all till date. There are deep impacts in global economy due to the lockdown and decreased productivity can leave its effect for years to come.[1]

The pandemic has posed a greater challenge in health sector and has triggered many ethical issues as well. Medical ethics is a set of moral principles which guides the clinical practice in the management of patients. It also applies to scientific research in the field of health care. Medical ethics is based on a cluster of principles which are referred to in case of any ethical confusion during management of patients. The "Principles of Biomedical Ethics" proposed by Tom L. Beauchamp and James F. Childress in 1977 has been regarded as the most practical in the field of medical practice and research particularly in Anglophone scholarship.[2] The four principles of health care ethics are autonomy, beneficence, non-maleficence, and justice. Though the principles have widely been accepted, some shortcomings have also been commented by authors, such as 'thick in status but thin in content' by Lee MJH.[2]

Health care providers deal with human suffering and there might be ethical dilemmas even under normal conditions, but they can be solved with minor workouts. There will be greater challenges when dealing with life threatening diseases and conditions, particularly when the patients are unable to make their own decisions. Whenever there is ethical dilemma during the management of a patient, healthcare providers can seek opinion from the ethical committee of the institutes whosoever can analyze the case in the particular context in team and provide best solution to the scenario. The service can also be extended to the family members or legal guardians in making informed choices to avoid harm to the patient and fair action.[3]

Though the ethical care is patient-centered, the conditions during the public health emergency like COVID 19 pandemic may disrupt or at least alter the usual process of ethically serving a patient.[3] During the pandemics, protection of a larger population gets preference over individual rights and privileges and prudent use of resources need to be done. When the resources are limited, the patients who could be protected during the normal conditions might not get adequate treatment and care as the patient-centered medical care is diverted to public-centered during the public health emergencies. In such a crisis, Public health ethics guide health care providers to

balance the challenging issues between the needs of the individual and of the public at large.

There have been concerns as the patients who have higher risk such as elderly and those having several underlying diseases and conditions are getting refused for treatment in favor for those whose prognosis is predicted to be better. This problem has been already experienced by health care providers in countries where the serious cases have been very high in number.

There have been recent developments in the health care facilities and availability of trained manpower in Nepal, but it is mainly restricted to major urban areas only. The unequal distribution of hospitals is an alarming situation in Nepal even for the regular provision of health care during normal conditions. In the current pandemic crisis, the situation can be grave if there is drastic surge of critical COVID 19 patients. There would be serious ethical and as well managerial challenge in our context.

The medical care is focused to protect greater population with strategic changes during the pandemics like COVID 19. But the rights of individuals who are in serious need of health care facilities due to other health reasons should not be neglected. Patients are afraid to go to hospitals in a fear of infection and opting traditional measures of treatment at home. It has mainly affected the pregnant mothers who fear to visit hospitals for regular antenatal care. The chances of infection could be more when medical or surgical procedures need to be done. The patients themselves might contract disease in the hospital from other patients and health care providers. The situation is important and should be dealt tactfully.

Health care providers are also vulnerable to distress due to increased workload and risk of contagious infection in the context of COVID 19 pandemic. The management of health institutions should be cautious to protect the frontline workers. The scarcity of proper Personnel Protective Equipment (PPE) poses risk of disease transmission to them. It is also challenging ethically in terms of putting one's own health into risk to care for others. Many health care providers have been infected with COVID 19 and even succumbed to the disease in different parts of the world.

Nepal Medical Council(NMC) has prepared interim guideline for infection prevention and control when COVID-19 is suspected. The document mentions the safety that the health care providers need during the management. It also includes organizational preparedness and the safety of visitors, attendants, care of instruments and laundry. The possible scarcity of PPEs, including the N95 face masks and the ways to reuse of the mask are also mentioned. The guideline needs to be followed by the health care professionals in order to keep themselves and other persons directly or indirectly involved in the management of the known or suspected COVID 19 patients safe. The guideline has been instrumental as an important document to follow in the present context. It needs to be updated with the addition of latest evidence of the nature of the disease.[4]

Nepal Medical Council has also published professional ethical guidelines in the management of patients in the context of COVID 19 in Nepal on 20th March, 2020. The challenges may differ in different context based on the availability of resources and trained manpower. Therefore, the local guideline in the context of Nepal was essential. The guideline mentions the need of public health ethics in order to protect greater community at large. Along with, the ethical aspects and rights of individual patients

in the context of current pandemic is included. The guidelines recommend avoiding elective medical and dental procedures during the pandemic as far as possible. This could breach the rights of individual patients but is needed to prevent the greater public getting infected. The document is also in favor of the lockdown conducted by the government of Nepal. The protection of health care professionals is emphasized and their responsibilities for protection of public is also highlighted. Although the document lacks in comprehensiveness in application of principles of public health ethics, the publication of guideline itself is praiseworthy in the current crisis. It recommends following the national and international guidelines in infection control as well to follow the established standard operating procedures in case basis. The health care providers should refer to the guideline to solve ethical dilemmas in the local context.[5]

The characteristics of COVID 19 is still getting explored and many facts are yet to come, but the current pandemic has already posed a great threat to the people globally. Everyone is responsible from their aspects to face the global crisis. Health care providers are highly responsible to heal the sufferers and to make critical decisions. The ethical aspects in management of patients should be followed properly to make rational decisions.

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Evaluation of agreement between clinical and histopathological diagnosis of subtype of leprosy by skin punch biopsy

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ABSTRACT

Introduction: Leprosy is chronic granulomatous disease caused by Mycobacterium leprae. Clinically diagnosed cases of leprosy can be evaluated by punch biopsy. It can be classified according to Ridley and Jopling Classification. **Objective:** To study the different spectrum of leprosy and its Bacillary Index (BI), evaluate the agreement between clinical and histopathological diagnosis. **Materials and Methods:** This is a hospital based cross sectional retrospective study done for the period of 6 months from January to June 2019. The data were subjected to kappa analysis by using SPSS version 24 to see the agreement between clinical and histopathological diagnosis. **Results:** Out of 62 cases of clinically diagnosed cases of leprosy three (4.83%) cases turned out to be other granulomatous diseases. According to Ridley and Jopling classification, clinical and histopathological agreement was seen in 38 (61.29%) cases. Agreement of 100% was seen in Indeterminate leprosy (IL) and Borderline tuberculoid leprosy (BT). The BI was more in Lepromatous leprosy (LL), Borderline lepromatous (BL) whereas IL, BT and Tuberculoid leprosy (TT) showed less BI. Overall there was moderate agreement between clinical and histopathological diagnosis (kappa- 0.505) which was statistically significant (p value <0.05). **Conclusion:** There was moderate agreement between the clinical and histopathological diagnosis of subtype of leprosy. The Bacillary load was high in LL whereas least in TT and is determined by immune system.

Key words: Bacillary index, Leprosy, Ridley and Jopling Classification

INTRODUCTION

Leprosy has become a major health problem in Nepal. It has been endemic in tropical countries, mainly in underdeveloped or developing countries like Nepal.[1] Hansen's disease is a chronic granulomatous disease caused by *M. leprae*, an acid fast bacillus. The clinical, pathological and immunological criteria of the Ridley and Jopling classification provide the basis for the classification of the various forms of the disease, including indeterminate (IL), tuberculoid (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL), and lepromatous (LL) form. The spectrum of disease as such depends upon the immune status of the patient.[2] The cellular response is seen in TT whereas humoral response is seen in LL.[3]

The complications and nerve damage can be prevented by early diagnosis of the disease.[4] Though clinical examination is essential, many skin lesions may share the common clinical findings. In difficult cases, for good management

of patients, histopathological studies are essential.[5] However, it requires good correlation between clinical variables like site, duration, number, nature of lesions and histopathological features to come to final diagnosis. Also histopathological examination is required to separate different types of granulomatous skin lesions like lupus vulgaris, Hansen's disease, sarcoidosis and fungal infection.[6]

In this present study, we study subtypes of leprosy, their BI and assess the agreement between clinical and histopathological diagnosis among the skin punch biopsy we received in Gandaki Medical College Teaching Hospital (GMC-TH), Pokhara.

MATERIALS AND METHODS

This is a cross sectional retrospective study performed in the Department of Pathology, GMC-TH, Pokhara for the period of 6 months from 1st January to 30th June 2019. Skin punch biopsies were done from clinically diagnosed skin lesion of leprosy from the Department of Dermatology of GMC-TH and Greenpastuer's Hospital, Pokhara. The well labelled samples were received, processed and stained with Hematoxylin-Eosin (H&E) and Fite Faraco (FF) stain wherever required. The prepared slides were evaluated by consultant pathologists. The H&E stained sections were studied for the morphology of disease and to make diagnosis of different types of leprosy. The histopathological typing of leprosy was done according to Ridley and Jopling classification.[3] In case of only nerve biopsy with features of leprosy, the different term, pure neuritic leprosy (PNL) was used. The FF stained sections were evaluated to see the Bacillary Index (BI) of the disease. The BI was calculated as given below:[15]

BI = 0: no bacilli observed

BI = 1: 1 to 10 bacilli in 10 to 100 high-power fields (hpf, oil immersion)

BI = 2: 1 to 10 bacilli in 1 to 10 hpf

BI = 3: 1 to 10 bacilli per hpf

BI = 4: 10 to 100 bacilli per hpf

BI = 5: 100 to 1000 bacilli per hpf

BI = 6: >1000 bacilli per hpf

The history and clinical examinations of patients regarding the nature of lesion was recorded with clinical differential diagnoses. The clinically diagnosed case of leprosy that differed on histopathology were categorised as different entity (others).

The data were collected filling the proforma and subjected to kappa analyses using Statistical Package for Social Sciences (SPSS version 24) to see the agreement between clinical and histopathological diagnosis. The kappa values and their interpretations were as follows: <0, no agreement; 0 to 0.19, very weak agreement; 0.20 to 0.39, weak agreement; 0.40 to 0.59, moderate agreement; 0.60 to 0.79, substantial agreement; and 0.8 to 1.0, excellent agreement.[14] The significance level used for the analyses was 5% (p < 0.05).

The ethical clearance for the study was obtained from GMC-Institutional Review Committee

RESULTS

Among the total of 62 cases, predominant of skin biopsies were received from male 39 (62.90%) and remaining were from female patients.

Table 1: Frequency of cases with respects to age group and sex

Age Range (Years)	Sex		Total
	Female	Male	
0-10	1 (1.6%)	0 (0%)	1 (1.6%)
11-20	7 (11.3%)	2 (3.2%)	9 (14.5%)
21-30	8 (12.9%)	13 (21.0%)	21 (33.9%)
31-40	4 (6.5%)	6 (9.7%)	10 (16.1%)
41-50	1 (1.6%)	5 (8.1%)	6 (9.7%)
51-60	1 (1.6%)	2 (3.2%)	3 (4.8%)
61-70	1 (1.6%)	5 (8.1%)	6 (9.7%)
71-80	0 (0%)	3 (4.8%)	3 (4.8%)
81-90	0 (0%)	3 (4.8%)	3 (4.8%)
Total	23 (37.1%)	39 (67.9%)	62(100%)

The age group is shown in table 1. We received more skin biopsies from the patients aged 21 to 30 years i.e. 21 (33.9%), followed by 31 to 40 years 10(16.1%) and least from the age group 0 to 10 years. The variable percentage of biopsies were received from other age groups. In all age groups there were male predominance except in 11 to 20 and 0 to 10 years .

Table 2: Clinical diagnosis of leprosy

Clinical Diagnosis	Frequency	Percent
IL	1	1.6
BT	6	9.7
TT	8	12.9
BL	13	21.0
LL	29	46.8
PNL	5	8.1
Total	62	100.0

Note: *IL: Indeterminate Leprosy, BT: Borderline Tuberculoid, TT: Tuberculoid Leprosy, BL: Borderline Lepromatous Leprosy, LL: Lepromatous Leprosy, PNL: Pure Neuritic Leprosy*

The most common clinical diagnosis in our study was LL

29 (46.8%) followed by BL 13(21%). The least common clinical diagnosis was IL One(1.6%) as shown in Table 2.

Table 3 : Histopathological diagnosis of leprosy

Histopathological Diagnosis	Frequency	Percent
IL	10	16.1
BT	11	17.7
TT	1	1.6
BL	14	22.6
LL	19	30.6
PNL	4	6.5
others	3	4.8
Total	62	100.0

In our study, LL was commonly diagnosed case19 (30.6%) followed by BL 14 (22.6%). TT was diagnosed only in one (1.6%) case. (Table 3)

Table 4: Bacillary index with subtype of leprosy

Histopathological Diagnosis	Bacillary Index (BI)							Total
	0	1	2	3	4	5	6	
IL	8	2	0	0	0	0	0	10
BT	1	7	3	0	0	0	0	11
TT	1	0	0	0	0	0	0	1
BL	0	0	5	2	6	1	0	14
LL	0	0	0	0	4	8	7	19
PNL	0	1	2	0	1	0	0	4
Others	3	0	0	0	0	0	0	3
Total	13	10	10	2	11	9	7	62

It was noted that IL, BT, TT had lower BI while LL and BL had higher BI. But the PNL had variable bacillary load. In the other granulomatous diseases besides leprosy, the BI was not applicable. (Table 4)

Table 5: Overall agreement and kappa values between clinical and histopathological diagnosis

Clinical Diagnosis	Histopathological Diagnosis										P-value
Types of Leprosy	No. of Patients	IL	BT	TT	BL	LL	PNL	BB	Others	Agreement	Percentage (%)	Kappa Measure of Agreement
IL	1	1	-	-	-	-	-	-	-	1/1	100	
BT	6	-	6	-	-	-	-	-	-	6/6	100	
TT	8	-	5	1	-	-	-	-	2	1/8	12.5	
BL	13	4	-	-	8	1	-	-	-	8/13	61.5	
LL	29	5	-	-	6	18	-	-	-	18/29	62.1	0.505
PNL	5	-	-	-	-	-	4	-	1	4/5	80	
BB	0	-	-	-	-	-	-	-	-	-	-	0.001
Others	0	-	-	-	-	-	-	-	-	-	-	
Total	62	10	11	1	14	19	4	0	3	38/62	61.29	

As shown in table 5, there was 100% agreement between clinical and histopathological diagnosis of IL and BT. There was only 12.5% agreement on diagnosis of TT. Overall there was moderate agreement (61.2%) between clinical and histopathological diagnosis (kappa- 0.505) which was statistically significant (p value <0.05).

Fig.1: Hypopigmented patch in Hansen's disease. The lesion is located in extremity.
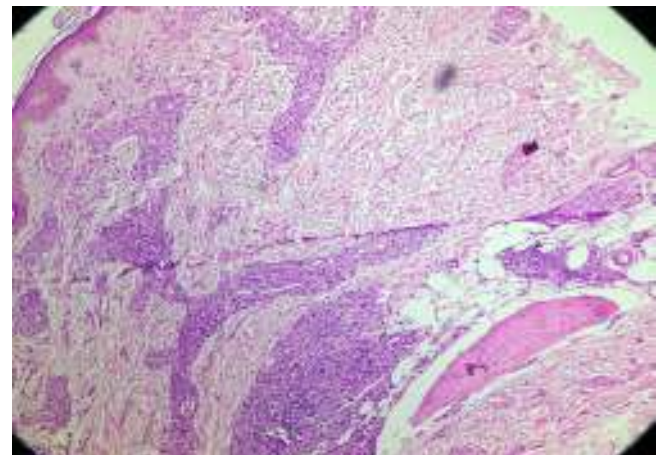
Fig. 2: Borderline Lepromatous Leprosy (BL) H&E sections (100 X). Section shows scattered epithelioid cells and foamy macrophages infiltrating dermal nerve and adenexal structures.
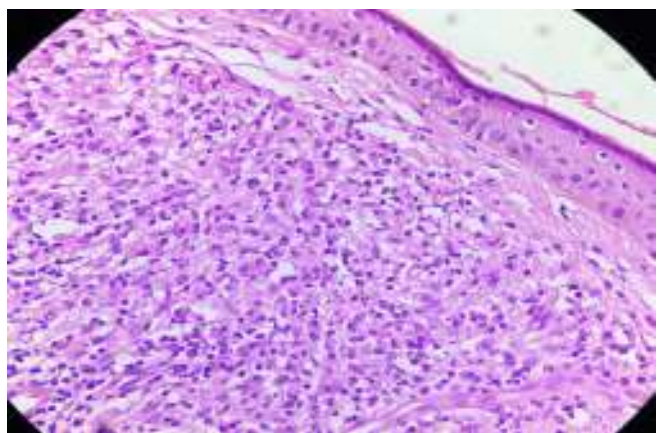
Fig.3: Lepromatous Leprosy (LL) H&E Sections (400X). Section shows sheets of foamy macrophages in dermis with clear grenz zone in dermoepidermal junction.
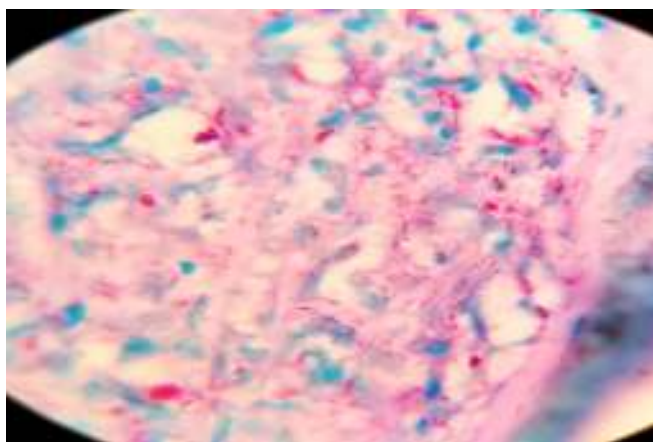
Fig.4: Bacillary Index- 6 (FF stain 1000X) Section shows globi of bacilli within leprae cells. This is the confirmatory findings of LL.
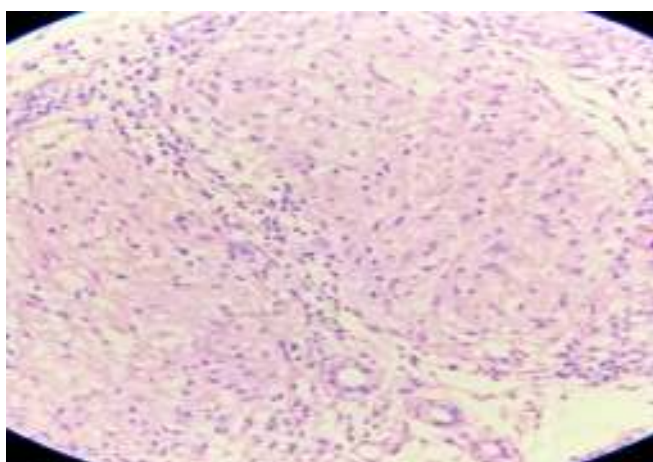
Fig. 5: Borderline Tuberculoid leprosy (H&E 400X). Section shows epithelioid granuloma. Giant cells are not seen.

DISCUSSION

Leprosy is a chronic granulomatous disease which is common in countries like Nepal. It has two polar forms ranging from TT to LL based on the immune system of the host. The disease primarily affects the skin and nerve.[7]

The present study showed male preponderance for the disease (62.90%). The higher male-to-female ratio (1.7:1) in our study could be due to increased number of males migrating to cities in search of employment. Similar findings were seen in the study conducted by Semwal et al.,[7] Karki et al.[8] and Ramos et al.[9] Male predominance is thought to be because of factors such as industrialization and urbanization which increases incidence for contact in males.[10]

In our study, the majority of patients belonged to the age group of 21 to 30 years (33.9%) followed by 31 to 40 years (16.1%) (Table1). This is similar to findings reported by Mathan R et al.[16] with common age group of 21 to 40 years.

In contrast, the study done by De Vares et al.[11] showed peak at 35 to 44 years. Though the percentage of children below 10 years in the study was only 1.6% and from 11 to 20 years was 14.5%, it indicated that there has been a community transmission.

The histopathological diagnoses are categorized according to Ridley and Jopling Classification of leprosy.[3] In our study, histopathologically, LL is found to be common lesion 19 (30.6%) cases, followed by BL in 14 (22.6%) cases whereas TT was less commonly diagnosed in 1(1.6%) case (Table 3). In contrast, the study done by Bijarragi et al. [17] showed BT (38%) and IL (6.4%). The morphology of two poles of spectrum of leprosy is different. In LL, dermis is infiltrated with sheets of foamy macrophages with clear grenz zone (Fig.3). The TT shows non caseating granuloma with Langhan's type giant cells encroaching the papillary dermis. Similarly, BT shows ill defined granuloma comprising of epithelioid histiocytes, but giant cells may be absent (Fig.5).

In the present study, three (4.8%) clinically diagnosed cases of leprosy turned out to be other granulomatous disease histopathologically, similar findings were seen in study done by Lockwood et al. (7.1%).[13] This is because various granulomatous diseases can share the similar clinical features with similar type of skin lesions.

It is found that the BI is maximum in LL and BL where as IL, BT and TT had low BI (Table 4). The study conducted by Premalatha et al.[10] and Semwal et al.[7] also showed similar features of multibacillary in BL, LL and paucibacillary in TT and BT. The FF stained sections of BL and LL showed globi of bacilli within leprae cells with BI five to six (Fig.4) whereas IL, BT and TT had BI of upto three. This polarization of leprosy from TT to LL is due to impact of a differential immune response of B and T lymphocytes on the form of disease in the individual.

There was 100% agreement between clinical and histopathological diagnosis in IL and BT whereas it was least (12.5%) in TT. Clinically diagnosed cases of TT are found to be BT. This disagreement might be because of similar clinical findings in the tuberculoid pole of the disease. In contrast to this, the study done by Moorthy B N et al.[12] showed maximum clinico-pathological agreement in LL (80%), followed by BL (70%), TT (46.15%) and least agreement was seen in IL (20%).

The present study showed that overall there was moderate agreement (61.29%) between clinical and histopathological diagnosis (kappa- 0.505) which was statistically significant (p value <0.05) (Table 5). Similar type of moderate agreement was seen in other studies

done by Semwal et al.[7] (62%) and Moorthy B N et al.[12] (62.63%).

CONCLUSION

There is overall moderate agreement between the clinical and histopathological diagnosis of subtype of leprosy. The histopathological evaluation is essential to differentiate leprosy from other granulomatous diseases.

Acknowledgements

Conflicts of Interest

There are no conflicts of interest.

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Serum Electrolyte in Acute Exacerbation of Chronic Obstructive Pulmonary Disease

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ABSTRACT

Introduction: Chronic Obstructive Pulmonary Disease (COPD) is one of the leading causes of mortality and morbidity worldwide. Though COPD is mainly a chronic disease, many patients experience exacerbations that are related to worst survival outcome, especially with abnormal serum electrolyte level. The objective of this study was to evaluate serum electrolyte levels among the patients with acute exacerbation of COPD. **Methods:** Structured questionnaire and patients' charts were used to collect data. Data was analyzed using Statistical Package for the Social Sciences (SPSS) software version 16.0 and descriptive statistics were used to generate the research findings. **Results:** The mean age of the patients with Acute exacerbation of COPD was 69.57± 9.765 years. Among 100 patients, (83%) belonged to the age group of 60 years and above, (54%) were male, (74%) were married, (52%) were illiterate and (41%) were engaged in agriculture, (41%) consumed alcohol and (67%) were smokers. Dyspnoea (90%) was the most common symptom. The mean level of sodium and potassium were 133.8±4.830 mEq/L, 3.6±0.533 mmol/L, respectively. Fifty seven percent patients had electrolyte disorder. More than half (51%) had hyponatremia and (40%) had hypokalemia. The average value of pH, $PaCO_2$ and PaO_2 are 7.34 ± 0.727, 46.64 ± 9.787 mm Hg and 69.38 ± 9.255 mm Hg respectively. Among them, (18%) were in respiratory failure. **Conclusion:** This study concluded that hyponatremia and hypokalemia are prevalent electrolyte disorder with AE of COPD patients. Therefore, we recommend routine monitoring of the serum electrolytes for better outcomes of patients.

Keywords: Chronic Obstructive Pulmonary Disease, Potassium, Sodium

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common preventable and treatable disease characterized by persistent respiratory symptoms and airflow limitation usually caused by significant exposure to noxious particles or gases.[1] The Global Burden of Disease Study reports a prevalence of 251 million cases of COPD globally in 2016.[2] Latest global estimates illustrate 3.2 million deaths from COPD and more than 90% of COPD deaths occur in low and middle income countries. Disease burden due to COPD was highest in Papua New Guinea, India, Lesotho and Nepal in 2015.[3] COPD is likely to increase in coming years due to higher smoking prevalence and aging populations in many countries and it is a major public health problem and its prevalence varies according to country, age and sex.[4] Estimation of Global Initiative for Chronic Obstructive Lung Disease (GOLD) suggest that COPD will be the third most common cause of death worldwide by 2020.[1]

COPD is complicated by frequent and recurrent acute exacerbations (AE) that compromise quality of life, diminish

respiratory functions and increased enormous health care expenditures and high morbidity. An exacerbation of COPD is an acute worsening of respiratory symptoms beyond normal day-to-day variations. It is a significant cause of mortality and morbidity and categorized in terms of clinical presentations or healthcare utilization.[5-7] Exacerbations may cause increased dyspnea, productive cough with altered sputum, and fever. The symptoms may be more nonspecific, such as malaise, fatigue, insomnia or sleepiness, depression and might be associated with impaired lung function, it is estimated that only 50% of all exacerbations are reported to physicians.[5]

Exacerbations are the most common cause of hospitalization among COPD patients. The economic and social burden created by acute execrations of COPD is extremely high.[8] Majority of the patients experience a temporary or permanent decrease in the quality of life due to acute exacerbation of COPD. Though the COPD patients mostly present with the features of acute respiratory infections, there may be a number of metabolic derangements arising out of the disease process or as a consequence of the therapy instituted like hyponatremia, hypokalemia, hyperbilirubinemia, elevated transaminases, elevated blood urea and elevated serum creatinine etc.[9] Thus, it is important to identify factors associated with exacerbation. Since electrolytes are important for nerve conduction as well as smooth muscle and skeletal muscle contraction, it may significantly alter the outcome of COPD exacerbation if untreated.[10]

A study conducted in Mysore, India among 100 COPD patients and 100 healthy controls shows that serum electrolyte levels were significantly low among patients of acute exacerbation of COPD. Hence screening for these abnormalities may improve outcome.[10] Similarly study conducted in Kerala among 100 COPD patients (50 stable COPD and 50 acute exacerbation of COPD patients) also demonstrates, there were changes in levels of sodium, potassium and magnesium in acute exacerbation of COPD patients compared to stable COPD patients. Timely screening with earlier detection and intervention would be useful in controlling acute exacerbation of COPD patients.[5]

In spite of such alarming outcomes, very less data are available there regarding the precipitating factors and predictors of prognosis in patients with acute exacerbation of COPD especially from the developing countries. Thus, this study aimed at evaluating serum electrolyte levels in acute exacerbation of COPD to assess the occurrence of dyselectrolytemia.

MATERIALS AND METHODS

Descriptive cross sectional research design was used to evaluate the serum electrolyte level in the patient with AE of COPD. The study population was the patient with AE of COPD admitted in Manipal Teaching Hospital, Pokhara. Consecutive sampling technique was used in this study and consisted of 100 samples. All cases with AE of COPD admitted through the emergency or outpatient department were included in the study. COPD patients admitted for causes other than COPD exacerbation, COPD patients requiring mechanical ventilation, and the patients with pre-existing renal, hepatic, endocrinal or cardiac illness were excluded in the study. The serum electrolytes (sodium, potassium) and Arterial Blood Gas (ABG) of the admission day were analyzed in this study. Other data were collected through interview technique and from patients chart. The data was collected within the period of March, 2019 to August, 2019. The obtained data was edited, classified and coded. Then, the data was analyzed using SPSS software version 16 and descriptive statistics were used to generate the research finding and result. Informed consent was taken from each participant and an approval was obtained from Institutional Review Committee of Manipal Teaching Hospital, Pokhara, Nepal.

RESULTS

Hundred patients diagnosed with acute exacerbation of COPD admitted in the Medicine ward of Manipal Teaching Hospital were included in the study. The age of the patients ranged from 49 to 90 years with the mean age of 69.57±9.76 years. Most of the patients (83%), belonged to the age group of more than 60 years and above. More than half (54%) of the patients were male. Regarding religion, majority of them (78%) were Hindu and 36% belonged to relatively advantaged Janajati. Majorities (74%) of the patients were married, 52% of the patients were illiterate and 41% of them were engaged in agriculture. Regarding family type, 73% belonged to joint family (Table 1). Among them, 41% consumed alcohol and 67% were smokers (Table 2).

During the time of admission, patients with AE of COPD had clinical symptoms of dyspnea (90%), cough (89%) and sputum (38%). Regarding the duration of hospital stay, majority (73%) of the patients had stayed hospital for 5 days and above and 27% of them stayed for less than 5 days (Table 3). Average serum Na$^+$ and K$^+$ levels in patients with COPD were 133.8 ± 4.83 mEq/L, 3.6±0.53 mmol/L, respectively. Minimum sodium level was 121 mEq/L and maximum was 143 mEq/L and for potassium, minimum level was 2.5 mmol/L and maximum was 5.9 mmol/L. In

this study, it was seen that 57% patients had electrolyte disorder and 43% were in normal range. It was found that 51% patients had hyponatremia and 49% had normal level of sodium. Likewise, 40% patients had hypokalemia and (2%) had hyperkalemia and more than half (58%) were in normal range (Table 4).

The mean value of pH, $PaCO_2$ and PaO_2 are 7.34 ± 0.72, 46.64 ± 9.78 mm Hg and 69.38 ± 9.25 mm Hg respectively (Table 5). Among patients with AE of COPD, 82% of them were without respiratory failure and 18% of them were in respiratory failure. Out of 100 patients with AE of COPD, 85% were discharged from hospital, 11% had left the hospital against medical advice as well as on request and 4% were expired (Table 6).

Table 1: Socio-demographic Characteristics of the Participants (n=100)

Characteristics	Frequency (Percentage)
Age (in years)	
Below 60	17 (17.0)
60 and above	83 (83.0)
Sex	
Female	46 (46.0)
Male	54 (54.0)
Religion	
Hindu	78 (78.0)
Buddhist	14 (14.0)
Christian	8 (8.0)
Ethnicity	
Dalit	19 (19.0)
Disadvantaged Janajati	11 (11.0)
Relatively advantaged Janajati	36 (36.0)
Upper caste groups	34 (34.0)
Marital Status	
Married	74 (74.0)
Unmarried	7 (7.0)
Widow/widower	19 (19.0)
Educational Status	
Illiterate	52 (52.0)
Can read and write	19 (19.0)
Primary education	14 (14.0)
Secondary education	15 (15.0)
Occupational status	
Agriculture	41 (41.0)
Homemaker	23 (23.0)
Service	29 (29.0)
Business	7 (7.0)
Type of family	
Nuclear	27 (27.0)
Joint	73 (73.0)

Table 2: Alcohol consumption and smoking habit (n=100)

Characteristics	Frequency (Percentage)
Alcohol consumption	
Yes	41(41.0)
No	59(59.0)
Smoking habit	
Yes	67(67.0)
No	33(33.0)

Table 3: Clinical symptoms during admission and hospital stay(n=100)

Variables	Frequency (Percentage)
Symptoms*	
Dyspnoea	90 (90.0)
Cough	89 (89.0)
Sputum	38 (38.0)
Hospital Stay	
Less than 5 days	27 (72.0)
5 days and more	73 (73.0)

*Multiple Responses

Table 4: Serum electrolytes among AE of COPD patients (n=100)

Electrolytes	Frequency (Percentage)
Sodium (Na⁺)	
Hyponatremia (less than 135 mEq/L)	51 (51.0)
Normal (135-145 mEq/L)	49 (49.0)
Hypernatremia (more than 145 mEq/L)	0 (0.0)
Potassium (K⁺)	
Hypokalemia (less than 3.5 mmol/L)	40 (40.0)
Normal (3.5-5 mmol/L)	58 (58.0)
Hyperkalemia (more than 5 mmol/L)	2 (2.0)
Electrolyte disorder	
Yes	57 (57.0)
No	43 (43.0)

Table 5: Arterial Blood Gas parameters among AE of COPD patients

Parameters	Mean ± SD	Range
pH	7.34 ± 0.72	7.00-7.50
$PaCO_2$(mmHg)	46.64 ± 9.78	35-88
PaO_2(mmHg)	69.38 ± 9.25	38-92

Table 6: Respiratory failure among AE of COPD patients and Outcome (n=100)

Variables	Frequency (Percentage)
Respiratory failure	
With respiratory failure	18 (18.0)
Without respiratory failure	82 (82.0)
Outcomes	
Discharge	85 (85.0)
LAMA/DOR	11 (11.0)
Expired	4 (4.0)

LAMA=Leave against medical advice   DOR= Discharge on request

DISCUSSION

Mean age of the patients with AE of COPD was 69.57±9.76 years. Most of the patients (83%) belonged to the age group of more than 60 years and above. More than half (54%) of the patients were male. Among them, 41% consumed alcohol and 67% were smoker. The finding is consistent with the study done by Adiody et al.[4] revealed by Keralastate of India which showed that 68% of the patients with AE of COPD were smoker.[4]

At the time of admission, patients with AE of COPD had clinical symptoms of dyspnoea, cough and sputum i.e. (90%), (89%) and (38%) respectively. The study conducted in India depicted that cent percent of the patient with AE of COPD had dyspnoea and 40 percent of them had cough.[4] Regarding the duration of hospital stay, majority (73%) of the patients had stayed hospital for 5 days and above and 27 percent of them stayed for less than 5 days.

Mean levels of Na+ and K+ in patients with COPD were 133.8±4.83 mEq/L, 3.6±0.53 mmol/L, respectively. This finding is consistent with the study done in Bangladesh which showed the mean values of serum sodium and potassium were 133.9(±9.18)mEq/L and 3.6(±0.84) mmol/L respectively.[11] Minimum sodium level was 121mEq/L and maximum was 143mEq/L and for potassium minimum level was 2.5 mmol/L and maximum was 5.9 mmol/L. The finding of the study done by Md Haroon ur Rashid[12] revealed minimum level of Na+ 118 mEq/L and maximum level 138 mEq/L and for K+, minimum level was 2.1 mEq/L and maximum level was 3.7 mEq/L. In this study, it was seen that (57%) patients had electrolyte disorder and (43%) were in normal range. Similar finding was found in the study conducted in Egypt which showed that (58.5%) patients had electrolyte disorder.[13] It was found that 51% patients had hyponatremia and 49% had normal level of sodium. Likewise, 40% patients had hypokalaemia and 2% had hyperkalemia and more than half (58%) were in normal range. According to the study done in Bangladesh, 45.4% patients had hyponatremia, 49.6% had normal level of sodium and 5% had hypernatremia, likewise, 41.2% patients had hypokalemia, 54.6% were in normal range and 4.2% had hyperkalemia.[11]

The mean value of pH, $PaCO_2$ and PaO_2 are 7.34 ± 0.72, 46.64 ± 9.78 mm Hg and 69.38 ± 9.25 mm Hg respectively. The study done by Md Haroon ur Rashid showed similar finding of mean value of pH 7.35± 0.06 and mean value of PaCO2 47.52± 10.49 whereas the mean value of PaO2 was 58.44±8.20 mm Hg.[12] Among patients with AE of COPD, 82% of them were without respiratory failure and 18% of them were in respiratory failure.

CONCLUSION

From this study it can be concluded that hyponatremia and hypokalemia are common electrolyte disorder with exacerbation of COPD patients. As electrolytes are predictor of outcome of critically ill patients, on time identification and abrupt management could lessen the sufferings of the patients with AE of COPD. Therefore, routine monitoring of the serum electrolytes level can be helpful to prevent poor outcomes among the patients with AE of COPD.

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Association of Preoperative mid-stream urine culture, Renal pelvic urine culture and Renal stone culture in the detection of Systemic inflammatory response syndrome/urosepsis post Percutaneous Nephrolithotomy

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ABSTRACT

Introduction: Urosepsis post Percutaneous Nephrolithotomy (PCNL) is a dreaded complication with high mortality rate. Objective: To find the association of preoperative midstream urine culture (PMUC), renal pelvic urine culture (RPUC) and renal stone culture (RSC) in the post-operative development of Systemic Inflammatory Response Syndrome (SIRS) and urosepsis. **Methods:** It was a prospective cross-sectional observational study. The study included all symptomatic patients meeting the inclusion criteria who underwent Percutaneous Nephrolithotomy from 18th August, 2019 to 28th March, 2020. PMUC, RPUC and RSC were done and analyzed accordingly. **Results:** A total of 140 (73 males, 67 females) patients underwent PCNL. PMUC was positive in 15% (21/140) as compared to RPUC and RSC which were 7.9 % (11/140) and 4.3% (6/140) of total cases. None of the patients had simultaneous culture positivity in all the three types of specimens. Only two (1.42%) patients had simultaneous positivity in pelvic urine culture and stone culture. The organisms obtained in pelvic urine culture and stone culture were same i.e. Klebsiella and Escherichia coli respectively. Only two (1.42%) patients developed SIRS post PCNL, where in both the cases stone culture were positive but PMUC and RPUC were negative. Urosepsis was found in none of the patients. In the Fischer Exact test PMUC and RPUC were not statistically significant in the detection of SIRS post PCNL. Whereas only RSC showed statistical significance in the detection of SIRS. **Conclusion:** Stone culture has high prediction for SIRS and it might be considered for patients undergoing PCNL in order to prevent stone related infective complications.

Keywords: Percutaneous nephrolithotomy, Sepsis, Urine culture

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INTRODUCTION

Percutaneous Nephrolithotomy (PCNL) is considered as the procedure of choice for a complex and large volume nephrolithiasis in all age groups.[1-3] But the procedure is not devoid of complications as fever occurs in 21% to 39.8% of patients undergoing PCNL.[4] The incidence of urosepsis is 0.3% to 9.3% and the septic shock is 1% in which the mortality reaches up to 66 to 80% post PCNL.[5-8] Hence, the factors leading to urosepsis in PCNL should be considered seriously in order to prevent the dreaded complications.

Factors responsible for post-operative urosepsis are the number of stones, duration of operation, bleeding and presence of residual stones.[9] Despite these factors, preoperative Urinary Tract Infection (UTI) also leads to post-operative urosepsis if urine is not made sterile before PCNL. Antibiotic prophylaxis is advised by the American

Urological Association (AUA) for the prevention of post-operative urosepsis but despite prophylaxis the incidence of urosepsis is 8-10%.[10] Thus control of urinary tract infection (UTI) is needed which is best correlated by Renal Pelvic Urine Culture (RPUC) and Renal Stone Culture (RSC) as compared to Pre-operative Midstream Urine Culture (PMUC).[11]

It is imperative to control UTI to prevent post-operative urosepsis and reduce the morbidity and mortality. Hence, this study aimed to find the association of PMUC, RPUC and RSC in the post-operative development of Systemic Inflammatory Response Syndrome (SIRS) and urosepsis.

MATERIALS AND METHODS

This was a prospective cross-sectional observational study conducted at the Department of Surgery, Lumbini Medical College and Teaching Hospital effectively from 18th August, 2019 to 28th March, 2020. Ethical approval was taken from the Institutional Review Committee of Lumbini Medical College and Teaching Hospital (IRC-LMC 06-G/019).

The sample size was calculated using the formula: Sample Size (S)= Z^2Pq/d^2, where: Z= Confidence Level of 95% (Standard Value – 1.96), P= Estimated Prevalence of Renal Stones (That corresponds to around 10%)[12], q= 1-P, d= Margin of error (0.05), Calculating sample size from above formula, the minimal sample required was 140. So, we decided to take the number of samples of 145.

All patients with renal stone size more than 10 mm in the largest dimension were included in the study, whose stone burden was calculated by multiplying the largest two dimensions of the stone. Patients not giving consent for the study, those having persistent preoperative midstream urine culture positivity and untreated coagulopathy were excluded from the study. Five cases were excluded from the study as three cases did not give the consent and the rest of the two had persistent coagulopathies. Routine preoperative and diagnostic investigations including ultrasonography of abdomen and pelvis, intravenous urogram and the preoperative midstream urine culture (PMUC) were done in all patients along with Computed Tomography –Intravenous urogram (CT-IVU) in selected cases. All patients were treated with empirical antibiotics for three days before the PMUC report arrived and the PMUC negative cases were taken for surgery, whereas for PMUC positive cases, culture specific antibiotics were administered for two weeks prior to the surgery and the PMUC was repeated. In every patient surgery was performed only when the PMUC report came out to be negative. All patients received intravenous antibiotics i.e.

third generation cephalosporin (Cefoperazone Sulbactum combination) of dose 1.5gms for adults and 750mg for children along with aminoglycosides (Amikacin) of dose 15mg/kg/day a day prior to the surgery according to the local hospital protocol which was carried out till the third postoperative (POD) day which was then subsequently converted into oral antibiotics continued up to seven days post-operatively.

All patients received the above mentioned intravenous antibiotics one hour before the procedure. Five French (Fr) ureteric catheter was introduced into ipsilateral ureter via 20 Fr cystoscope (Karl Storz, Germany) in lithotomy position and foley's catheterization was done. Later, the position was changed into prone and under fluoroscopic guidance using C-arm (Allengers, India) after the instillation of urografin 76% via ureteric catheter, targeted calyceal puncture was done using 18 Gauge (G) needle. Pelvic urine was aspirated from the puncture site and was sent for the culture in 3ml sterile urine within one hour of collection. Subsequent dilatation of the track with the help of fascial dilators (Cook, Germany) was done using Seldinger's technique with the introduction of 0.035 inches guide wire. After the dilatation of track Amplatz sheath of size 20 Fr were used. Nephroscope (Wolf, Germany) of size 18 Fr was introduced and the stones were visualized and broken down into fragments using lithotripter (Nidhi lith, India). The stones were taken out using forceps. The fragmented stones were washed with normal saline and sent in fresh normal saline for the culture. Double J stent (DJ stent) was routinely kept in all patents. Nephrostomy drain of size 18 Fr was also placed routinely at the end of the procedure. Nephrostomy drains were removed on the third postoperative day while DJ stents were removed three weeks after the procedure. Patients were closely monitored for any signs of SIRS (Systemic Inflammatory Response Syndrome) and urosepsis postoperatively. UTI was defined as urine test having pus cells equivalent to or more than five in number per high power field in microscopic examination.[13] SIRS was defined as patients having two or more of the following criteria i.e. hypothermia (body temperature less than 36 degree Celsius) or hyperthermia (body temperature more than 38 degree Celsius), leukocytosis (total leucocyte count more than 12,000/mm^{3}) or leucopenia (total leucocyte count less than 4,000/mm³), tachycardia (heart rate more than 90 beats/min) or tachypnea (respiratory rate more than 20 beats/min). Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection.[14] Sepsis secondary to urinary tract infection was considered as urosepsis.

All the pre, intra and post-operative data were collected in a preformed proforma and entered into Microsoft Excel spreadsheet 2013. The data were checked for consistency and validity. They were then imported to Statistical Package for Social Sciences (SPSS™) software version 16 for statistical analysis. Categorical variables were analysed as the Fisher exact test and chi-square tests to determine associations among the various groups and subgroups. Sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and association risks were calculated. P value less than 0.05 was considered statistically significant.

RESULTS

There were 140 patients which included 73 males and 67 females. The mean age of the patient was 39.9 ± 14.8 years. The mean operation time was 40.61 ± 13.4 minutes. Table 1 shows mean age, mean operation time and stone burden of the patients under study.

Table 1: Patient and stone demographics

Mean Age ± SD(in years)	39.97±14.87
Mean Stone Burden± SD(in mm^2)	190.97±177.5
Mean Operative Time± SD ((in minute)	40.61 ±13.4

PMUC was the most prevalent culture positive specimen with 21 cases (15%) with Escherichia coli (7.1%) being the commonest organism Isolated. RPUC was positive in 11 cases (7.9%) with Proteus mirabilis (3.6%) infection being the commonest organism cultured. Stone culture was positive in six cases (4.3%) where Proteus mirabilis (1.4%) was the commonest organism cultured. Table 2 depicts the same.

Table 2: Types of organism obtained in PMUC, RPUC and RSC

PMUC positive Organisms cultured (N=21)	Frequency	Percent
Staphylococcus aureus	1	0.7
Mixed growth	1	0.7
Klebsiella	4	2.9
Proteus mirabilis	5	3.6
E.coli	10	7.1
RPUC positive Organisms cultured (N=11)	Frequency	Percent
E.coli	3	2.1
Klebsiella	3	2.1
Proteus mirabilis	5	3.6
RSC positive Organisms cultured (N=6)	Frequency	Percent
E.coli	1	0.7
Klebsiella	1	0.7
Staphylococcus aureus	1	0.7
Pseudomonas	1	0.7
Proteus mirabilis	2	1.4

None of the patients had simultaneous culture positivity in all the three types of specimens. Only two (1.42%) patients had simultaneous positivity in pelvic urine culture and stone culture. The organisms obtained in pelvic urine culture and stone cultures were same i.e. Klebsiella and E.coli respectively. Only two (1.42%) patients developed SIRS post PCNL, where in both the cases stone culture were positive but PMUC and RPUC were negative. Urosepsis was found in none of the patients.

Detection of infection of preoperative midstream urine culture and renal pelvic urine culture were correlated with renal stone culture for the detection of infection in renal stones. True and false positivity along with true and false negativity were calculated for the same, which is depicted in the table no.3

Table 3: Table showing parameters of PMUC and RPUC for the detection of infection in renal stones.

Parameters	Preoperative midstream urine c/s	Renal pelvic urine c/s
True Positive	1	2
True Negative	114	125
False Positive	20	9
False Negative	5	4

PMUC and RPUC didn't show significant association with RSC on Fisher exact test where p value was 1 and 0.071. PMUC in our study showed 16.67% sensitivity, 85.07% specificity, positive predictive value of 4.76%, and negative predictive value of 95.8% in 95% confidence interval (CI), in the detection of infection in renal stones. Likewise, renal pelvic urine showed 33.33% sensitivity, 93.28% specificity, 18.18% of positive predictive value, 96.9% of negative predictive value in 95%CI in the detection of infection in renal stones which is shown in table 4.

Table 4: Predicting infected stones with preoperative midstream urine and renal pelvic urine

Diagnostic accuracy	Preoperative midstream urine c/s (%)	Renal pelvic urine c/s (%)
Sensitivity	16.67	33.33
Specificity	85.07	93.28
Positive predictive value (PPV)	4.76	18.18
Negative predictive value (NPV)	95.80	96.90

PMUC had 0% sensitivity, 84.7% specificity, 0% positive predictive value and 98.3% of negative predictive value in 95% CI for the detection of SIRS in post PCNL patient, where as it was 0% sensitivity, 92.03% specificity, 0% of positive predictive value and 98.45% of negative predictive value in 95% CI in RPUC for the detection of SIRS post PCNL. RSC on the other hand had sensitivity of 100%, specificity of 97.10%, and positive predictive value of

33.33% and negative predictive value of 100% with 95% of CI in the detection of SIRS in post PCNL patients. In the Fischer Exact test PMUC and RPUC were not statistically significant in the detection of SIRS post PCNL the p value was 1 in both the cases respectively. Whereas RSC showed statistical significance in the detection of SIRS in the Fischer Exact test p value is 0.002. Table 5 depicts the same.

Table 5: Predicting SIRS using various specimens

Diagnostic accuracy	PMUC (%)	RPUC (%)	RSC (%)
Sensitivity	0.00	0.00	100
Specificity	84.78	92.03	97.10
PPV	0	0	33.33
NPV	98.32	98.45	100
P value(95% CI)	1	1	0.002

DISCUSSION

Postoperative fever in PCNL is bothersome which ranges from 10-15%.[15,16] Fever post PCNL can be due to underlying infection, hence we routinely gave antibiotic prophylaxis three days prior to surgery according to our local hospital protocol. And in cases where pre-operative midstream urine culture was positive, antibiotics were given unless the urine culture report came out to be negative.

Positive preoperative midstream urine culture in our study was 15% and E.coli was the commonest organism detected which is comparable to the study conducted by Gutierrez et al.[1] But in a study conducted by Patel et al[17], PMUC was 51% which is much higher than our study and the reason for it might be due to the absence of patients in our study who were under long-term catheters, nephrostomy tubes.[1] We also aimed to make preoperative urine sterile even in stag horn stones.

Preoperative midstream urine culture is not a good test for the presumption of SIRS or urosepsis post PCNL which is shown by Mariappan et al[18] and Paonessa et al.[19] Sensitivity of preoperative midstream urine culture and the pelvic urine culture for the detection of infected stones were 16.67% and 33.33% respectively in our study which is comparable to the study conducted by Mariappan et al.[18] Likewise, stone culture has the highest value in detecting SIRS/urosepsis as according to the above mentioned studies, which is comparable to the findings of our study.

SIRS in our patient was only 1.42% and no urosepsis was noted, which is much less compared to the study done by Waltondiaz et al.[11] The reason might be due to stringent use of antibiotics preoperatively and continuation of the same postoperatively for seven days in our study.

The present study has few limitations as the surgeries were performed by different consultants. Robust methods for the calculation of stone burden was not carried out. It was a single center study.

CONCLUSION

Stone culture has high prediction for SIRS and it should be considered for patients undergoing PCNL in order to prevent stone related infective complications.

Conflict of interest

Authors declare no conflict of interest

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Knowledge of conjunctivitis among high school students in Pokhara valley of Western Nepal

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ABSTRACT

Introduction: Conjunctivitis is a common health problem in Nepal. It is inflammation of conjunctiva presenting as red eye. It is a communicable eye disease. Health education to the students and proper management can help spread of the disease. Knowledge of conjunctivitis among school students will help in prevention of the disease. So the study was carried out to assess the knowledge of conjunctivitis among high school students in Pokhara valley of Western Nepal. **Materials/ Methods:** A school based cross sectional study was performed among six government schools in western Nepal from May 2019 to June 2019. Students from grade eight, nine and ten were included in the study. Data was collected using structured questionnaire including demographic data, knowledge regarding sign symptoms of conjunctivitis, treatment, prevention, complication of conjunctivitis. Data was analysed using SPSS version 11.6. **Result:** A total of 523 students were included in the study. The mean age of the students was 14.7 ± 1.2 years with majority of females (53%). Nearly 2/3rd of the students (61.6%) had heard of conjunctivitis. Majority of the students (87.4%) of the students mentioned that it is communicable while 80.3% of the students correctly mentioned its etiology. Majority of the students (97.9%) mentioned that it is curable with treatment as first option (97.5%). Majority of the students (98.3%) correctly responded to the preventive measures but majority of the students (83.2%) wrongly mentioned mode of transmission. **Conclusion:** Secondary school students in Western Nepal have poor knowledge of conjunctivitis. Appropriate eye health education should be given to school students to prevent rapid spread of infective conjunctivitis.

Keywords: Conjunctivitis, Knowledge, School students

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INTRODUCTION

Conjunctivitis is a common problem worldwide.[1] Annually estimated 6 million new cases of viral conjunctivitis occur in the United States.[2] It is inflammation of conjunctiva characterized by hyperemia and edema of the conjunctiva typically associated with discharge.[3] It can be classified as viral, bacterial, fungal, parasitic, chlamydial, allergic and toxic. Viral conjunctivitis is more common than bacterial[2,4] and incidence of viral conjunctivitis increases in the summer and rainy season.

Infective conjunctivitis is a common, self-limiting condition. Topical antibiotic may shorten symptoms by one to two days in bacterial infection. Most cases would resolve without treatment within one week.[5] Viral conjunctivitis resolves in two to three weeks.

Most causes of conjunctivitis are benign, with a self-limited process; however, depending on the immune status of the patient and the aetiology, conjunctivitis can progress to sight-threatening complications like keratitis, uveitis, retinitis, optic neuritis and extra-ocular muscle palsy.[6,7]

The disease spreads through tears, eye discharge,

contaminated fingers, fomites and aerosols. If one member of family is affected usually whole family members will be infected. Likewise, if one student in class is affected, whole class will be affected. Infected students should refrain from attending school if their behavior is such that they cannot avoid close contact with other students.[8] Practicing good hygiene is the best way to control the spread of the disease.

Treatment is symptomatic and supportive unless complications occur. Bacterial conjunctivitis usually improves within three to four days of treatment, but viral conjunctivitis takes two weeks to resolve.

Conjunctivitis is a common health problem in Nepal. It is a communicable eye disease. Health education to the students regarding the disease and proper management can help spread of the disease. It is also common in school students. Knowledge develops good habit and good habit stays with person throughout their whole life. No such studies have been performed in our part. So the study was carried out with the objective to assess the knowledge of conjunctivitis among high school students in Pokhara valley of Western Nepal.

MATERIALS AND METHODS

This is a school based cross sectional study conducted from May 2019 to June 2019. Six government schools were randomly selected among the list of 35 schools of Pokhara. Only government schools were selected because we have recently performed school health programmes with education of common eye problems in some private schools. All students from grade eight, nine and ten were included in the study. All the students present at the time of survey were only included in the study. Absentees were excluded from the study.

Sample size calculation- Using the formula for cross sectional studies and assuming a prevalence of 50% (since no previous study has been carried out on the subject matter in our part of world), a sample size of 384 was calculated by using the formula, $n = z^2 p(1-p)/d^2$, where z=1.96, p= 0.5 and d= 5%. However, this was exceeded, and 523 students were recruited into the study.

The dependent variable was knowledge and independent variables were age and gender, class grade of the student. Data was collected using structured questionnaire. The questionnaire was prepared after reviewing articles and standard books. The questionnaire included (i) demographic data, (ii) having heard of conjunctivitis, (iii) cause of conjunctivitis, (iv) disease communicable or not, (v) symptoms of conjunctivitis, (vi) disease curable or not, (vii) treatment modalities, (viii) preventive measures, (ix)

necessity of treatment, (x) complication of conjunctivitis, (xi) mode of transmission.

The students were asked to fill the questionnaire completely. They were informed only to fill only one best option in the question. Questions (ii), (iii), (iv), (v), (vi), (ix), (x), (xi) reflected knowledge of conjunctivitis while questions (vii) and (viii) reflected students practice in conjunctivitis. The data was collected by ophthalmologist visiting the schools. The data was analysed using SPSS version 11.6. Mean and standard deviation were calculated. All the result was expressed in frequency and percentage.

Ethical approval was taken from ethical review committee (IRC) of Gandaki Medical College (GMC).We had also taken permission from educational office and school personnel. Verbal assent was taken from the participants.

RESULT

A total of 523 students were included in the study. The minimum age of the students was 10 years and maximum was 15 years with the mean age of 14.7±1.2 years. In our study, 47% (246) of the students were males while 53.1% (277) were females. Details of the students age and sex distribution is shown in table 1.

Table1: Age and sex distribution of the students

Sex	Age (10-15 yrs) n (%)	Age (> 15 yrs) n (%)	Total n (%)
Male	183 (35%)	63 (12%)	246(47%)
Female	200(38%)	77(15%)	277(53%)

Out of 523 students, nearly 2/3rd of our students (61.6%) mentioned that they had heard of conjunctivitis while 38.4% had not heard of conjunctivitis.

In our study, 87.4% (457/523) of the students mentioned that conjunctivitis is communicable while 12.6% (66/523) mentioned it as non communicable.

Most of the students 80.3% (420/523) mentioned organism as the cause of conjunctivitis followed by hot climate 13.8 %(72/523) and water 3.8% (20/523). Details of students knowledge regarding cause of conjunctivitis is shown in table 2.

Table 2: Cause of conjunctivitis

Cause of conjunctivitis	Number	Percentage
Organism	420	80.3
Ghost	10	1.9
Water	20	3.8
Hot climate	72	13.8
Don't know	1	0.2
Total	523	100

Majority of the students correctly mentioned the symptoms of the conjunctivitis. Details are shown in table 3.

Table 3: Symptoms of conjunctivitis

Symptoms	Numbers	Percentage
Redness	89	17
Discharge	15	2.9
Itching	47	9
All of the above	363	69.4
Others	9	1.7
Total	523	100

Majority of the students 97.9 % (512/523) responded that the conjunctivitis was curable. Regarding health seeking behavior of the students, 97.5 % (510/523) of the students mentioned that one should go to the hospital. Details are shown in table 4.

Table 4: Health seeking behavior of the students

Health seeking behaviour of students	Number	Percentage
Go to hospital	510	97.5
Put gazal	2	0.4
Put milk	8	1.5
Go to traditional healers	3	0.6
Total	523	100

For prevention of conjunctivitis, 98.3% of the students mentioned that one should go to the hospital or clean eyes with cold water. Details are shown in table5.

Table 5: Knowledge regarding prevention of conjunctivitis

Prevention of conjunctivitis	Frequency	Percentage
Clean eyes with water	92	17.6
Go to hospital	66	12.6
Both of the above	356	68.1
Others	9	1.7
Tota1	523	100

Majority of the students 98.5%(515/523) mentioned complications of conjunctivitis correctly. Only 1.5% said there will be no complication of conjunctivitis. Details are shown in table 6.

Table 6: Knowledge regarding complication of conjunctivitis

Complications	Frequency	Percentage
Visual acuity decreases	107	20.5
Other ocular diseases occurs	33	6.3
Both of the above	375	71.7
Nothing happens	8	1.5
Total	523	100

Majority of the students wrongly replied the mode of transmission of conjunctivitis as they mentioned it is transmitted by viewing eyes of patients with conjunctivitis (83.2%). Details are shown in table 7. This showed the necessity of health education to the high schools students as conjunctivitis is a communicable disease which has become endemic.

Table7: Mode of spread of conjunctivitis

Mode of transmission of conjunctivitis	Frequency	Percentage
Tears	29	5.5
Blood	25	4.8
Discharge	34	6.5
By viewing eyes of patients with conjunctivitis	435	83.2
Total	523	100

DISCUSSION:

Conjunctivitis is a communicable disease. It occurs throughout the year but more cases are seen in summer and rainy season in our country. Infective conjunctivitis is highly contagious and can easily spread in schools and at home. An outbreak of any communicable disease can be prevented by eliminating or reducing the source of infection, interrupting the transmission and protecting the person at risk.

In Nepal, majority of our community is literate. High school students are the future of our country and they are given basic knowledge of different common communicable diseases. Conjunctivitis is such a disease which can be controlled if properly treated and proper hygiene and habits practiced. So, if high school students have proper knowledge regarding the disease, they will tell their parents about the disease which help to change the knowledge and attitude regarding the disease.

No studies have been done in Nepal, regarding knowledge of conjunctivitis though similar studies have been done abroad.[9, 10] In our study though majority of the students correctly responded saying they have heard of conjunctivitis, only 80.3% of them reported that organism is the cause. In a similar study done in Africa[10], 81% of the students said that they have heard of conjunctivitis.

In our study, 69.4% of the students mentioned redness, discharge and itching as the symptoms of the study. But other studies that have been done by Bhat et al[9] and Prabhu et al[11] showed redness as the most common symptom. Likewise, study by Everitt et al[12] showed redness as the most common symptom. Majority of the students said that conjunctivitis was curable and they have to go to hospital in case of conjunctivitis. But some said that they put gazal or milk or go to traditional healer. This reflects our community's belief towards conjunctivitis. Other studies also showed people putting different things like onion, breast milk, sugar water, urine etc as traditional treatment methods.[10, 13, 14]

In our society there is myth that conjunctivitis is transmitted by viewing eyes of patients with conjunctivitis. Our study showed its reflection as majority of the students mentioned same cause. Health education together with

written materials, such as pamphlets, is safe and cost-effective with high rates of patient satisfaction and compliance.[15] So, if students are provided proper health education, it will help to change their attitude and practice towards the disease. This will help to reduce the burden of the disease and possible complications.

CONCLUSION

In Nepal, majority of our community is literate. High school students are the future of our country and they are given basic knowledge of different common communicable diseases. Secondary school students in Western Nepal have poor knowledge of conjunctivitis. Appropriate eye health education should be given to school students to prevent rapid spread of infective conjunctivitis. Also early presentation to eye care centers for its treatment should be encouraged.

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Prevalence of Extended Spectrum Beta Lactamase Producing and Carbapenems Resistance Isolates from Hands of Health Care Workers in a Health Care Setting of Nepal

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ABSTRACT

Background: Hands of healthcare workers (HCWs) could be colonized by potential drug resistant bacteria like Extended Spectrum Beta Lactamase producers (ESBLs) and Carbapenems-resistant (CR) isolates and could become vectors of nosocomial pathogens in healthcare facilities that are associated with an increase of morbidity, mortality and healthcare costs. This study aimed to investigate the prevalence of ESBLs and CR isolates from hands of HCWs with their antibiotic susceptibility pattern. **Materials and methods:** This was a cross-sectional study that included a total of 150 hand swabs collected from March, 2018 to September, 2018 in Gandaki Medical College and Teaching Hospital. Isolation, identification and antimicrobial susceptibility tests were done using standard microbiological procedures. **Results:** Among the total isolates of 219 obtained from growth positive samples 92/219(42.01%) were Gram negative bacteria (GNB) and the most common were *Klebsiella spp* 32(34.78%) followed by *Escherichia coli* 17(18.48%), *Pseudomonas aeruginosa* 12 (13.04%), *Acenetobacter spp* 11(11.96%), *Proteus spp* 9(9.78%), *Citrobacter spp* 7(7.61%) and *Enterobacter spp* 4(4.35%). The prevalence of ESBLs, CR and ESBLs with Co-resistant to Carbapenems were 19.56%, 14.13% and 9.78%, respectively. The most effective drugs for isolates were Nitrofurantoin followed by Amikacin, Tetracycline and Gentamycin. Distribution pattern of the ESBLs and CR isolates among doctors, nurses, laboratory technicians, helpers and basic science faculties were not significant (p>0.05). **Conclusions:** This report revealed the emerging and moderately high prevalence of ESBLs, CR and ESBLs with Co-resistant to Carbapenems GNB with their antibiotic susceptibility patterns found on hands of HCWs in Nepal. Thus, this study could be helpful in developing proper guidelines on hand hygiene and implementation of infection control measures including contact precautions against the spread of infections by such pathogens in healthcare settings.

Key Words: Carbapenems-resistance, Extended Spectrum Beta Lactamase producers, Healthcare workers

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INTRODUCTION

β-lactamases are a heterogeneous group of enzymes able to inactivate penicillins, cephalosporins and monobactams. These enzymes, frequently produced by aerobic and anaerobic Gram-negative bacteria, hydrolyze the β-lactam ring by irreversible hydroxylation, consequently inactivating the antibiotic.[1-3] *Enterobacteriaceae* that produce extended-spectrum β-lactamases carry plasmid-encoded enzymes that can efficiently hydrolyze and confer resistance to a variety of β-lactam antibiotics.[4] ESBL-producing organisms are responsible for a significant proportion of infections in health care settings. Treatment of these infections can place an added constraint on already overburdened health systems in developing countries.[5]

Infections with carbapenem-resistant *Enterobacteriaceae* (CRE) are increasingly being reported from patients in healthcare settings. They are associated with high patient

morbidity, attributable mortality and hospital costs. The management of infections has been complicated by the emergence of carbapenems resistance pathogens. Carbapenemases are β-lactamases that hydrolyse carbapenems, usually along with other β-lactams.[6] The most frequently occurring species of *Enterobacteriaceae* which are found to be carbapenem-resistant and that produce carbapenemases, are *Klebsiella pneumoniae* and *Escherichia coli.*[7]

Enterobacteriaceae can also produce β-lactamase enzymes such as AmpCs (chromosomal or acquired) and ESBLs that do not readily inactivate carbapenems on their own but can confer carbapenem resistance when combined with chromosomal porin mutations that prevent accumulation of β-lactam agents in the bacteria. Finally, the production of carbapenemase enzymes, typically found on mobile genetic elements that inactivate carbapenem and other β-lactam antibiotics is increasingly common.[8,9] These carbapenemase-producing CRE (CP CRE) frequently carry multiple resistance mechanisms, which can include redundant β-lactamases such as AmpCs and ESBLs and genes conferring resistance to other antimicrobial classes. While *Proteus*, *Providencia*, and *Morganella species* demonstrate intrinsically elevated MICs to Imipenem.[10] β-lactams drugs are often the primary therapeutic option for serious infections, and carbapenems in particular areoften considered agents of last resort. Thus, the emergence and spread of ESBLs and carbapenem-resistant *Enterobacteriaceae* are significant clinical and public health concern.

Hand hygiene before patient contact is strongly recommended by the World Health Organization[11] and by Hand hygiene guidelines[12] as a measure to prevent cross-transmission of micro-organisms. Moreover, findings in different studies strongly recommend the implementation of multimodal campaigns to improve Hand hygiene compliance in HCWs and the use of sinkless alternatives such as alcohol based hand rub.[12, 13]

HCWs hands might be colonized with ESBLs and carbapenem-resistant Enterobacteriaceae isolates and contaminated hands of healthcare providers may cause threat to not only the life of hospital staffs, patients and visitors but also to the people in the community. Hand hygiene is one of the most important preventive interventions against the spread of infections in healthcare settings. There is a paucity of documented literature on investigation of ESBLs and Carbapenems resistance Gram negative organisms on hands of HCWs from Nepal. To the best of our knowledge this is the first report from Nepal with an exclusive focus on investigating the current prevalence and of ESBL producing, Carbapenems resistance and ESBLs with Co-resistance to Carbapenems Gram negative organisms carriage among hands of HCWs from Nepal. Therefore, the study aimed to investigate the prevalence of ESBLs and CR isolates from hands of HCWs with their antibiotic susceptibility pattern.

MATERIALS AND METHODS

Study design and setting

This cross-sectional study was carried out at the Microbiology Laboratory from March, 2018 to September, 2018 after obtaining ethical clearance from Institutional Ethical Committee of Gandaki Medical College and Teaching Hospital. Verbal consent was taken from each participant and all samples were collected after he/she accepted and knew that they were participating in clinical study. The total of 150 hand swab samples were collected from the hands of Health care workers which include doctors, nurses, laboratory technicians and helpers working at various wards and departments of Gandaki Medical College and Teaching Hospital, Prithivichowk, Pokhara, Nepal.

Collection and Processing of Samples

HCWs working at various wards and departments and basic science faculties of Gandaki Medical College and Teaching Hospital were enrolled in this study who were apparently healthy and not taking any antibiotics two weeks prior to this study. The health care worker's hand swab samples were collected by means of sterile cotton swabs moistened in sterile saline water (0.85%). The sterilized cotton buds were rotated onto the overall surface area of palms of both hands and in between of the fingers too. The cotton bud swabs after swabbing the hands were kept in the sterile small tube containing Brain Heart Infusion (BHI) broth separately, labeled and was immediately transported to the microbiology laboratory of Gandaki Medical College and Teaching Hospital (GMC) for further processing.

All the swabs were cultured directly on MacConkey agar, Blood agar and Nutrient agar (Himedia). All cultured plates were incubated aerobically at 37°C for 24 hours. Bacterial isolates were identified using standard microbiological techniques.[14]Antimicrobial susceptibility testing of the isolates was performed by Kirby-Bauer disc diffusion technique and the interpretation was done according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2017).[15]

Screening and Confirmation of ESBL

The ESBL phenotype of identified colonies of *Enterobacteriaceae* were detected by confirmatory double disk diffusion test according to the CLSI guidelines (2017).[15] For this, Cefotaxime (30 µg) or Ceftazidime (30 µg), discs were placed on Mueller-Hinton agar plates, 20 mm apart (centre to centre) from a Cefotaxime plus Clavulanic acid (30+10 mcg) and ceftazidime plus clavulanic acid (30+10 mcg).The zones of inhibition for the Cefotaxime and Ceftazidime alone was compared to that of combined disc of cefotaxime and Cefotaxime plus Clavulanic acid and Ceftazidime plus Clavulanic acid respectively. An increase of ≥ 5mm in a zone of inhibition for either antimicrobial agent tested in combination with clavulanate vs zone of the inhibition of the agent when tested alone, was confirmed to be the ESBL producer. Plates were incubated overnight (18-20 h) at 35°C.

Carbapenem Susceptibility Testing

Susceptibility of all the isolates to carbapenems was tested in accordance with the current CLSI guidelines (CLSI 2012).[16]Carbapenem antibiotics Meropenem and Imipenem were used. The discs were placed on the surface of inoculated Mueller Hinton Agar (MHA) (Hi media, India) plates using sterile forceps. The discs were placed about 25mm apart, and the plates were incubated for 24hours at 37ºC after which zones of inhibition in diameter were recorded according to CDC guidelines (CDC 2009).[17] Isolates that showed a zone of inhibition <21mm in diameter for Meropenem or <23mm in diameter for Imipenem were considered as carbapenem resistant (CDC 2009).[17]

Data Analysis

All the data were entered into a computer database using standard format, checked for errors and verified. Data maintained in the computer sheets were organized and analyzed by using GraphPad Prism software for Windows (version 8). A value of p≤0.05 was assumed wherever applicable and 95% confidence intervals along with the exact p-values were presented. Data were presented in appropriate table, figures by calculating percentage, rate etc. Appropriate statistics were applied wherever applicable.

RESULTS

The total of 150 hand swab samples were collected from the hands of health care workers which include doctors 33.33% (50/150), nurses 33.33% (50/150), helpers 13.33% (20/150), laboratory technicians 6.66% (10/150) working at various wards and departments of hospital and 13.33% (20/150) from hands of basic science faculties working at basic science blocks, Rithhepani Lekhnath Pokhara who does not have regular exposure to hospital environment of Gandaki Medical College and Teaching Hospital.

The frequency of bacterial growth positive among the cultivated hand swabs were 60% (90/150) and 40% (60/150) hand swab samples didn't show any growth. In most of the positive samples, they showed polymicrobial growth. Among the total isolates of 219 obtained from overall growth positive samples in this study 92/219 (42.01%) isolates were Gram negative bacteria and 127/219 (57.99%) were Gram positive bacteria. Bacterial isolates were identified using standard microbiological techniques.

Distribution Pattern of Gram-Negative Bacteria Isolated From Hand Swab Samples

The total numbers of Gram-negative bacteria isolated in this study was 92 isolates from 90 positive hand swab samples. The most common Gram-negative organism isolated in this study were *Klebsiella spp* 32 (34.78%) followed by *Escherichia coli* 17 (18.48%), *Pseudomonas aeruginosa* 12 (13.04%), *Acenetobacter spp* 11 (11.96%), *Proteus spp* 9 (9.78%), *Citrobacter spp* 7 (7.61%) and *Enterobacter spp* 4 (4.35%) (Table 1, figure 1).

Table 1. Distibution of Gram-negative bacteria isolated from various hand swab samples.

Organism Identified	Number	Frequency
Klebsiella spp	32	34.78%
Escherichia coli	17	18.48%
Pseudomonas aeruginosa	12	13.04%
Acenetobacter spp	11	11.96%
Proteus spp	9	9.78%
Citrobacter spp	7	7.61%
Enterobacter spp	4	4.35%
Total	92	100%

Distribution Pattern of ESBLs Isolated Gram Negative Bacteria

Out of total 92 Gram negative isolates, 18 were found to be ESBLs. The overall prevalence of ESBLs in this study was (18/92) 19.56%. Out of total isolates *Klebsiella spp* 6/92(6.52%); *Escherichia coli*, 4/92(4.35%); *Pseudomonasaeruginosa* 3/92 (3.26%); *Acenetobacter spp* 3/92 (3.26%) and *Proteus spp* 2/92 (2.17%) were found to be Extended B-Lactamase Producers. None of the isolated *Citrobacter spp* and *Enterobacter spp* was found to ESBLs producers (Table 2, figure 1).

Table 2. Distribution of ESBLs among the isolated Gram negative bacteria

Organism	Number of Isolates Tested	ESBL Producer N (%)
Klebsiella spp	32	6(6.52%)
Escherichia coli	17	4(4.35%)
Pseudomonas aeruginosa	12	3(3.26%)
Acenetobacter spp	11	3(3.26%)
Proteus spp	9	2(2.17%)
Citrobacter spp	7	-
Enterobacter spp	4	-
Total	92	18 (19.56%)

Distribution Pattern of Resistance among the Isolated Gram Negative Bacteria to Carbapenems

Out of total 92 Gram negative isolates, 12 were found to be Carbapenems resistance (Resistant to both Meropenem and Imipenem). The overall prevalence of Carbapenem resistant to both Meropenem and Imipenem in this study was (13/92) 14.13%. Out of total isolates *Klebsiella spp* 4/92(4.35%); *Escherichia coli*, 3/92 (3.26%); *Pseudomonas aeruginosa* 3/92(3.26%); *Acenetobacter spp* 2/92 (2.17%) and *Proteus spp* 1/92 (1.09%) were found to be Carbapenems resistant. None of the isolated *Citrobacter spp* and *Enterobacter spp* was found to Carbapenems resistant (Table 3, figure 1).

Table 3. Distribution of Resistance among the isolated Gram negative bacteria to carbapenems

Organism	Number of Isolates Tested	No. resistant to Meropenem (Zones<21mm) N (%)	No. resistant to Imipenem (Zone<23mm) N (%)	No resistant to both N (%)
Klebsiella spp	32	4 (4.35%)	6 (6.52%)	4 (4.35%)
Escherichia coli	17	5 (5.43%)	3 (3.26%)	3 (3.26%)
Pseudomonas aeruginosa	12	3 (3.26%)	3 (3.26%)	3 (3.26%)
Acenetobacter spp	11	3 (3.26%)	2 (2.17%)	2 (2.17%)
Proteus spp	9	1 (1.09%)	1 (1.09%)	1 (1.09%)
Citrobacter spp	7	-	-	-
Enterobacter spp	4	-	-	-
Total	92	16 (17.39%)	15 (16.30%)	13 (14.13%)

Distribution Pattern ESBLs Producers and Carbapenems Resistance Isolates

Out of total 92Gram negative isolates, 9 were found to be ESBLs Producers and Carbapenems resistance. The overall prevalence of ESBLs producers and Co-resistant to Carbapenems in this study was (9/92) 9.78%. Out of total isolates *Klebsiella spp* 3/92 (3.26%); *Escherichia coli*, 2/92 (2.17%); *Pseudomonas aeruginosa* 2/92 (2.17%); Acenetobacter spp 1/92(1.09%) and *Proteus spp* 1/92 (1.09%) were found to be ESBLs producers and Co-resistant to Carbapenems. None of the isolated *Citrobacter spp* and *Enterobacter spp* was found to ESBLs producers and Co-resistant to Carbapenems (Table 4).

Table 4. Distribution of ESBLs Producers and Carbapenems Resistance Isolates

Organism	Number of Isolates Tested	ESBL Producer + Carbapenems Resistance N (%)
Klebsiella spp	32	3 (3.26%)
Escherichia coli	17	2 (2.17%)
Pseudomonas aeruginosa	12	2 (2.17%)
Acenetobacter spp	11	1 (1.09%)
Proteus spp	9	1 (1.09%)
Citrobacter spp	7	-
Enterobacter spp	4	-
Total	92	9 (9.78%)
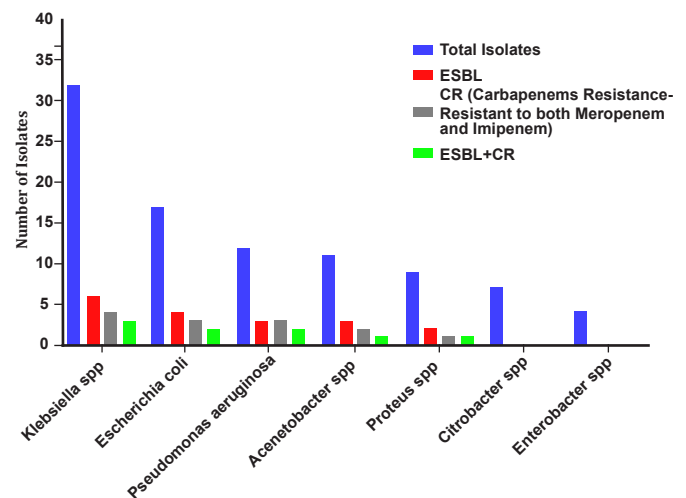
Figure1: Distribution of ESBLs Producers, Carbapenems Resistance and ESBLs producers with Co-resistance to Carbapenems among the isolated Gram negative bacteria.

Antibiotic Susceptibility Test of the isolated Gram-negative bacteria

Various antibiotics were used for antibiotic susceptibility pattern determination using Kirby Bauer disc diffusion method. The most effective drug of choice for different isolates were Nitrofurantoin followed by Amikacin, Tetracycline and Gentamycin. All the isolates were 100% resistant to ampicillin and Amoxicillin+Clavulanate. Neither isolates of *Citrobacter spp* and *Enterobacter*

spp obtained in this study were resistant to Cefotaxime, Ceftazidime, Meropenem and Imipenem (Table 5).

Table 5. Antibiotic Susceptibility Test of the Isolated Gram-Negative Bacteria from Hand Swab Samples

Pathogens	Klebsiella spp	Escherichia coli	Pseudomonas aeruginosa	Acenetobacter spp	Proteus spp	Citrobacter spp	Enterobacter aerogens
Total no. of isolates	32	17	12	11	9	7	4
	No. of isolates resistant to						
CTX	6 (18.75%)	4 (23.52%)	3 (25.0%)	3 (27.27%)	2 (22.22%)	0	0
CAZ	6 (18.75%)	4 (23.52%)	3 (25.0%)	3 (27.27%)	2 (22.22%)	0	0
MEM	4 (12.50%)	5 (29.41%)	3 (25.0%)	3 (27.27%)	1 (11.11%)	0	0
IPM	6 (18.75%)	3 (17.64%)	3 (25.0%)	2 (18.18%)	1 (11.11%)	0	0
AMP	0	0	0	0	0	0	0
AMC	0	0	0	0	0	0	0
CRO	26 (81.25%)	14 (82.35%)	10 (83.33%)	10 (90.90%)	8 (88.88)	6 (85.71%)	3 (75.0%)
NX	25 (78.12%)	12 (70.58%)	9 (75.0%)	9 (81.81%)	7 (77.77%)	5 (71.42%)	2 (50.0%)
GEN	17 (53.12%)	7 (41.17%)	4 (33.33%)	4 (36.36%)	4 (44.44%)	3 (42.85%)	2 (50.0%)
COT	24 (75.0%)	14 (82.35%)	8 (66.66%)	7 (63.63%)	7 (77.77%)	6 (85.71%)	3 (75.0%)
TE	10 (31.25%)	6 (35.29%)	5 (41.66%)	4 (36.36%)	3 (33.33%)	2 (28.57%)	2 (50.0%)
AK	8 (25.0%)	5 (29.41%)	3 (25.0%)	3 (27.27%)	2 (22.22%)	1 (14.28%)	1 (25.0%)
NIT	3 (9.37%)	3 (17.64%)	2 (16.66%)	2 (18.18%)	1 (9.09%)	1 (14.28%)	1 (25.0%)

CTX, Cefotaxime; CAZ, Ceftazidime; MEM, Meropenem; IPM, Imipenem; AMP, Ampicillin; AMC, Amoxicillin+Clavulanate ; CRO, Ceftriaxone; NX, Norfloxacin; GEN, Gentamycin; COT, Cotrimoxzole; TE, Tetracycline; AK, Amikacin; NIT, Nitrofurantoin.

Distribution of Isolates of ESBLs Producers, Carbapenems Resistance and ESBLs producers with Co-resistance to Carbapenems in Relation to Samples Group

Among the total 92 Gram negative isolates obtained from different study groups total isolates were 18/92 (19.56%) from doctors, 29/92 (31.52%) from nurses, 19 (20.65%) from helpers, 15 (16.30%) from laboratory technicians and 11 (11.96%) from basic science faculties.Out of those tested 92 isolates 3 (3.26%) from doctors, 6 (6.52%) from nurses, 3 (3.26%) from helpers, 4(4.35%) from laboratory

technicians and 2 (2.17%) from basic science faculties that produced ESBLs, Among the 92 isolates tested, 2 (2.17%) from doctors, 5 (5.43%) from nurses, 2(2.17%) from helpers, 3 (3.26%) from laboratory technicians and 1 (1.09%) from basic science faculties wereresistant to Carbapenems (Resistant to both Meropenem and Imipenem). Out of 92 isolates tested 2(2.17%) from doctors, 3(3.26%) from nurses, 1(1.09%) from helpers, 2(2.17%) from laboratory technicians and 1 (1.09%) from basic science faculties were ESBLs producers with Co-resistance to Carbapenems. These data were not found in significantly different percentage from different study groups with p>0.05 (ESBLs, p=0.37; CR, p=0.40; ESBLs+CR, p=0.43) (Table 6).

Table 6. Distribution Pattern of Isolates of ESBLs Producers, Carbapenems Resistance and ESBLs producers with Co-resistance to Carbapenems in Relation to Sample Groups

	Doctors N (%)	Nurses N (%)	Helpers N (%)	Laboratory Technicians N (%)	Basic Science Faculties N (%)	Total N (%)	"p" value
	ESBLs Producers N (%)						
Total Isolates Tested	18	29	19	15	11	92	0.37
ESBL Positive (%)	3 (3.26)	6 (6.52)	3 (3.26)	4 (4.35)	2 (2.17)	18 (19.56)	
	Carbapenems Resistance (CR=Resistance to both Meropenem and Imipenem) N (%)						
Total Isolates Tested	18	29	19	15	11	92	0.40
CR	2 (2.17)	5 (5.44)	2 (2.17)	3 (3.26)	1 (1.09)	13 (14.12)	
	ESBLs+CR (ESBLs Producers with Co-Resistance to Carbapenems) N (%)						
Total Isolates Tested	18	29	19	15	11	92	0.43
ESBL+CR	2 (2.17)	3 (3.26)	1 (1.09)	2 (2.17)	1 (1.09)	9 (9.78)	

DISCUSSION

Hand washing may not usually be performed often enough and many HCWs may not wash their hands properly in the course of their work throughout the day and contaminated hands of healthcare providers play a major role in spreading infections in healthcare settings. The emergence and spread of ESBLs and Carbapenems resistant bacteria through hands of HCWs is a public health threat as they usually associated with an increase of morbidity, mortality and healthcare costs. Hand hygiene is one of the most important preventive interventions against the spread of infections in healthcare settings.

In this study, the frequency of growth positive for bacteria among the cultivated hand swabs were 60% (90/150) and 40% (60/150) hand swab samples didn't show any growth. In most of the positive samples, they showed polymicrobial growth. The isolation rate of the pathogens was lower than previous study carried out by Kumari et al[18] but comparable with the findings of Vedavati and Halesh[19]. Among the total isolates of 219 obtained from overall growth positive samples in this study 92/219 (42.01%) isolates were Gram negative bacteria and 127/219 (57.99%) were Gram positive bacteria. In this study, Gram positive bacteria were found to occur more than Gram negative bacteria. Most skin flora bacteria are Gram positive, which would account for their predominance on hand swab samples which was in accordance with the findings of Vedavati and Halesh[19] who also demonstrated that the Gram positive bacteria predominate over Gram negative bacteria from the growth positive hand samples.

This study also highlighted the presence of potential pathogenic Gram-negative bacteria in hands of HCWs like Klebsiella spp 32(34.78%) followed by *Escherichia coli* 17(18.48%), *Pseudomonas aeruginosa* 12(13.04%), *Acenetobacter spp* 11(11.96%), *Proteus spp* 9(9.78%), *Citrobacter spp* 7(7.61%) and *Enterobacter spp* 4(4.35%). Similar types of pathogens were found on hands of health care workers in studies done by Vedabati and Halesh[19] and Kumari et al[18]. The presence of these pathogenic bacteria on hands poses a potential risk to vulnerable, immune-compromised individuals. The fact that bacteria of the *Enterobacteriaceae* found on hands may indicate feacal contamination of the hands.

The overall prevalence of ESBLs in this study was (18/92) 19.56%. The frequency of ESBL producer in other studies was higher than our studies.[20,21] It may be due to steadily increasing the incidence of ESBL producing strains among the clinical isolates, also the prevalence of ESBLs among clinical isolates varies from country to country and from institution to institution.[22] In the present study, *Klebsiella spp* was found to be the most common ESBL producer which was in concordance with the previous studies of Nepal and other countries.[23-26] who had also reported that *Klebsiella spp* were the predominant ESBL producers among the clinical isolates. However, another study from Nepal has reported that *Escherichia coli* were the predominant ESBL producers among the clinical isolates.[20] In this study, none of the isolates of Citrobacter spp and Enterobacter spp were found to ESBLs positive which was in agreement with the findings of Lohani et al[20] who also reported none of the isolates of *C. freundii* were

ESBL positive in their study. It might be because of the less number of isolates.[27]

The overall prevalence of Carbapenem resistant to both Meropenem and Imipenem in this study was (13/92) 14.13% which was lower as compared to prevalence of Carbapenem resistance from the family *Enterobacticeae* by Pokhrel et al[28]. Among which most common bacteria was Klebsiella spp followed by Escherichia coli which was in agreement with the findings of previous studies done in Nepal by Pokhrel et al[28] and Bora et al[29] among the clinical isolates. None of the isolated *Citrobacter spp* and *Enterobacter spp* was found to be resistant to Carbapenems. In this study *E. coli* was more resistant to Meropenem followed by *Klebsiella spp*, *Pseudomonas aeruginosa*, *Acenetobecter spp* and *Proteus spp*. While, K. pneumoniae was more resistant to Imipenem followed by *E. coli*, Pseudomonas aeruginosa, *Acenetobacter spp* and *Proteus spp*. Poor hygienic practices by HCWs are some of the driving forces of ESBLs producers and Carbapenems resistance in this study. This is in accordance with the findings of Yusuf et al[30] who also demonstrated that E. coli was more resistant to Meropenem than to Imipenem while, in K. pneumoniae, the reverse was the case. The resistant genes in these pathogens could be easily transmitted not only to other HCWs, patients and visitors but also to the community if proper care is not taken.

The most effective drugs of choice for different isolates were Nitrofurantoin followed by Amikacin, Tetracycline and Gentamycin. A comparable finding was obtained by Chander and Shrestha[23] who also reported Nitrofurantoin and Aminoglycosides as optimal drugs of choice for isolated pathogens. Similarly Pokhrel et al[28] reported the increased rate of sensitivity of Amikacin in Carbapenem resistant enterobacteriaceae. All the isolates were 100% resistant to Ampicillin and Amoxicillin+Clavulanate. Neither isolates of *Citrobacters pp* and *Enterobacter spp* obtained in this study were resistant to Cefotaxime, Ceftazidime, Meropenem and Imipenem. The overall prevalence of ESBLs producers and Co-resistant to Carbapenems in this study was (9/92) 9.78%. Out of total isolates *Klebsiellaspp* 3/92 (3.26%); *Escherichia coli,* 2/92 (2.17%); *Pseudomonas aeruginosa* 2/92 (2.17%); A*cenetobacter spp* 1/92 (1.09%) and Proteus spp 1/92 (1.09%) were found to be ESBLs producers and Co-resistant to Carbapenems. None of the isolated *Citrobacter spp* and *Enterobacter spp* was found to ESBLs producers and Co-resistant to Carbapenems. Though the prevalence is moderately high in this study, the simultaneous resistance to ESBL and Carbapenems by the isolates in

hands of HCWs make the whole scenario more alarming.

The health care workers harboring the ESBLs and Carbapenems resistant strains might be asymptomatic and could serve as carriers of these strains. Hand hygiene is one of the most important preventive interventions against the spread of infections by such pathogens not only to hospital settings but also to the community

Strengths and limitations

This study will be a useful reference for future studies, to explore and expand on the wider prevalence of ESBLs, Carbapenems resistant and ESBLs with Co-resistant to Carbapenems Gram negative organism found on hands of health care workers in Nepal. Since our study was based on phenotypic detection of ESBL production and Carbapenem resistance genotypic characterization is recommended in future studies.

CONCLUSION

The study identified a baseline of data on types of Gram negative bacterial isolates along with their antibiotic susceptibility patterns. This report revealed the emerging and moderately high prevalence of ESBLs, Carbapenems resistant and ESBLs with Co-resistant to Carbapenems Gram negative bacteria found on hands of health care workers in Nepal. Thus, this study could be helpful in developing proper guidelines on hand hygiene and implementation of infection control measures including contact precautions against the spread of infections by such pathogens in healthcare settings.

Conflict of Interest

The authors declare that they have no competing interests.

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Abortion Law awareness and Abortion Services Utilization among reproductive age women of Inarwa municipality of Eastern Nepal

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ABSTRACT

Introduction: Globally, reproductive health and quality of life among female population has been found to be unsatisfactory in developing world. Until 1963, Nepal's 1854 legal code known as MulukiAin was revised numerous times that banned abortion exempting risk of women's life. Enactment of New Abortion Policy from 2003 brought landmark reforms to the women's choice in family planning methods and ended the sufferings of lengthy prison sentences for abortion crimes. This study was conducted to explore the level of awareness about abortion law with regard to health care utilization among women of reproductive age group along with its association with socio-demographic characteristics. **Methodology:** A cross-sectional study was conducted in March 2014, in Inarwa Municipality among women of reproductive age group (16 to 49 years). Convenient sampling was done to collect data from households. Data was entered and analyzed in SPSS 11.5 and presented in tabular form. Chi-square test was used to show association of awareness of abortion law and socio-demographic variables. **Results:** education, age at first marriage and age at 1st pregnancy was found to be significant (p value: <0.005). **Conclusion:** As media was the most used source of information, more education on awareness of abortion law and health care utilization should be disseminated through this medium.

Key words: - Abortion Law, Reproductive health, Utilization

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INTRODUCTION

Globally, reproductive health and quality of life among female population has been found to be unsatisfactory in developing world.[1] Estimates of unsafe abortions in 2008 accounted for a death of 47000 worldwide with decline in deaths by 50 in 1990 to 30 in 2008.[2] About 97% of all unsafe abortions are in developing countries while 55% are in Asia (South Central Asia).[3] It is difficult to extract relevant data regarding unsafe abortion from countries where access to abortion is legally restricted.

Until 1963, Nepal's 1854 legal code known as Muluki Ain was revised numerous times that banned abortion exempting risk of women's life. About $1/4^{th}$ of women were branded as murderers on the basis of pregnancy termination under charges of infanticide and homicide. Most of these victimized women belonged to poor communities and were illiterate, reported to police mostly by their relatives while women from affluent families resorted to neighboring countries like India for abortion practices.[4]

Enactment of New Abortion Policy from 2003, brought landmark reforms to the women's choice in family planning methods and ended the sufferings of lengthy prison sentences for abortion related 'crimes' representing Nepal globally as a model for change.[5]

Being a patrilineal country with many patriarchal societies, Nepal bears cultural and social norms where women are restricted to silence and dominance. Premarital pregnancy is considered culturally unacceptable in developing country like Nepal[6,7] leading to unsafe abortions while bearing a son is considered culturally acceptable irrespective of number of children[8,9]. Preference for son over daughter has led to sex selective abortion despite being charged with imprisonment for such crime. Such practices have led to unsafe abortion which is underreported keeping the lives of these women at stake.[10]

As we embark in Sustainable Development Goal (SDG 2016-2030), Nepal has experienced a tremendous achievement with decline in Maternal Mortality Rate (MMR) from 1990 (850/100000 Live Births) to 2011(229/100000 Live births). The pillars for the success in decline in MMR in achieving the target for Millennium Development Goal (MDG) 2015 lies not only with the improvement in healthcare services but also with the Amendment of Abortion Law 2002 to certain extent.[11]

The Abortion law has given women liberty with permission of abortion upto 12 weeks of gestation. Under the new policy, which went into effect from 2003, women are permitted abortion for up to 12weeks of gestation on request and under certain medical/legal conditions thereafter specifications of Nepal's 2002 Abortion Law, pregnancy termination is available under these circumstances: up to 12 weeks' gestation for any indication, by request, up to 18 weeks' gestation in the case of rape or incest, at any time during pregnancy if mental/physical health or life of the pregnant woman is at risk with approval from a medical practitioner, if fetus is deformed and incompatible with life with approval from medical practitioner. Additional considerations includes punishment for sex selective abortion, right of pregnant women to choose or discontinue pregnancy, minors (<16 years) with mental incompetence, a consent by legal guardian is a must and only providers certified in safe abortion care are eligible to provide induced abortion services[12]

Nepalese women in patriarchal society are facing many challenges in battling Right to reproductive and Sexual Health[5]. Despite the legalization of abortion law, there lies a challenge of unsafe abortion as a study in 2008 in

Nepal showed that the number of abortions performed by unregistered providers was likely equal to those done by registered providers [13] indicating that Nepalese women were lacking utilization of health care facilities for safe abortion.This study was conducted to explore the level of awareness about abortion law and to assess the utilization of abortion services among women of reproductive age group.

METHODS

A cross-sectional study was conducted in Inarwa Municipality of Sunsari district of Eastern Nepal using non-probability convenient sampling based on household sample of reproductive aged women (15-49 years) purposively. Data was collected for a month in March, 2014.

Inarwa Municipality has 6195 population household with 7946 female of reproductive age group 15-49 years.[14] There are 10 wards in Inarwa Municipality[15]. Among 10 wards, the ward number 1, 2, 7,8, 9 and 10 were randomly selected by lottery method using simple random sampling.

The population of interest were all women of reproductive age group (16-49) years regardless of their marital status at the time of the survey living in the Inarwa Municipality of eastern Nepal.

This Municipality was chosen for a number of reasons. Firstly, the aim of the study was to get a local community's perspective on the research questions so the semi-urban areas of Inarwa were relevant. Secondly, no similar study had previously been conducted in the area. Thirdly, the area was also chosen on the basis of convenience as Inarwa hospital stands as one of the teaching hospital of B.P. Koirala Institute of Health Sciences.

For ethical reasons the age limit was set to 16 years and older as the topic of interest was within the field of sexual and reproductive health and rights, which could be perceived as a culturally sensitive subject for Nepalese female youths. For the survey, a non-probability sampling technique in the form of convenient sampling was used to reach the respondents.

In total, 375 of 391 respondents who were eligible participated in this study: reasons for non- participation were that women were not healthy to participate (n=6), women were busy with household chores (n=8) and women refused (n=2). Sample size calculation was based on the reference taken by study done by Thapa S and Sharma SK[16] where 32% of women were aware about the legalization of abortion law using single proportion

formula.

Written ethical clearance was taken from institutional review committee of BPKIHS. Informed consent was obtained from each participant prior to interviewing them by signing a consent form. In order to ensure confidentiality, the questionnaires were anonymous, which meant that no names were noted but instead the questionnaire were coded.

Data was coded, entered in excel program and was analyzed by using Statistical Package for Social Sciences (SPSS) software version 11.5. Finally, descriptive data was presented in table and association of knowledge with sociodemographic data was shown by using chi-square test.

RESULTS:

A total of 375 respondents out of 391 women of reproductive age group (16-49 years) participated in this study. This gave a response rate of 95.91%.

I) Sociodemographic Characteristics

Majority (65.6%) of respondents belonged to age group 26-45 years with mean ±SD:30.75±7.884 (Min-Max=17-49 years) with 94.9% of them belonging to Hindu religion. The maximum, 48% of the respondents had secondary level education. Among the total married women (93.3%), there were 44 respondents (13%) who were married at less than 16 years of age while only 15 (4%) were married above 24 years with mean age at marriage ± SD: 17.52± 5.649 (Minimum age: 4 years-Maximum age: 29 years). More than half (63.53%) of married respondents of had 1-2 children while eight respondents (2.27%) had more than 5 children. About three-fourth (80.5%) of the respondents were homemakers by occupation as shown in table 1.

Table 1. Socio-demographic characteristics of women of reproductive age group of Inarwa Municipality, 2014

Characteristics	Categories	n (%)
Age of the respondent (in years) (n=375)	≤25	118 (31.4)
	26-45	246 (65.6)
	≥46	11 (2.9)
Mean ±SD:30.75±7.884 (Min-Max=17-49 years)		
Religion (n=375)	Hindu	356 (94.9)
	Muslim	2 (0.5)
	Kirat	5 (1.3)
	others	12 (3.2)

Characteristics	Categories	n (%)
Education (n=375)	Illiterate	55 (14.7)
	Primary	35 (9.3)
	Secondary	183 (48.8)
	Higher Secondary	72 (19.2)
	Above Higher Secondary	30 (8)
Marital Status (n=375)	Married	350 (93.3)
	Unmarried	24 (6.4)
	Divorced/ Widow	1 (0.26)
Age in years at Marriage (n=349)	≤13	14(4.01)
	>13 -<16	30 (8.6)
	16-24	290 (83)
	>24	15 (4)
Mean ±SD= 17.52±5.649 (Min-Max=4-29 years)		
Number of children(n=349)	No children	31 (9.40)
	1-2 children	223 (63.53)
	3-4	87 (24.78)
	≥5	8 (2.27)
Occupation of respondents (n=375)	Homemaker	302 (80.5)
	Labour	3 (0.8)
	business	25 (6.7)
	Farmer	43 (11.5)
	others	2 (0.5)

II) Abortion and Misconceptions

Among the total participants, 345 (92%) of respondents had an idea about abortion and the most (82.6%) used source to gather information was found to be mass media as shown in table 2.. One fourth (25%) respondents had misconception regarding abortion as a taboo. Out of 93 (25%) respondents, 82 of them believed it to be a curse.

Table 2. Misconception regarding abortion among women of reproductive age of Inarwa Municipality, 2014

Characteristics	Categories	n(%)
Idea about abortion (n=375)	Yes	345 (92)
	No	30 (8)
Sources of information about abortion *	Friends/ neighbour	119 (31.7)
	Mass media	310 (82.6)
	Health Professionals	73 (19.46)
Presence of taboo regarding abortion (n=375)	Yes	93(25)
	No	282 (75)
Type of taboo regarding abortion (n=93)	Curse	82 (88)
	Karma	10 (11)
	Others	1 (1)

*multiple responses

III) Knowledge Regarding Abortion Law

With regard to abortion, larger proportion (65%) had knowledge on abortion law. The most well-known condition among 242(65%) respondents were two under

which abortion is legal under some grounds in Nepal (81.40%) and abortion is illegal on basis of sex selection (76.03%). However, it was found that least knowledge (13.22%) was regarding abortion legalization upto 18 weeks on rape/incest. Larger proportion (87%) among 242 respondents reported of self-decision regarding right to abortion, while 6.6% reported as right of in-laws (Table 3).

Table 3. Knowledge regarding abortion law among women of reproductive age of Inarwa Municipality, 2014

Characteristics	Categories	n (%)
Knowledge regarding abortion law (n=375)	Yes	242 (65)
	No	133 (35)
Specific knowledge regarding abortion law* (n=242)		
Abortion is legal on some grounds in Nepal		197 (81.40)
Abortion is legal upto 12 weeks upon request		109 (45.04)
Abortion is legal if pregnancy poses danger to mothers health		134 (55.37)
Abortion is not legal on the basis of sex selection		184 (76.03)
Abortion is not legal without the consent of women		114 (47.10)
Abortion is legal incase of fetal abnormality		146 (60.33)
Abotion is legal upto 18 weeks on rape/incest		32 (13.22)
Opinion regarding right to make decision on abortion * (n=242)	Self	210 (87)
	husband	180 (86.77)
	In-laws	16 (6.6)
	Others	5 (2.47)

*multiple responses

IV) Utilization of Abortion Related Health Services

Out of total respondents, 52 (14%) had history of abortion and among those respondents, more than half (60%) had gone to hospitals for abortion.While 24 (45%) had surgical abortion, 19 (36%) had spontaneous abortion with majority (50%) belonging to 12-28 weeks of period of gestation at time of abortion. Reason for abortion was marked as maternal/fetal complications by 28(54%) respondents and one participant gave the reason as gender preference. Nearly half (42.3%) of respondents who underwent abortion had 2 children. These finding have been shown in table 4.

Table.4 Utilization of abortion related health services among women of reproductive age of Inarwa Municipality, 2014

Characteristics	Categories	N (%)
History of Abortion (n=375)	Yes	52 (14)
	No	323 (86)
Place of abortion (n=52)	hospital	31 (60)
	Private clinic	12 (23)
	others	9 (17)
Type of abortion (n=52)	medical	10 (19)
	surgical	24 (45)
	spontaneous	19 (36)
Number of children of termination seekers(n=52)	No	1 (1.92)
	1	10 (19.2)
	2	22 (42.3)
	≥3	18 (34.61)
Period of gestation at time of abortion (n=52)	<12 weeks	23 (44)
	12-28 weeks	26 (50)
	>28 weeks	3 (6)
Reason for abortion (n=52)	Maternal/Fetal complication	28 (54)
	Unplanned Pregnancy	15 (29)
	Gender Preferences	1 (2)
	Financial burden	1 (2)
	Others (Limit family number etc.)	7 (13)

Table 5 shows that education, age at marriage and age at birth of first child were found to be significantly related with the awareness about abortion.

Table 5. Association between having awareness of legal abortion in Nepal and socio-demographic characteristics

Characteristics	Categories	Abortion law awareness		p- value
		Yes (%)	Non (%)	
Age	≤25 years	74(62.7)	44(37.3)	0.617
	≥26 years	168(65.4)	89(34.6)	
Religion	Hindu	230 (64.6)	126(35.4)	0.898
	Others	12(63.2)	7(36.8)	
Education	Illiterate	15(27.3)	40(72.7)	<0.001**
	Literate	227(70.9)	93(29.1)	
Occupation of female	Home-maker	193 (63.9)	109(36.1)	0.417
	others	49(69.0)	22(31.0)	
Age at 1st marriage	≤15 years	18(40.9)	26(59.1)	0.001*
	≥16 years	202(66.2)	103(33.8)	
Age at birth of 1st child	≤16 years	5(26.3)	14(73.7)	<0.001**
	>16 years	198(66.2)	101(33.8)	

*highly significant (p value<0.01); ** very highly significant (p<0.001)

DISCUSSION

This study was carried out to describe abortion related awareness of law and hospital services utilization among women of reproductive age group (16 to 49 years) of Inarwa Municipality.

This study was conducted in similar setting as Makwanpur district[6] where the majority (36.4%) of female participants were of similar age of 26 to 45 years group as in our study (65.6%). Likewise, a hospital based study in Kathmandu found that the participants belonged to 30-34 years irrespective of their complains and symptoms.[17] In a study conducted at Kapan VDC, the education among the respondents was found to be Post higher secondary (47%) which is comparable to our study where the highest level of education was found to be secondary (48.8%).[18] It was surprising to see that 4.01% of respondents in this study were married by 13 years of age and 8.6% were married before 16 years which can also be seen as a consequence of abortion in studies in India [19] where 2.6% were married before 13 years of age and 22.6% were married before age 16 . Another study in Northern Ethiopia showed that by age 15 almost 50% girls were married.[20] The legal age for marriage is taken to be 18 years in Nepal for women.

Misconception regarding abortion was seen among 93 (25%) participants as taboo which is in resonance with studies conducted among marginalized underserved community of 6 districts under FPAN of Nepal[21] where 23 (19.65%) respondents thought abortion was sin. Mass media (82.6%) comprised most used source of information in our study while least source of information was friends/ neighbours (31.7%) which has also been reflected by a study at Nepal Medical College[17] with friends (6%) and media (92%). As abortion is a stigmatized issue in our country though legalization, this might be the reason for neighbours/friends not to be much source of information. However, a contradictory finding has been reported in study in Rupandehi District.[22]

More than half (65%) of respondents had a knowledge about abortion law with 81.40% knowing that abortion was legal in our country. In addition, a study done at Kathmandu Marie Stopes clinic[23] showed that among the abortion seekers 59% knew that abortion was legal and two most conditions for abortion was mentioned as health status of mother (43.4%) and incase of rape/incest (32.2%). Likewise, a study by Hald SC et al.[6] showed that knowledge regarding sex selection being illegal was most known condition (89.1%), a finding that mirrors our study with response of 76.03%.

Nepal like other developing country is a patriarchal country where husbands hold decision making for the family. Such is the case seen even when it comes to reproductive health and right of women even in neighboring country India.[24] Our study shows that decision for abortion by self and husband is comparatively similar (87% vs 86.77%).

History of abortion was given by 3.2% respondents in a study by Anderson et al[22] while our study had a 14% respondents with history of abortion. The increase in our respondents may be due to the fact of inclusion of women of reproductive age group 16-49 years, as in earlier study the sample included women of 16-24 years only. As one of the most noted reason for pregnancy was maternal and fetal complication (54%), this might be the reason for most (50%) termination of pregnancy between 12 -28 weeks of gestation as seen in our study. Unplanned pregnancy that accounted for 29% of cause was second reason for abortion as compared with other study[23] where it was first cause for termination accounting for 53.3% responses. Other reasons for abortion was found to be limiting of family size (13%) where the respondents were found to larger proportion (42.3%) of children at the time of survey. Similar study had been conducted in 2001 in urban Nepal[25] where the most given reason (34.4%) for abortion was too many children with most (43%) of women having 2 children. Such finding is also supported by study in Srilanka.[26] Among the total respondents who had undergone abortion (52), the reason for gender preference was only seen among 1 participant. There might have been social desirability bias and the respondents might have feared punishment for crime if reported to police as it is illegal. However, preferences for son still exist among the society as son is considered culturally valuable for performing various death rituals and continuing generation.

It was found that there was an association between literacy and awareness on abortion law (p value<0.001) which was also seen in similar other studies.[23, 27] Report from national data also highlights that nearly two thirds of women with higher Secondary education and half with secondary education believed that abortion was legal[28]. The reason for better knowledge on abortion law might be the fact of exposure to information on abortion as inclusion of reproductive health in school curriculum. Age at 1st marriage in Nepal was reported by18 years (55%) and by 20 years (70 %)[28] but in this study age at marriage was found to be among aged ≤13 years(4.01%).A study reported that with increase in education level there was a gradual decline in early age at marriage.[29] This might be

the reason the women who marry above 16 years in our study have better knowledge than the women who resort to marriage at early age of less than 15 years as education engulfs studies related to sexual and reproductive health. This study findings of better abortion law awareness were found more among women who were married and had their first children when they were 16 years and above than those who had early marriages and were pregnant before 16 years of age.

The reasons for this could be the fact that after marriage, women are bound by social responsibilities and has limited independence forcing her to drop out of school as well.

Lack of this knowledge regarding abortion law has made the vulnerable teenagers and youths more prone to unsafe abortion and lesser utilization of healthcare services.[30]

CONCLUSION

This study highlights the need to focus education on abortion law among illiterate women and females of adolescent age group. The overall awareness level was satisfactory and use of media was effective for dissemination of information. Of total,17% respondents had got abortion services from others places than the hospital and clinics. So, more campaigning and health education program should be conducted in the community where early marriages are prevalent.

Conflict of interest: None

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Establishing surface projection of Temporo-Mandibular Joint using tragus of ear as landmark

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ABSTRACT

Introduction: The temporo-mandibular joint (TMJ) is a synovial joint between the articular fossa of the temporal bone and the mandibular condyle. It is condylar variety of joint. The most important functions of the TMJ are mastication and speech and are of great interest to anatomists, dentists, orthodontists and oro-maxillo-facial surgeons. The study was conducted with objective to establish the surface projection of Temporo-mandibular joint (TMJ) using tragus of ear as land mark. **Materials and methods:** Twenty five cadavers dissected in pre auricular area on both right and left side were studied. Out of 25 cadavers, 18 were male and seven were females. The temporo-mandibular joints were exposed on both sides, keeping the tragus of the ear intact. Altogether fifty temporo-mandibular joints were studied. On living persons, condylar head of TMJ were palpated while the subjects were carrying out side to side movement of lower jaw. The distance between the summit of the tragus and the marking on condylar head was measured with the help of divider and scale. **Result:** The mean distance in millimeter (mm) from midpoint of condylar head to the summit of tragus in all living subjects and cadavers (n=150) was 12.5 ± 3.5 mm and the mean length of distal phalanx of fore finger in all living subjects and cadavers (n=150) was 22 ± 4 mm. **Conclusion:** The mandibular condyle can be palpated at 12.5 ± 3.5 mm distance from summit of tragus of ear (i.e. Half-length of distal phalanx of fore finger which is 22 ± 4 mm) just below the inferior border of zygomatic arch.

Key words: Surface projection, Temporo-mandibular joint, Tragus of ear.

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INTRODUCTION

The temporo-mandibular joint (TMJ) is a synovial joint between the articular fossa of the temporal bone above and the mandibular condyle below.[-6] It is a ginglymo-arthrodial joint, a term that is derived from ginglymus, meaning a hinge joint, allowing motion only backward and forward in one plane, and arthrodia, meaning a joint which permits a gliding motion of the surfaces.[1,] Its joint cavity is divided into two by an articular disc.[1-6] The articular eminence, a transversely elliptical region sinuously curved in the sagittal plane and tilted downwards anteriorly at approximately 25 degree to the occlusal plane, forms most of the articular surface of the mandibular fossa. The articular surface of the mandibular condyle is slightly curved and tilted forward at approximately 25 degree to the occlusal plane.[1] The right and left TMJ form a bicondylar articulation and ellipsoid variety of the synovial joints similar to knee articulation.[1-]

The common features of the synovial joints exhibited by this joint include a disk, bone, fibrous capsule, fluid, synovial membrane, and ligaments. However, the features that

differentiate and make this joint unique are its articular surface covered by fibrocartilage instead of hyaline cartilage. Movement is not only guided by the shape of the bones, muscles, and ligaments but also by the occlusion of the teeth, since both joints are joined by a single mandible bone and cannot move independently of each other.[1-6] The most important functions of the TMJ are mastication and speech and are of great interest to dentists, orthodontists and oro-maxillo-facial surgeons. This research is aimed to find the surface marking of TMJ using the tragus of ear as the landmark.

Rationale for the study: This type of study had not been conducted so far. The outcome of the study will be very informative for anatomist and oro maxillo facial surgeons, thus, this study was undertaken.

Importance of this study: This study will be highly useful for oral and maxillo- facial surgeon while operating the temporomandibular joint and during arthoscopic surgery and ortho centers of TMJ. As this study is establishing new easy method of surface marking of TMJ, it will be significant for anatomist also.

AIM & OBJECTIVES

Aim (General objective) of study

To establish the surface projection of Temporo-mandibular joints (TMJ) using tragus of ear as land mark.

Specific Objectives

1. To find the mean distance in mm from midpoint of condylar head to summit of tragus in living male and female

2. To find the mean distance in mm from midpoint of condylar head from summit of tragus in cadaveric male and female

3. To find the mean length of distal phalanx of fore finger in living male and female.

4. To find the mean length of distal phalanx of fore finger in cadaveric male and female

5. To compare the mean distance in mm from midpoint of condylar head to summit of tragus between living and cadaveric male and female and test the significant difference between them.

6. To compare the mean length of distal phalanx of fore finger between living and cadaveric male and female and test the significant difference between them.

METHODOLOGY

It is a crosss-sectional study conducted in Gandaki Medical College, Pokhara, Kaski from March 2014 to April 2019

Sample size and Sampling method for cadaveric study: Non-probability convenient sampling technique was adopted for cadaveric study. Whatever cadavers were available during study period in dissection hall, anatomy department were studied. **Cadaveric study procedure:** Twenty -five cadavers dissected in pre auricular area on both right and left side were studied. Out of 25 cadavers, 18 were male and seven were females. The temporo-mandibular joints were exposed on both sides, keeping the tragus of the ear intact. Altogether fifty temporo-mandibular joints were studied.

The distance between the summit of the tragus and the midpoint marking on condylar head was measured with the help of divider and scale. The distal phalanx length of fore finger was measured in a similar way. It is the distance between the crease on junction of distal and middle phalanx (distal interphalangeal joint) and tip of distal phalanx.

Sample size and Sampling method for living subjects study: For the living sample subjects, sample size was calculated by formula, sample size (n) = s^2/e^2 where 's' is standard deviation and 'e' is standard error. From the previous pilot study, value of standard deviation(s) and standard error (e) were determined as two and 0.28 respectively. Thus sample size was calculated as 50. Fifty living person (25 male and 25 female) were studied. Simple random sampling method was adopted to select the research subjects. **Study procedure on living subjects:** On living persons, condylar head which is one of the articular surface located by temporo-mandibular joints were palpated while the subjects were carrying out side to side movement of lower jaw. The TMJ was marked as point after condylar head was being palpated. The distance between the summit of the tragus and the marking on condylar head was measured with the help of divider and scale. The distal phalanx length of fore finger was measured in a similar way. It is the distance between the crease on junction of distal and middle phalanx (distal interphalangeal joint) and tip of distal phalanx. The procedure is shown in figure 1, 2 and 3.

Reliability and validity of research: To ensure the high reliability and validity of research bias was eliminated and measurements were taken by single observer who was principal investigator. **Inclusion and Exclusion criteria:** We included 25 to 35 years male and female with normally developed pinna of ear. Those with abnormally

developed pinna were excluded. **Statistical analysis:** The data collected entered into excel and data were analysed by using statistical software SPSS version 16. Student's 't' test was applied to compare the statistical difference between male and female and living and cadaveric data. For the data quality control during present research study, measures taken were adopting correct method of data collection; screening and verifying the collected data; selecting appropriate computer software; coding and entering the data into computer and rechecking it; processing the data; constructing a data base for data management and appropriate data analysis and review over it. **Ethical consideration:** There was no ethical issue for the cadaveric study. For the study on living subjects, consent was taken from every subject prior to conducting the study. Ethical clearance was obtained from the institutional ethical committee.

RESULTS

The dissection study of twenty-five cadavers on right and left side i.e. fifty temporo-mandibular joints (TMJs) were carried out where as one hundred TMJs on fifty living persons (25 male and 25 female) were studied. The findings are presented in table 1 to 10.

Table 1: The mean distance of midpoint of condylar head from summit of tragus and length of distal phalanx of fore finger of all cadavers (n=50 TMJs):

sn	The mean distance of midpoint of condylar head from summit of tragus and length of distal phalanx of fore finger	measurement
1.	The mean distance in mm from midpoint of condylar head to summit of tragus in all cadavers(n=50)	11.5+_2.5 mm
2.	The mean length of distal phalanx of fore finger in all cadavers(n=50)	21+_3 mm

Table 2: The mean distance of midpoint of condylar head from summit of tragus and length of distal phalanx of fore finger in male and female cadavers:

sn	The mean distance of midpoint of condylar head from summit of tragus and length of distal phalanx of fore finger	measurement
1.	The mean distance in mm from midpoint of condylar head to summit of tragus in male cadavers(n=36)	12+_2 mm
2.	The mean distance in mm from midpoint of condylar head to summit of tragus in female cadavers(n=14)	11+_2 mm
3.	The mean length of distal phalanx of fore finger in male cadavers(n=36)	22+_2 mm
4.	The mean length of distal phalanx of fore finger in female cadavers(n=14)	20+_2 mm

Table 3: Level of significance while comparing male and female cadaver parameters in the study:

S.n	Level of significance while comparing following male and female cadavers parameters	P value
1.	The mean distance in mm from midpoint of condylar head to summit of tragus in male cadavers(n=36)=12±2 mm	0.08
2.	The mean distance in mm from mi point of condylar head to summit of tragus in female cadavers(n=14)=11±2 mm	
3.	The mean length of distal phalanx of fore finger in male cadavers(n=36)=22±2 mm	0.06
4.	The mean length of distal phalanx of fore finger in female cadavers(n=14)=20±2 mm	

Statistical significance (p<0.05)

Table 4: The mean distance of midpoint of condylar head from summit of tragus and length of distal phalanx of fore finger of all living subjects (n=100 TMJs):

S.n	The mean distance of midpoint of condylar head from summit of tragus and length of distal phalanx of fore finger	measurement
1.	The mean distance in mm from midpoint of condylar head to summit of tragus in all living subjects(n=100)	13.5±2.5 mm
2.	The mean length of distal phalanx of fore finger in all living subjects(n=100)	23±3 mm

Table 5: The mean distance of midpoint of condylar head from summit of tragus and length of distal phalanx in living male and female:

S.n	The mean distance of midpoint of condylar head from summit of tragus and length of distal phalanx	measurement
1.	The mean distance in mm from midpoint of condylar head to summit of tragus in living male (n=50)	14±2 mm
2.	The mean distance in mm from midpoint of condylar head to summit of tragus in living female (n=50)	13±2 mm
3.	The mean length of distal phalanx of fore finger in living male (n=50)	24+_2 mm
4.	The mean length of distal phalanx of fore finger in living female (n=50)	22+_2 mm

Table 6: Level of significance while comparing living male and female parameters in the study:

S.n	Level of significance while comparing living male and female parameters in the study	P value
1.	The mean distance in mm from midpoint of condylar head to summit of tragus in living male(n=50)= 14±2 mm	0.07
2.	The mean distance in mm from midpoint of condylar head to summit of tragus in living female(n=50)=13±2 mm	
3.	The mean length of distal phalanx in living male(n=50)=24±2 mm	0.06
4.	The mean length of distal phalanx in living female(n=50)=22±2 mm	

Statistical significance **(p<0.05)**

Table 7: Level of significance while comparing the mean distance in mm from midpoint of condylar head to summit of tragus between living and cadaveric male and female.

S.n	Level of significance while comparing the mean distance in mm from midpoint of condylar head to summit of tragus between living male and female	P value
1.	The mean distance in mm from midpoint of condylar head to summit of tragus in living male (n=50)= 14±2 mm	0.07
2.	The mean distance in mm from midpoint of condylar head to summit of tragus in cadaveric male(n=36)= 12±2 mm	
3.	The mean distance in mm from midpoint of condylar head to summit of tragus in living female(n=50) =13±2 mm	0.06
4.	The mean distance in mm from midpoint of condylar head to summit of tragus in cadaveric female(n=14)= 11±2 mm	

Statistical significance **(p<0.05)**

Table 8: Level of significance while comparing the mean length of distal phalanx between living and cadaveric male and female:

Sn	Level of significance while comparing living male and female parameters in the study	P value
1.	The mean length of distal phalanx in living male(n=50)= 24±2 mm	0.07
2.	The mean length of distal phalanx in cadaveric male (n=36)= 22±2 mm	
3.	The mean length of distal phalanx in living female(n=50) =22±2 mm	0.06
4.	The mean length of distal phalanx in cadaveric female (n=14)= 20±2 mm	

Statistically significance **(p<0.05)**

Table 9: Level of significance while comparing the mean distance in mm from midpoint of condylar head to summit of tragus and the mean length of distal phalanx between overall living subjects and cadaveric bodies:

Sn	Level of significance while comparing overall living subjects and cadaveric parameters in the study	P value
1.	The mean distance in mm from midpoint of condylar head to summit of tragus in all cadavers(n=50)= 11.5±2.5 mm	0.07
2.	The mean distance in mm from midpoint of condylar head to summit of tragus in all living subjects(n=100)= 13.5±2.5 mm	
3.	The mean length of distal phalanx of fore finger in all cadavers(n=50)= 21±3 mm	0.06
4.	The mean length of distal phalanx of fore finger in all living subjects(n=100)= 23±3 mm	

Statistically significance **(p<0.05)**

Table10: The mean distance in mm from midpoint of condylar head to summit of tragus and the mean length of distal phalanx in all living subjects and cadavers:

sn	The mean distance of midpoint of condylar head from summit of tragus and length of distal phalanx	Measurement
1.	The mean distance in mm from midpoint of condylar head to summit of tragus in all living subjects and cadavers (n=150)	12.5±3.5 mm
2.	The mean length of distal phalanx of fore finger in all living subjects and cadavers (n=150)	22±4 mm

DISCUSSION

The temporo-mandibular joint (TMJ), which is synovial joint of condylar variety is very important joint because it involves in chewing food. As this joint has function of mastication and during mastication there is wear and tear in structure of the joint which results several types of joint disorders. In TMJ disorders, from the examination i.e. palpation of condylar head to clinical operative procedures like TMJ arthroscopy and others, it is quite necessary to locate condylar head by surface marking. So far there was no established method to locate it by surface marking thus this study was undertaken.

The present study was designed to carry out both on living subjects and dead bodies (cadavers) so that the result obtained from cadaveric study would be compared with the results obtained from study on living subjects. The statistical tests were used to compare the data obtained from living subjects and cadaveric studies for statistical significance. While doing so data would be validated for living subject studies.

The mean distance in mm from midpoint of condylar head to summit of tragus in all cadavers (n=50) was 11.5±2.5 mm and the length of distal phalanx of fore finger in all cadavers (n=50) was 21±3 mm. The mean distance in mm from midpoint of condylar head to summit of tragus in male cadavers (n=36) was 12±2 mm and in female cadavers (n=14) was 11±2 mm. The mean length of distal phalanx of fore finger in male cadavers (n=36) was 22±2 mm and in female cadavers (n=14) was 20±2 mm. The level of significance while comparing male and female cadavers parameters in the study was followed: the mean distance in mm from midpoint of condylar head to summit of tragus in male cadavers (n=36) = 12±2 mm and in female cadavers(n=14) =11±2 mm was statistically insignificant **(p<0.05)** likewise length of distal phalanx of fore finger in male cadavers (n=36) = 22±2 mm and in female cadavers (n=14) = 20±2 mm was also statistically insignificant **(p<0.05)**.

The mean distance in mm from midpoint of condylar head to summit of tragus in all living subjects (n=100) was 13.5±2.5 mm and the length of distal phalanx of fore finger (n=100) was 23±3 mm. The mean distance in mm from midpoint of condylar head to summit of tragus in living male (n=50) was 14±2 mm and in living female (n=50) was 13±2 mm. The mean length of distal phalanx of fore finger in living male (n=50) was 24±2 mm and in living female (n=50) was 22±2 mm. The Level of significance while comparing living male and female parameters in

the study was followed: the mean distance in mm from midpoint of condylar head to summit of tragus in living male (n=50) = 14±2 mm and in living female (n=50) = 13±2 mm was statistically insignificant (p value <0.05) likewise the mean length of distal phalanx in living male (n=50) = 24±2 mm and in living female (n=50) = 22±2 mm was also statistically insignificant (p value <0.05).

The level of significance while comparing the mean distance in mm from midpoint of condylar head to summit of tragus between living and cadaveric male and female was followed: the mean distance in mm from midpoint of condylar head to summit of tragus in living male (n=50) was 14±2 mm and in cadaveric male (n=36) = 12±2 mm was statistically insignificant (p value <0.05). Likewise, the mean distance in mm from midpoint of condylar head to summit of tragus in living female (n=50) was 13±2 mm and in cadaveric female (n=14) = 11±2 mm was also statistically insignificant (p value <0.05). The level of significance while comparing the mean length of distal phalanx between living and cadaveric male and female was followed. The mean length of distal phalanx in living male (n=50) was 24±2 mm and in cadaveric male (n=36) was 22±2 mm was statistically insignificant (p value <0.05). Likewise, the mean length of distal phalanx in living female (n=50) was 22±2 mm and in cadaveric female (n=14) was 20±2 mm was also statistically insignificant (p value <0.05).

The level of significance while comparing the mean distance in mm from midpoint of condylar head to summit of tragus and the mean length of distal phalanx between overall living subjects and cadaveric bodies was followed: the mean distance in mm from midpoint of condylar head to summit of tragus in all cadavers (n=50) was 11.5±2.5 mm and in all living subjects (n=100) was 13.5±2.5 mm was statistically insignificant (p value <0.05). Likewise, the mean length of distal phalanx of fore finger in all cadavers (n=50) was 21±3 mm and in all living subjects (n=100) was 23±3 mm was also statistically insignificant (p value <0.05).

The mean distance in mm from midpoint of condylar head to summit of tragus in all living subjects and cadavers (n=150) was 12.5±3.5 mm and the mean length of distal phalanx of fore finger in all living subjects and cadavers (n=150) was 22±4 mm.

From all these observations, the present study proved that the midpoint of mandibular condyle from summit of tragus was found to be 12.5±3.5 mm just below the inferior border of zygomatic arch. The mean length of

distal phalanx of fore finger was found to be 22± 4 mm. It means mandibular condyle can be palpated at half length of distal phalanx of fore finger just below the inferior border of zygomatic arch.

There was no previous study of this kind in the world thus comparison of present study result with previous ones could not be done. The outcome of this study is very important for anatomist as it is establishing new easy method of surface marking of TMJ. This study will be highly useful for oral and maxillofacial surgeon during arthroscopic surgery and while surgically operating the temporo-mandibular joint by open method.

CONCLUSION

The midpoint of mandibular condyle from summit of tragus was found to be 12.5±3.5 mm just below the inferior border of zygomatic arch. The mean length of distal phalanx of fore finger was found to be 22± 4 mm. It means mandibular condyle can be palpated at half length of distal phalanx of fore finger just below the inferior border of zygomatic arch.

Conflict of interest: None

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Estimation of ventricles size of human brain by Magnetic Resonance Imaging in Nepalese Population: A retrospective study

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ABSTRACT

Background and Objective: Magnetic resonance imaging (MRI) provides image acquisition of three-dimensional data and measurement in any chosen imaging plane. Objective of this study is to assess the size of ventricles of the brain of normal Nepalese people and establish the range of size of the ventricular system and compute the ventricular dimensions among different age and gender. **Materials and methods:** This is a cross-sectional retrospective study done at Gandaki Medical College, Pokhara. A total of 106 MRI scan data of healthy individuals were collected over a period of seven months between March to September 2019. Patients ranged between eight and eighty years of age with 58 males and 48 females. Measurements of the mean of bifrontal diameter (BFD), bihemispheric diameter (BHD), third ventricle transverse dimension (TVTD), fourth ventricle antero-posterior dimension (FVAP), fourth ventricle width (FVW), and frontal horn ratio (FHR) were done. **Result:** The mean of BFD, BHD, TVTD, FVAP, FVW, and FHR were found to be 3.05 ± 0.10 cm, 10.11 ± 0.40 cm, 0.43 ± 0.11 cm, 0.90 ± 0.11 cm, 1.22 ± 0.12 cm, and 0.30 ± 0.01 cm, respectively. The mean width of fourth ventricle in males and females was observed to be 1.23 ± 0.12 cm and 1.19 ± 0.11 cm respectively. There was a significant correlation of TVTD, FVAP, FHR and BFD with age with Pearson correlation coefficient 0.393 (P value <0.01), 0.259 (P value <0.01), 0.34 (P value <0.01), and 0.219 (P value <0.05) respectively. However, BHD and FVW have no correlation with age. **Conclusion:** Third Ventricle Traverse Dimension, FVP, FVW and FHR show almost similar or slight difference in measurement according to gender. However, BFD shows larger difference in measurement according to gender. Similarly there is no such significant difference according to age in measurement of BFD, BHD, FVAP, FVW and FHR, while TVTD measurement shows slight increased measurement according to age.

Keywords: Brain, Brain ventricles, Magnetic resonance imaging, Ventricular size

INTRODUCTION

MRI is a non-invasive medical test that helps to diagnose and treat medical conditions. MRI scanners use strong magnetic fields, magnetic field gradients, and radio waves to generate images of the organs in the body. The brain ventricular system is a cavity filled with cerebrospinal fluid, the fluid which protects the brain and spinal cord from trauma, supplies nutrients to nervous system tissue, and removes waste products from cerebral metabolism. Understanding the normal and abnormal anatomy of the ventricular system of the brain is helpful for the clinician, neurosurgeon, and radiologist in day-to-day practices. The abnormal anatomy of the lateral ventricle may be great academic interest regarding cerebrospinal fluid circulation and also important for surgical intervention.[1] Morphometric analysis of cerebral

ventricular system is important for evaluating changes due to growth, ageing, intrinsic, ad extrinsic pathology.[2] The advantages given by the modern imaging methods and minimally invasive technique motivated many of the researchers to study the anatomy of the ventricular system and measure the distances in individuals.[3]

The cerebral ventricles are of a relatively fixed shape and size and in the absence of diseases, do not vary significantly, and systematic study is desirable to establish this point.[4] to understand these changes the knowledge of normal morphometry and size of normal ventricular system of brain is important.[5] The advantages given by the modern imaging methods and minimally invasive technique motivated many of the researchers to study the anatomy of ventricular system and measure the distances in individuals.[3] Morphometric analysis of brain structure such as volume, shape, and size of ventricular system especially lateral ventricle, recently has become the main focus of interest in studies of some neuropsychiatric diseases like Alzheimer's.[6] Further, the knowledge of measurement of the ventricular system of the brain by MRI is of use in the diagnosis of some diseases like hydrocephalus, schizophrenia and surgical intervention like endoscopic neurosurgery.[7]

Very few studies have been done to study the ventricular size using MRI technique as well as there is limited study in the Nepalese population. In this study, an attempt has been made to establish the ranges of normal values for the measurement of the ventricular system by use of MRI. The objective of the study was to establish the size range of ventricular system in normal brain MRI and compute the FHR of healthy Nepalese people. After the study, we will be able to compare the ventricular size and FHR of the Nepalese population with other Asians and other ethnicities.

MATERIALS AND METHODS

The Retrospective cross-sectional study was conducted at Department of Radio diagnosis and imaging, Gandaki Medical College Teaching Hospital & Research Centre (GMCTHRC), Pokhara metropolitan city, Kaski with the approval of the Ethical committee of Institutional Review Board of GMC.

A total of 106 healthy individuals patient MRI scan were collected from MRI workstation which was performed between March to September 2019, aged between eight to 80 years of which 58 were males and 48 were females. Inclusion criteria for this study were MRI scan of Brain and patients with no known pathologies or abnormalities of brain. Exclusion criteria for the study were evidence of abnormality i.e. space occupying lesions, cerebral hemorrhage, brain tumor, head injuries, previous intracranial surgeries, metal implants, image with significant noise, and uncooperative patients. Only Nepalese patients were included, any foreigner or non-Nepalese patients were also excluded from the study.

The total number of patients was divided into four groups, all patients less than 20 years of age were kept in the first group, age between 20 to 39 years were kept in the second group, 40 to 59 years in the third group, and age of 60 and above were kept in the fourth group. MRI was conducted using Seimens Magnetom Essenza, 1.5 tesla machine and measurement of the ventricles were done in and T2 axial spin-echo (CSF differentiation is best seen in T2 images) sequence having slice thickness of 5mm, Distance factor= 40% of slice thickness, repetition time (TR) 3600 (milliseconds) and Echo time (TE) 106 ms, Bandwidth= 190.0, Field of View (FOV) = 194X230, FOV phase= 84.4%, Concancenations = 1. The patient was placed in MRI table and centering was done in correlation with brain coil. All the measurements were taken under the standard protocol made by the radiological society with the help of an inbuilt measuring tool from viewing option of MRI console computer. Data were collected in Performa and was kept in Microsoft excel 2016.

Measurement of the lateral ventricle was done at the level of the bilateral caudate nucleus, third ventricle below the level of the interventricular foramen of Monro and fourth ventricle at the level of pontomedullary junction.

Measurement of BFD: (Figure 1)

The maximum bifrontal diameter is the transverse distance defined by a line connecting two anterior corners of the frontal horns 'a' and 'b'.

Measurement of bihemispheric diameter: (Figure 2)

The brain width or bihemispheric diameter is the distance measured along the line of bifrontal diameter to the brain edge 'c' and 'd'.

Calculation of FHR:

Frontal horn ratio is the greatest distance between the tips of the frontal horn divided by the first transverse diameter of the brain along the same level. Frontal horn ratio = BFD divided by BHD

Fig. 1: MRI Scan of brain showing TVD of frontal horn (BFD) (a-b)

Fig. 2: MRI Scan of brain showing BHD (c-d) along the BFD (a-b)

Measurement of third ventricle transverse dimension: (Figure 3)

The transverse dimension of third ventricle is the greatest distance between lateral margins of the third ventricle from 'e' to 'f'.

Measurement of anterior-posterior dimension and width of the fourth ventricle: (Figure 4)

The greatest anterior- posterior dimension of fourth ventricle is the distance from anterior aspect of vermis to the posterior aspect of pons, 'a' line connecting 'I' and 'j' and the width is the maximum transverse distance along the coronal plane connecting 'g' and 'h'.

Statistical analysis:

Statistical analysis was carried out with the help of SPSS version 25. The mean, standard deviation, and correlation between size of ventricles of brain among different age groups and gender were expressed in tables and figures. Pearson correlation test was conducted to compare two variables.

Fig. 3: MRI Scan of brain showing greatest diameter of third ventricle (e-f)

Fig. 4: MRI Scan of brain showing greatest ventricle diameter (i-j) and greatest transverse diameter (g-h) along coronal plane of axial section.

RESULT

The data was collected from 106 normal individuals, 58 (54.7%) males and 48 (45.3%) females with age range from eight years old to 81 years old. Data were presented as mean and standard deviation of all variables. Detailed results are shown in the table 1

Table 1: Sample distribution on the basis of age group

Age group	Frequency	Percent
less than 20	16	15.1
20-39	35	33.0
40-59	26	24.5
60 and above	29	27.4
Total	106	100.0

Table 2: Comparison of different parameters of ventricle size of Human brain according to gender

Sex		BFD	BHD	TVTD	FVAP	FVW	FHR
Male	Mean (±SD)	3.123±0.204	10.241±0.398	0.443±0.114	0.907±0.119	1.236±0.127	0.305±0.017
	N	58	58	58	58	58	58
Female	Mean (±SD)	2.966±0.156	9.963±0.349	0.420±0.108	0.895±0.100	1.199±0.113	0.297±0.013
	N	48	48	48	48	48	48
Total	Mean (±SD)	3.052±0.199	10.115±0.400	0.433±0.111	0.902±0.111	1.220±0.121	0.301±0.016
	N	106	106	106	106	106	106

Legends: Bifrontal diameter (BFD), bihemispheric diameter (BHD), third ventricle transverse diameter (TVTD), fourth ventricle antero-posterior diameter (FVAP), fourth ventricle width (FVW) and frontal horn ratio (FHR)

Values are measured in centimetre (cm)

Table 2 explains the mean and standard deviation of the BFD, BHD, TVTD, FVAP, FVW, and FHR based on gender.

Mean BFD was observed to be larger in male (3.12 ± 0.20) cm than compared to (2.96 ± 0.15) cm in female. Similarly, mean BHD was observed to be (10.24 ± 0.39) cm in male patients and (9.96 ± 0.34) cm in female patients. Mean of TVTD was (0.44 ± 0.11) cm for male and (0.42 ± 0.10) cm for female. The mean of FVAP of male and female was observed to be (0.90 ± 0.11) cm and (0.89 ± 0.10) cm respectively. Similarly, the mean of FVW of male and female was observed to be (1.23 ± 0.12) cm and (1.19 ± 0.11) cm respectively.

The mean FHR was found to be (0.30 ± 0.01) cm in male and (0.29 ± 0.01) cm in female.

Table 3: Comparison of different parameters of ventricle size of human brain according to age group

Age Group		BFD	BHD	TVTD	FVAP	FVW	FHR
less than 20	Mean (±SD)	3.058±0.111	10.253±0.348	0.391±0.066	0.847±0.096	1.222±0.106	0.298±0.011
	N	16	16	16	16	16	16
20-39	Mean (±SD)	2.993±0.183	10.100±0.414	0.400±0.064	0.893±0.076	1.215±0.108	0.296±0.013
	N	35	35	35	35	35	35
0-59	Mean (±SD)	3.016±0.211	10.071±0.420	0.428±0.084	0.903±0.138	1.182±0.134	0.299±0.013
	N	26	26	26	26	26	26
60 and above	Mean (±SD)	3.153±0.213	10.096±0.395	0.500±0.161	0.941±0.117	1.257±0.126	0.312±0.019
	N	29	29	29	29	29	29
Total	Mean (±SD)	3.052±0.199	10.115±0.400	0.433±0.111	0.902±0.111	1.220±0.121	0.301±0.016
	N	106	106	106	106	106	106

Legends: bifrontal diameter (BFD), bihemispheric diameter (BHD), third ventricle transverse diameter (TVTD), fourth ventricle antero-posterior diameter (FVAP), fourth ventricle width (FVW) and frontal horn ratio (FHR)

Values are measured in cm.

Table 3 explains the mean of BFD, BHD, TVTD, FVAP, FVW, and FHR was found to be 3.05 ± 0.10 cm, 10.11 ± 0.40 cm, 0.43 ± 0.11 cm, 0.90 ± 0.11 cm, 1.22 ± 0.12 cm and 0.30 ± 0.01 cm respectively.

As seen in the table, the measured BFD was almost equal in all age groups with slight variation and also similar results were observed in case of BHD. It can be summarized that the mean values in case of TVTD were slightly increasing according to increase in age. The FVAP and FVW were observed to be high at sixth decades of life.

Table 4: Correlation of different parameters of ventricle size of human brain with age

		BFD	BHD	TVTD	FVAP	FVW	FHR
Over all Age	Pearson Correlation	0.219*	-0.102*	0.393**	0.259**	0.122*	0.340**
	P value	0.024	0.300	0.000	0.007	0.212	0.000

*. Correlation is significant at the 0.05 level
**. Correlation is significant at the 0.01 level

Legends: bifrontal diameter (BFD), bihemispheric diameter (BHD), third ventricle transverse diameter (TVTD),   fourth ventricle antero-posterior diameter

(FVAP), fourth ventricle width (FVW) and frontal horn ratio (FHR)

Table 4 explains that there was significant correlation of TVTD, FVAP, and FHR with overall age at level of significance 0.01. There was significant correlation of BFD with age at level of 0.05. BHD and FVW have no significant correlation with age.(P value > 0.05)

DISCUSSION

The objectives of this study were to establish range of size of ventricular system in normal brain MRI and compute the ventricular dimensions among different age and gender. The adult human brain has size of around 1130 cm[3] in women and 1260 cm[3] in men. Male brains are about 10% larger than female brain in volume and weighs 11 to 12% more than female.[8]

According to Hahn and Rimet et al.[9] the cerebroventricular index (FHR) seems to be a reliable indicator of ventricular size and emphasizes the study of normal ventricles. Another study done by Cala et al.[10] found ventriculo-internal cranial ratio (FHR) of (0.31 ± 0.08) in female and (0.33 ± 0.06) in male i.e. genders were not statistically significant, however our study, found the FHR at level of tips of frontal horn varied from minimum value of 0.29 to 0.31 from age of 8 to 80 years averaging (0.30 ± 0.16). The male result was found to be 0.30 ± 0.01 cm, which was almost equal while female result was found to be 0.29 ± 0.01 cm that was quite less than their study. Our study showed slightly different result than that of D'Souza and Natekar et al.[11] in terms of third ventricle transverse diameter (TVTD) who studied on morphometry of ventricular system for the large sample size and found the TVTD in male and female were (0.45 ± 0.29 cm) and (0.39 ± 0.17 cm) respectively, but in our current study, male width was found to be (0.44 ± 0.11 cm) comparable to their finding, whereas female width was 0.42 ± 0.10 cm which was higher than they observed. Singh et al.[5] found that the TVTD was slightly lower in male (0.34 ± 0.10 cm) than in female (0.33± 0.09 cm), which is higher than our study, 0.44 ± 0.11 cm in male and 0.42 ± 0.10 cm in female. Satapara et al.[7] suggests that TVTD increases with increase in age which is statistically significant, their study shows the value for age group less than 20 to be (0.34± 0.05) and for the maximum of age group 60 and above (0.92± 0.42) giving a mean of (0.52 ± 0.24).Similarly, our study shows that TVTD increases slightly with age. Our study shows that there is statistically significant differences (P value <0.05), for age group less than 20 years be (0.39± 0.06) and for the age group 60 and above (0.50± 0.16) giving a mean of (0.43± 0.11).

Study by D'Souza et al.[11] found the FVAP to be 0.11 ± 0.002 cm in male and 0.11 ± 0.002 cm in female. In our study, mean FVAP in male and female was higher than the study done by D'Souza and Natekar et al.[11]. Fourth ventricle in male and female was similar to the study done by D'Souza and Natekar et al.[11] Also, it was concluded that FVW was observed to be larger than its antero- posterior diameter in both male and female as found in their study.

Duffner et al.[3] measured the FVW with a mean of 1.25 ± 0.17 cm which was comparable with present calculation of width with overall mean of 1.22 ± 0.12 cm.

CONCLUSION

We can conclude that TVTD, FVP, FVW and FHR shows almost similar or slight difference in measurement according to gender. However, BFD shows larger difference in measurement according to gender. Similarly there is no such significant difference according to age in measurement of BFD, BHD, FVAP, FVW and FHR. While TVTD measurement shows slight increased measurement according to age, there changes are also commented by other researcher with small sample and large sample size. However, in order to say exact range of ventricles size in Nepalese population we recommend measurement with large sample size as well for comparison in Nepalese population. There may be personal variation in measurement as it's done manually.

Limitations of the study

Because of the time limitation and experimental difficulties for age variation of ventricle size data were taken from different subjects with different ages instead from the single subject at different age structural changes of human brain with age is expected to be normal.

Recommendations

Further studies for measurement of size of ventricles with larger sample size are recommended for more accuracy in results. Volumetric measurement is recommended as it provides more information than linear measurement. Further studies are recommended to be done with more body characteristics for more and meaningful information.

Conflict of Interest:

None declared

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Mesio-distal crown width in permanent dentition amongst adolescent population of Province II of Nepal

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ABSTRACT

Background: The purpose of this study was to gather normative data on the mesio-distal crown dimensions amongst adolescent population of Province II, Nepal, to make an accurate diagnosis and treatment planning in orthodontics. It will also be useful in various clinical disciplines of dentistry including basic dental and anthropological research. **Materials and Methods:** Samples were selected Full form OPD of M B Kedia Dental College, Birgunj, Nepal. Total numbers of participants were 120, out of which 60 males and 60 females were selected after initial examination aged between 11 to 23 years. Subjects with all permanent teeth erupted (except second and third molar) without any history of previous orthodontic treatment and with no dental anomalies were included in this study. The alginate impressions were made by the well trained dental surgeon. Digital vernier calliper providing measurements to ± 0.01millimeter(mm) was used to measure the mesio-distal dimension of all teeth. **Results:** The mean, range and standard deviation were calculated for the size of the teeth. Independent t-test was used to compare between male and female population. The significance level was set at p value <= 0.05. The population of Province II, Nepal shows greater sexual dimorphism in mesio-distal crown dimension which was exhibited by the maxillary molars (0.88 mm) followed by mandibular molars (0.38 mm). Similarly in anterior tooth segment the maxillary canines (0.29 mm) followed by the mandibular canines (0.27 mm). **Conclusion:** The mean mesio-distal crown dimensions of the permanent dentition of males were larger than that of females for each type of tooth except maxillary central and lateral incisor.

Keywords: adolescent, mesio-distal width, sexual dimorphism

INTRODUCTION

Variation of tooth size is influenced by genetic and environmental factors. Many studies reported that variation of tooth size exists within the race and in subjects with different racial origin.[1] According to Proffit and Fields[2], tooth space analysis should be done accurately before any Orthodontic treatment. Tooth size discrepancy is defined as a disproportion among the sizes of individual teeth and amongst the jaws.[2] In order to achieve optimal occlusion maxillary to mandibular tooth width ratios must be proportionate in size.

Many authors have evaluated factors associated with differences in the tooth width. The relationship between malocclusion type and tooth size proportions has also been reported.[1, 3-7] Since differences in tooth size have been reported with ethnic groups,[3, 8-11] most of the practitioners now disagree to apply the tooth size reported from the other ethnic groups. Bishara et al.[11] conducted a study which includes the population of Egypt, Mexico, and the United States. They

noticed that there was a difference in the mesio-distal dimensions of teeth among the three populations. There was sexual dimorphism where males had larger teeth than females in all three groups of population. There were no statistical significant differences between the right and left sides for all the three study groups.

Till date, few studies on the mesio-distal dimensions of teeth of the Nepalese population are found in the literature. No such studies have been reported in the population of province II. This study was carried out to gather data from province II on the mesio-distal widths of the permanent teeth and to see the gender differences in tooth width. This will help Orthodontist to diagnose the case properly and finish the case with precise occlusion.

MATERIALS AND METHODS

This was a hospital based cross-sectional study conducted at M B Kedia Dental College, Birgunj, Nepal, within the duration of August 2018 to November 2018. The study was conducted after getting ethical clearance from institutional review board, M B Kedia Dental College Birgunj, Nepal. Sample size calculation was based on 80% power and significance level of 5% [12] and considering 0.70 as maximum tolerable error rate and based on standard deviation of 2.5. $N=[Z +(1-ß)]^2 X\ SD^2/L^2$,where, Z=Confidence interval (95%,CI=1.96), ß=probability of type II error= 0.16 , Standard Deviation= 2.5 ,L= tolerable error= 0.70 and N=Sample size. The sample size came to be 100. We included 120 samples in our study. Informed consent was obtained from parents or patients before recording data as applicable. The patients were examined in a clinical set up of dental OPD with illuminated light using mouth mirror, probe and tweezers. The inclusion criteria were 1) Participants must be a resident of province II 2) 11-23 years of age 3) All set of permanent teeth erupted (except second and third molars). The exclusion criteria were subjects with 1) Proximal or occlusal wear, inter-proximal caries or restorations 2) Crowding, spacing, cross bite, over retained deciduous tooth, missing permanent tooth, any dental anomalies 3) Cleft lip and palate or any other syndromes 4) Past history of orthodontic treatment. After obtaining the consent from patients meeting the inclusion criteria, alginate impressions of both maxillary and mandibular teeth were made by a trained dental surgeon. The impressions were poured with dental stone, Type 3 gypsum product. Total numbers of participants were 120; 60 males and 60 females. Digital vernier calliper [0-150 mm, 799A-6/150, Starrett tools (Suzhou) Co. Ltd., China] providing measurements to ± 0.01mm was used to measure the mesio-distal dimension of all teeth. The

teeth measured included the mandibular and maxillary permanent incisors, permanent canines, first premolars, second premolars and first molars. All measurements were made by a single investigator. Maximum 10 pairs of casts measured per day to avoid eye fatigue. All measurements were done directly on study models. Maximum mesio-distal width of each tooth was measured and recorded to 0.1mm. All measurements were taken perpendicular to the long axis of the tooth with the calliper beak entering the inter-proximal area from the buccal or occlusal side.[13] Repeated measurements were performed to minimize the possible errors. Intra-examiner reliability was predetermined at 0.2mm as mentioned by Bishara et al.[11] Bishara[11, 14] recommended that measurements that varied by 0.2mm or less to be averaged and re-measurement was done for measurements that varied more than 0.2 mm and the three measurements were averaged.

 Statistical analysis was performed with IBM SPSS Statistics Version 16 for Windows. Data were presented as mean, standard deviation (SD), frequency and percentage where appropriate. Independent t-test was used to compare between males and females. The significance level was set at p value <= 0.05.

RESULTS

The data on mesio-distal crown dimensions of the permanent maxillary and mandibular teeth of the Nepalese population studied are summarized in table 1 and 2 respectively. The mean mesio-distal crown dimensions of the permanent dentition of males were larger than that of females for each type of tooth except maxillary central and lateral incisor. Comparison was done between the mean mesio-distal dimensions of right and left side of both maxillary and mandibular arches amongst females and males as shown in table 5 and 6. The differences between the mean mesio-distal dimension of individual tooth on the right and left sides were comparable.

Table 1: Mesio-distal width of individual tooth in females

Tooth	Min width (mm)	Max width (mm)	Mean+SD (mm)
Mandibular right first molar	10.00	10.99	10.57±0.24
Mandibular right second premolar	5.76	7.87	6.82±0.51
Mandibular right first pre-molar	5.31	7.71	6.91±0.48
Mandibular right canine	5.44	7.93	6.65±0.45
Mandibular right lateral incisor	4.63	6.82	5.87±0.42
Mandibular right central incisor	4.13	6.33	5.35±0.44
Mandibular left central incisor	4.12	6.44	5.33±0.42

Mandibular left lateral incisor	4.82	6.90	5.87±0.41
Mandibular left canine	5.50	7.96	6.62±0.47
Mandibular left first premolar	5.31	7.70	6.89±0.48
Mandibular left second premolar	5.73	8.03	6.86±0.52
Mandibular left first molar	10.11	11.12	10.59±0.24
Maxillary right first molar	9.15	9.97	9.59±0.22
Maxillary right second premolar	5.36	7.52	6.45±0.47
Maxillary right first premolar	5.22	7.77	6.84±0.48
Maxillary right canine	6.48	8.43	7.57±0.42
Maxillary right lateral incisor	6.08	7.23	6.62±0.27
Maxillary right central incisor	8.09	8.97	8.51±0.23
Maxillary left central incisor	8.13	9.09	8.53±0.25
Maxillary left lateral incisor	6.12	7.30	6.64±0.27
Maxillary left canine	6.65	8.40	7.59±0.40
Maxillary left first premolar	5.21	7.77	6.83±0.48
Maxillary left second premolar	5.45	7.52	6.44±0.45
Maxillary left first molar	9.17	10.23	9.64±0.26

Table 2: Mesio-distal width of individual tooth in males

Tooth	Min width (mm)	Max width (mm)	Mean+SD (mm)
Mandibular right first molar	9.60	12.15	10.94±0.61
Mandibular right second premolar	5.79	8.15	6.92±0.48
Mandibular right first premolar	5.89	8.31	7.03±0.46
Mandibular right canine	6.20	7.76	6.89±0.35
Mandibular right lateral incisor	4.97	6.70	6.04±0.36
Mandibular right central incisor	4.67	6.26	5.36±0.33
Mandibular left central incisor	4.66	6.26	5.37±0.33
Mandibular left lateral incisor	4.97	6.80	6.03±0.36
Mandibular left canine	6.20	7.77	6.92±0.33
Mandibular left first premolar	5.92	8.30	7.05±0.46
Mandibular left second premolar	5.79	8.05	6.88±0.46
Mandibular left first molar	9.79	12.03	10.97±0.60
Maxillary right first molar	9.92	11.14	10.49±0.31
Maxillary right second premolar	5.49	7.32	6.49±0.39
Maxillary right first premolar	5.82	7.85	6.98±0.38
Maxillary right canine	7.15	8.82	7.88±0.37
Maxillary right lateral incisor	5.91	7.14	6.52±0.33
Maxillary right central incisor	7.80	9.13	8.38±0.38
Maxillary left central incisor	7.50	9.16	8.37±0.38
Maxillary left lateral incisor	5.88	7.10	6.53±0.32
Maxillary left canine	7.16	8.82	7.86±0.35
Maxillary left first premolar	6.07	7.85	6.97±0.38
Maxillary left second premolar	5.29	7.89	6.48±0.43
Maxillary left first molar	9.97	11.09	10.49±0.29

Table 3: Comparison of tooth width of mandibular arch between female and male

Tooth	Gender	Mean±SD (mm)	S.E. Mean	P Value
Mandibular right first molar	F	10.57±0.24	0.031	P<0.000*
	M	10.94±0.61	0.079	
Mandibular right second premolar	F	6.82±0.51	0.066	P =0.281
	M	6.92±0.48	0.061	
Mandibular right first premolar	F	6.91±0.48	0.061	P =0.156
	M	7.03±0.46	0.060	
Mandibular right canine	F	6.65±0.45	0.058	P =0.002*
	M	6.89±0.35	0.044	
Mandibular right lateral incisor	F	5.87±0.42	0.054	P =0.017*
	M	6.04±0.35	0.045	
Mandibular right central incisor	F	5.35±0.44	0.056	P =0.894
	M	5.36±0.34	0.043	
Mandibular left central incisor	F	5.33±0.42	0.054	P =0.556
	M	5.37±0.33	0.042	
Mandibular left lateral incisor	F	5.87±0.41	0.052	P =0.032*
	M	6.03±0.36	0.047	
Mandibular left canine	F	6.62±0.47	0.060	P<0.000*
	M	6.92±0.33	0.042	
Mandibular left first premolar	F	6.89±0.48	0.062	P =0.084
	M	7.05±0.46	0.058	
Mandibular left second premolar	F	6.87±0.52	0.066	P =0.788
	M	6.88±0.46	0.059	
Mandibular left first molar	F	10.59±0.24	0.031	P<0.000*
	M	10.97±0.60	0.077	
F=female, M= male, *p <0.05 significant				

Table 4: Comparison of tooth width of maxillary arch between male and female

Tooth	Gender	Mean±SD (mm)	S.E. Mean	P Value
Maxillary right first molar	F	9.59±0.22	0.028	P<0.001
	M	10.49±0.31	0.039	
Maxillary right second premolar	F	6.45±0.47	0.061	P =0.660
	M	6.49±0.39	0.051	
Maxillary right first premolar	F	6.84±0.48	0.061	P =0.087
	M	6.98±0.38	0.048	
Maxillary right canine	F	7.57±0.42	0.054	P<0.000*
	M	7.88±0.37	0.048	
Maxillary right lateral incisor	F	6.62±0.27	0.035	P =0.071
	M	6.52±0.33	0.042	
Maxillary right central incisor	F	8.51±0.23	0.029	P =0.020*
	M	8.38±0.38	0.048	
Maxillary left central incisor	F	8.53±0.25	0.032	P =0.005*
	M	8.37±0.38	0.049	
Maxillary left lateral incisor	F	6.64±0.27	0.035	P=0.032*
	M	6.53±0.32	0.041	
Maxillary left canine	F	7.59±0.40	0.052	P <0.001*
	M	7.86±0.35	0.045	

Maxillary left first premolar	F	6.84±0.48	0.062	P =0.098
	M	6.97±0.38	0.049	
Maxillary left second premolar	F	6.44±0.45	0.057	P =0.624
	M	6.48±0.43	0.055	
Maxillary left first molar	F	9.64±0.26	0.033	P <0.001*
	M	10.49±0.29	0.038	

F=female, M= male, *p <0.05 significant

Table 5: Comparison of tooth width between right and left side of both arches in females

Arch	Tooth notation	Right (mm) Mean+SD (n=60)	Left(mm) Mean+SD (n=60)	p-value
Maxillary	First Molar	9.64±0.26	9.59±0.23	0.315
	Second Premolar	6.44±0.45	6.45±0.47	0.924
	First Premolar	6.84±0.48	6.84+0.48	0.937
	Canine	7.59±0.40	7.57±0.42	0.783
	Lateral Incisor	6.64±0.27	6.62±0.27	0.670
	Central Incisor	8.53±0.25	8.51±0.23	0.577
Mandibular	First Molar	10.59±0.24	10.57±0.24	0.634
	Second Premolar	6.86±0.52	6.82±0.51	0.698
	First Premolar	6.89±0.82	6.91±0.48	0.909
	Canine	6.62±0.0.47	6.65±0.45	0.720
	Lateral Incisor	5.87±0.41	5.87±0.42	0.942
	Central Incisor	5.33±0.43	5.35±0.44	0.769

*p <0.05 significant

Table 6: Comparison of tooth width between right and left side of both arches in males

Arch	Tooth notation	Right(mm) Mean+SD (n=60)	Left(mm) Mean+SD (n=60)	p-value
Maxillary	First Molar	10.49±0.29	10.49±0.31	0.990
	Second Premolar	6.48±0.43	6.49±0.39	0.960
	First Premolar	6.97±0.38	6.98±0.38	0.880
	Canine	7.86±0.35	7.88±0.37	0.837
	Lateral Incisor	6.53±0.32	6.52±0.33	0.953
	Central Incisor	8.37±0.38	8.38±0.38	0.897
Mandibular	First Molar	10.97±0.60	10.94±0.61	0.813
	Second Premolar	6.88±0.46	6.92±0.48	0.663
	First Premolar	7.05±0.46	7.03±0.46	0.842
	Canine	6.91±0.33	6.89±0.35	0.674
	Lateral Incisor	6.03±0.36	6.04±0.36	0.843
	Central Incisor	5.37±0.33	5.36±0.34	0.891

*p <0.05 significant

The largest sexual dimorphism in mesio-distal crown dimension was exhibited by the maxillary molars (0.88mm) followed by mandibular molars (0.38 mm), similarly in anterior tooth segment the maxillary canines (0.29 mm) followed by the mandibular canines (0.27 mm).

In both genders the maxillary first premolars exhibits greater width than the maxillary second premolars,

averaging 0.42 mm. In the mandibular arch, the lateral incisors were wider than the central incisors, by an average dimension of 0.67 mm in males and 0.53 mm in females. In the mandibular arch, the mean mesio-distal crown dimension of the central incisors was less than that of the lateral incisors for both the genders. In mandibular arch, the mean mesio-distal crown dimension of the first premolars was greater than that of the second premolars for males but almost same for females. In both males and females, the SD of tooth size measurement showed that variability differed between individual teeth.

DISCUSSION

In the present study, the mean mesio-distal width of the individual tooth showed larger tooth size in males as compared to females except for maxillary central and lateral incisor. This is similar to the study done in Nepalese population by Shrestha R,[15] in Bangladeshi population by Khan et al.[16] The study showed that teeth in males, with the exception of the mandibular 2nd premolar, mandibular 1st premolar ,mandibular central incisors, maxillary 2nd premolar, maxillary 1st premolar ,maxillary right lateral incisors, had significantly greater mesio-distal crown width than those in females p value <= 0.05 table 3 and 4 ,which is similar as reported by Kundi [17] in his study.

There were no significant differences in the mesio-distal dimensions as compared to the right and left side in both females and males as shown in table 5 and 6. This was similar to the studies done by Khan et al[16] in Bangladeshi population. This result is in contrary to the study done by Shrestha R[15] in Nepalese population, which showed significant differences in the size of maxillary first molars, maxillary second molars, mandibular central incisors and mandibular lateral incisors, which were larger in right side. These differences could be due to the variation in distribution of different ethnic groups in samples collected. Mahmoud NM[4], Lavelle et al.[1] and Lundstoum[18] also found definite differences between right and left side teeth measurements.

The results obtained in this study will provide useful clinical information to diagnose and frame treatment planning for orthodontic patients of province II of Nepal. This will help orthodontist to achieve good treatment results. As Nepal is a country with ethnic diversity this study can be extended to other provinces with larger sample size.

CONCLUSIONS

From the findings, it could be concluded that, the differences between the mean mesio-distal dimension

of individual tooth on the right and left sides were comparable. The mean mesio-distal crown dimensions of the permanent dentition of males were larger than that of females for each type of tooth except maxillary central and lateral incisor.

CONFLICT OF INTEREST

None declared

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Outcome of diagnosed cases of appendicitis and diagnostic accuracy of ultrasound of the appendix

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ABSTRACT

Background: Acute appendicitis is the most common condition requiring an emergency ultrasound scan as well as surgery. Several scoring systems for acute appendicitis has been suggested to improve diagnostic accuracy and decrease the negative appendectomy rate. In this study, we have evaluated the diagnostic performance of ultrasound on the diagnosis of acute appendicitis, other appendicular pathology, and correlate with surgical outcome. **Material and methods:** This retrospective study included 776 cases of ultrasound scan reports in which the appendix was visualized and not visualized or equivocal. Data were collected from October 2014 to April 2019 from different department and wards of Gandaki Medical College Teaching Hospital and Research Center (GMCTHRC). All the cases with diagnosed acute appendicitis, appendicular lump, and appendicular abscess were included and were followed for its surgical outcome. The surgical note or post-operative findings served as the reference standard for determining whether perforation was present or not. **Result:** There were 776 ultrasound scans for suspected appendicitis out of which 423 (54.5%) were diagnosed as appendicular pathology. Out of 423 diagnosed cases, 192 (45.4%) were males and 231 (54.6%) were females, with age ranging from 24 months to 87 years. Sonographic findings, in these positive subjects, suggested acute appendicitis, appendicular lump, abscess, and amount of free fluid in right iliac fossa (RIF) and pelvic cavity. Frank acute appendicitis was present in 378 (89.3%) cases, eight (1.9%) cases had an appendicular abscess, 23 (5.4%) had an appendicular lump and 14 (3.3%) had perforated appendicitis. **Conclusion:** We conclude that ultrasound is a good modality for visualization of appendicitis with other appendicular pathology. We could follow a structured report and identify pathology more specifically. Ultrasound is an easy and non-invasive test to investigate.

Keywords: Acute appendicitis, Right Iliac Fossa, Ultrasound, Ultrasonography.

INTRODUCTION

The vermiform appendix is considered to be a vestigial organ, its importance in surgery is only due to its tendency for inflammation resulting in the syndrome called acute appendicitis. Acute appendicitis is the most common cause of an acute abdomen in children and adults that requires emergency surgery. Several scoring systems for acute appendicitis has been suggested to improve diagnostic accuracy and decrease the negative appendectomy rate.[1] Appendectomy is the most frequently performed emergency abdominal operation. The lifetime rate of appendectomy is 12% for men and 25% for females.[2] Acute appendicitis is relatively rare in infants, becomes increasingly common in childhood and early adult life, reaching a peak incidence in the teens & the early twenties. Obstruction of appendix lumen is important, some form of luminal obstruction by

either a fecolith or stricture is found in the majority of cases. Obstruction of the orifice by the tumor (carcinoma of the caecum) is a cause of acute appendicitis, in middle age and elderly.[3]

Inflammation of the appendix is associated with obstruction in 50 to 80% of cases, mostly due to fecolith, less commonly due to tumor, gall stone, or worms. There is a continuous secretion of mucinous fluid in an obstructed viscous which leads to an increase in intraluminal pressure sufficient to cause a collapse of draining veins, leading to ischemic injury to the appendix. Ischemia favors bacterial proliferation with additional inflammatory edema and exudation further hampering the blood supply.

Ultrasonography (US) is a preferred modality for diagnosing pediatric appendicitis because of its high diagnostic accuracy, ready availability, noninvasive nature, and lack of ionizing radiation or contrast medium administration.[4] However, recent studies in both adults and children report poor US accuracy for differentiating perforated from non-perforated appendicitis, with published sensitivities ranging from 23% to 48% and specificities ranging from 93% to 100%.[4-5] Although these studies support our hypothesis that US sensitivity for perforated pediatric appendicitis is poor, prospective studies with sufficient patient numbers to allow calculation of reliable test performance measures have not been performed.

MATERIALS AND METHODS

Patient data:

Ethical approval for this study was taken from the Institutional review board of Gandaki Medical College teaching hospital and Research Centre (GMCTHRC). The inclusion criteria were US diagnosed cases of acute appendicitis, appendectomy, conservative management of appendicitis, appendicular lump, and abscess. Data were collected from October 2014 to April 2019 from the radiological department, department of anesthesia, surgical postoperative ward, surgical ward, and cabin of GMCTHRC. A total of 776 US reports were available where Appendix measurements were done, which were suspicious of appendicitis in the selected duration of time. Out of 776 US scans, in 353 cases, the appendix was measured to be less than five millimeter or was equivocal at the time of the scan and no surgery was performed, therefore, these were excluded from the study. Thus, the study included 423 diagnosed cases of appendicitis.

Ultrasound examinations:

Ultrasound examinations were performed with two

scanners (GE C5 and GE logic PE6) by certified radiologists. The graded compression technique was used. The diagnostic criteria for appendicitis were based on previous reports and as per international practice.[6]

The surgical note or post-operative finding served as the reference standard for determining appendicular perforation. When surgical note or post-operative findings suggests it as perforated appendicitis, histopathology report was not taken in consideration to look for perforated appendicitis as it was assumed that pathological specimen may not have included the perforated part of appendix or perforation occurring during handling of the appendix during surgery and after surgery.

Statistical analysis

All the data were managed and kept in Microsoft excel in 2019. According to the dependent and independent variable, data were analyzed using predictive analytics software (SPSS version 25 IBM Corporation Chicago, IL USA). Descriptive statistics were expressed as a number, frequency, percentage for categorical variables, and as mean, median, minimum-maximum for age variables.

Result

The study included 423 diagnosed cases of appendicitis. The median age of patients with appendicitis was 26 years ranging from 24 months to 87 years.

Table 1: Distribution of cases according to age group

	Frequency	Percentage
Below 10	45	10.6
10-19	137	32.4
20-29	117	27.7
30-39	43	10.2
40-49	29	6.9
50-59	24	5.7
60-69	12	2.8
70 and above	16	3.8
Total	423	100.0

In our study, the maximum number of patients were children and teens between 10 to 19 years of age i.e., 137 (32.4%), followed by young adults in the second and third

decade of life respectively. In contrast, there were few cases in the upper age group (Table 1). There were 192 (45.4%) male and 231 (54.6%) female patient in our study.(Table 2).

Table 2: Distribution of disease according to gender

Present or Absent	Condition and pathology									
	RIF tenderness		Free fluid		Abscess		Lump		perforation	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Present n (%)	129	129 (30.49)	138 (32.6)	47 (11.1)	51 (12.0)	3 (0.7)	5 (1.1)	12 (2.8)	3 (0.7)	5 (1.1)
Absent n (%)	63	63 (14.8)	93 (21.9)	145 (34.2)	180 (42.5)	189 (44.6)	226 (53.4)	180 (42.5)	189 (44.6)	226 (53.4)
Total n (%)	192	192 (45.3)	231 (54.6)	192 (45.3)	231 (54.6)	192 (45.3)	231 (54.6)	192 (45.3)	231 (54.6)	192 (45.3) 231 (54.6)

Note: RIF: Right iliac fossa

Similarly, we also accessed Sonographic RIF tenderness (graded compression technique), free fluid in RIF and pelvic cavity, abscess formation, lump formation and perforation of the appendix.

Table 2 shows the presentation of disease in different forms in 423 patients based on their gender. Sonographic tenderness in RIF was absent in 156 (36.9%), 63 male and 93 females, but present in 267 (63.1%), 129 males and 138 females. Similarly, in 325 (76.6%) patients, 145 male, and 180 female, did not have free fluid in RIF while 98 (23.3%) patients, 47 male, and 51 females, had free fluid in RIF. Likewise, there were eight (1.9%), three males and five females, with appendicular abscess and 23 (5.4%), 12 males and 11 females, had an appendicular lump. Further, out of 423 cases of appendicular pathology diagnosed on ultrasound, 378 patients (89.3%) were frank, non-perforated appendicitis, however, 14 cases were perforated appendicitis (3.3%) which were confirmed by postoperative findings.

DISCUSSION

Acute appendicitis is the most common surgical emergency. Its clinical profile determines whether there is a need for emergent operative intervention or not. Appendicitis is considered as a disease of adolescent age groups.[7,8] In present study maximum number of patients belong to the first and the second decade of life which is in accordance with the study done by Hale et al.[9] where median age was 23 years while ours was 26 years.

Generally, appendicitis is common in children and young adults male as stated by other studies.[1,2,5,7,9] In our study, there were more children and the young adult female affected by appendicitis consistent with the study done by Lewis et al.[2] where appendectomy was more in female compared to male. In contrast to our findings, there are many studies which suggests that it affects male predominately than female, however there is no such huge difference, just a slight increase in proportion of men at adolescence. This is most likely the influence of active duty population.[2,4,9,10,11]

Once the diagnosis of appendicitis is made, differentiation of perforated from nonperforated appendicitis becomes important. Emergency surgery is indicated for perforated appendicitis, whereas the initial therapy for nonperforated appendicitis may be nonsurgical because nonsurgical treatment has a lower complication rate.[10,12,13,15]

Ultrasound finding of the patients is important to confirm the diagnosis of acute appendicitis. Majority of the patients had probe tenderness on ultrasound evaluation, this is sensitive but not specific of appendicitis, which can be present in all clinical stages of appendicitis.

In our study probe tenderness on ultrasound and free fluid in abdomen/RIF was 63% and 23% respectively. Free fluid in the abdomen or RIF may be due to reactive inflammatory process or it may be secondary to pus discharge, accumulation and rarely due to fecal matter spillage. We found that five percent of our patients had lump formation and 1.9% had abscess formation which is almost similar to Linam et al.[3], which also has 5.4% cases of lump formation and 2% of cases of abscess formation.

In children with acute appendicitis, the risk of appendiceal perforation ranges from 23% to 73%. Lee et al.[14] reported that perforation occurred with greater incidence in children younger than five years and that abscess formation at presentation occurred more commonly in children older than 10 years and old age more than 60 years. In our study, we found that all perforated appendicitis cased were equally distributed between children, second, fifth, and eighth decade.

There is no statistical correlation between the ultrasound finding to differentiate patients with perforated and non-perforated appendicitis.[3] However, we suggest a clinical correlation with Alvarado Score is mandatory and increases the value if coupled with ultrasound examination.[15]

CONCLUSION

Ultrasound is useful in the diagnosis of appendicitis and should suffice as the modality of choice whenever the appendix is identified. However, the appendix is not always visualized in all patients with pain at RIF. As the majority of patients had on probe tenderness i.e. graded compression

it is mandatory to perform while accessing for an appendix. The decision to perform an appendectomy or to treat a patient conservatively should be made in association with clinical findings.

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Conflict of Interest:
None declared

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Bacteriological profile and antimicrobial susceptibility patterns of wound infections among adult patients attending Gandaki Medical College Teaching Hospital, Nepal

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ABSTRACT

Introduction: Wound infections are significant group of infections in the hospitals worldwide. The wide spread uses of antimicrobial agents lead to emergence of resistant pathogens contributing to increased morbidity and mortality. Accurate and prompt antimicrobial therapy is required to reduce the complications. This study was aimed to investigate pyogenic bacterial pathogens and their susceptibility patterns. **Methods:** A cross sectional study was carried out at Gandaki Medical College Teaching Hospital from July to December 2018. Wound specimens obtained from adult patients were inoculated onto appropriate media and pathogens were identified using standard microbiological methods. Antimicrobial susceptibility patterns were determined by Kirby-Bauer disc diffusion method following the guidelines of Clinical and Laboratory Standard Institute (CLSI). **Results:** A total of 264 specimens were included in the study of which 167 (63.3%) were positive for bacterial growth. Of these, polymicrobial growth was observed in two specimens. Gram positive bacteria (119, 70.4%) were the leading cause of infections, *Staphylococcus aureus* (102, 85.7%) being the most dominant. Among the Gram negative pathogens (50, 29.6%), *Escherichia coli* (31,62%) was found to be the predominant followed by *Pseudomonas aeurogenosa* (10, 20%). Overall, the isolates were resistance to Ampicillin (90.7%), Amoxycillin (64.9%), Cloxacillin (68%), Ofloxacin (61.5%) and Cotrimoxazole (55.6%). Lesser rates of resistance were observed to Doxycycline, Erythromycin, Amikacin, Gentamicin and Imipenem. **Conclusion:** This study revealed the most common pathogens causing pyogenic wound infections in our setting. Again, these pathogens are resistance to commonly used antibiotics. Therefore, this study could be helpful to develop proper guidelines of antibiotics to be used for prophylactic and empiric treatment.

Key words: Wound Infection, Bacterial Pathogens, Antimicrobial Resistance, MDR

INTRODUCTION

Any abrasion or breach in the skin surface provides an open door for bacterial entry. Wound provides moist and nutritive enriched environment for colonization and proliferation of bacteria to establish an infection.[1] Entry of bacterial pathogens and their lodgment provoke host immune system, bringing defensive immune cells into the area and eventually formation of pus. Therefore, infected wounds are characterized by inflammation and pus formation with bacterial burden.[2] Some of the common bacterial pathogens causing pyogenic wound infection include *S. aureus, S. pyogens, Enterococcus* spp., *E. coli, Klebsiella* spp., *Pseudomonas aeurogenosa, Proteus* spp. and *Acinetobacter* spp.[3,4] However, the epidemiological and antimicrobial susceptibility pattern of these pathogens vary from one country to the other

and also in different geographical areas within the same country.[5] Therefore, investigation of bacterial pathogens associated with wound infection and their susceptibility pattern should be carried out in each setting to establish empirical therapy for pyogenic infections.

In developing countries like Nepal, wound infections, although preventable and curable remained one of the major public health problems. Moreover, emergence of multidrug resistance pathogens pose extra burden on management of pyogenic infections.[4] The crisis of antimicrobial resistance in developing countries has been attributed to misuse of antibiotic practices such as, over use, under use, and inappropriate use. Important drug resistant pyogenic infections causing pathogens in Nepal include Methicillin and Vancomycin resistance *S. aureus,* Vancomycin resistance *Enterococcus* and Extended-spectrum beta-lactamase (ESBL) producing Gram negative bacilli such as *E. coli, Klebsiella* spp. and *Pseudomonas aeurogenosa*.[6] Therefore, knowledge of causative agents and their resistance pattern would be helpful for management of pyogenic infections in each setting.

Although different studies, including in Kathmandu, had been conducted to access the bacterial profiles of pyogenic infections,[4,7,8] those data might not be consistent enough to describe the current trend of our region. Therefore, this study aimed to characterize the bacterial etiological agents causing pyogenic wound infections and to determine the antimicrobial susceptibility patterns of those isolates to commonly used antimicrobial agents.

MATERIALS AND METHODS

Study design and population: A cross sectional study was carried out from July to December, 2018 at Clinical Laboratory of Gandaki Medical College Teaching Hospital, Pokhara Nepal. All patients more than 15 years of age fulfilling the criteria of wound infection,[2] who visited the hospital during the study period were enrolled in this study. Patients undergoing antimicrobial therapy were excluded. Ethical approval for the study was obtained from Gandaki Medical College Institution Review Committee.

Sample collection, processing and culture methods: All clinical samples such as pus, pus aspirates and wound swabs collected aseptically were processed immediately in the laboratory. The specimens were inoculated onto MacConkeys agar, Blood agar and Chocolate agar (HiMedia Laboratories, India) plates. Inoculated MacConkeys and Blood agar plates were incubated in aerobic condition while Chocolate agar plates were incubated in 5-10% CO_2 atmosphere environment at 37^0C for 24-48 hours.

Bacterial isolates were identified using standard protocols of the WHO.[9] Antimicrobial susceptibility testing (AST) was carried out by Kirby-Bauer disc diffusion method on Mueller Hinton agar or Blood agar (HiMedia, India) as recommended by CLSI guideline.[10]AST was performed against antibiotics (HiMedia, India), Ampicillin (10 µg), Amoxycillin (10 µg), Cloxacillin (5 µg), Cotrimoxazole (25 µg), Ofloxacin (5 µg), Doxycycline (10 µg), Ceftriaxone (30 µg), Erythromycin (15 µg), Amikacin (30 µg), Gentamicin (10 µg) and Imipenem (10 µg). The zone of inhibition was measured and the result was interpreted according to the guideline.[10] *S. aureus* ATCC-25923 strain was used as reference organism for AST.

Determination of multidrug resistance and special resistance: Any bacterial isolate that showed resistant to at least one agent in three groups of antimicrobial drugs tested were considered as multidrug resistant.[11] To determine the prevalence of Methicillin resistance S. aureus (MRSA), phenotypic test was performed using Cefoxitin (30µg) disc as recommended by CLSI guideline.[10] Data Analysis: Patient's details and all the data related with isolation and characterization of bacterial isolates were also recorded and analyzed using Excel data analysis tools, Microsoft Excel Spreadsheet 2007.

RESULTS

A total number of 264 patients (142 male and 122 female) with wound infection were included in this study. The age range was 16 to 81 years with majority of patients (68.6%) of age group 16-40 years. Of 264 specimens included in the study, 167 (63.3%) were positive for bacterial growth; 89 samples (53.3 %) were from male and 78 samples (46.7 %) were from female patients. Polymicrobial growth was observed with two specimens. A total of 169 bacterial pathogens were recovered with predominance of Gram-positive bacteria (119, 70.4%). *Staphylococcus aureus* (102, 60.4%) was the most frequent isolate followed by E. coli (31,18.3%), Coagulase negative *Staphylococcus* (CoNS) (10, 5.9%) and *Pseudomonas aeurogenosa* (10, 5.9%), respectively (Table 1).

The antimicrobial susceptibility patterns of the pathogens are presented in Table 2 and 3. The leading pathogen *S. aureus* were highly resistant to Ampicillin (95.1%), Cloxacillin (65.7%), Ofloxacin (64.7%) and Cotrimoxazole (56.9%). The second predominant pathogen, *E. coli* showed 71%, 64.5%, 58.1%, and 51.9% resistance to Amoxycillin, Cloxacillin, Ofloxacin and Cotrimoxazole respectively. Similarly, isolates of CoNS, *E. coli* and *Pseudomonas aeruginosa* also showed higher rate of resistance to those antibiotics (Table. 2). However, the bacterial

pathogens were highly susceptible to Doxycycline (68.6%), Erythromycin (73.3%), Amikacin (73.4%), Gentamicin (79.9%) and Imipenem (90.0%) (Table.3).

In this study, we demonstrate high rate of MDR pathogens associated with pyogenic infections. Among Gram positive pathogens, 60% CoNs and 56.9% *S. aureus* were MDR. Of total 102 *S. aureus* isolates, 31(30.4%) were Methicillin resistant (MRSA). Similarly, *E. coli* (58.1%) followed by *P. aeurogenosa* (50.0%) constituted Gram negative pathogens with highest MDR strains. The additional finding, we observed was that the least common isolates like *E. fecalis, Acinetobacter* spp*., P. mirabilis, and Enterobacter* spp. were also resistant to commonly prescribed antibiotics (Table 2).

Table 1. Bacterial Isolates associated with wound infections in this study.

Bacterial Isolates	Frequency	Percentage (%)
Gram Positive isolates (n=119)		
S. aureus	102	60.4
CoNS	10	5.9
S. pyogenes	05	3.0
E. faecalis	02	1.2
Gram Negative isolates (n=50)		
E. coli	31	18.3
P. aeurogenosa	10	5.9
Klebsiella spp.	4	2.3
Acinetobacter spp.	2	1.2
Proteus mirabilis	2	1.2
Enterobacter spp.	1	0.6
Total	169	100.0

Table 3. Overall susceptibility pattern of bacterial isolates to various antimicrobial agents in this study.

Name of drug	Susceptible	Resistant
Ampicillin	11 (9.3%)	108 (90.7%)
Amoxycillin	13 (35.1%)	37 (64.9%)
Cloxacillin	54 (32.0%)	115 (68.0%)
Cotrimoxazole	75 (44.4%)	94 (55.6%)
Ofloxacin	65 (38.5%)	104 (61.5%
Doxycycline	116 (68.6%)	53 (31.4%)
Amikacin	124 (73.4%)	45 (26.6%)
Gentamicin	135 (79.9%)	34 (20.1%)
Ceftriaxone	106 (62.7%)	63 (37.3%)
Erythromycin	87 (73.3%)	32 (26.9%)
Imipenem	45 (90.0%)	05 (10%)

DISCUSSION

Among 264 specimens included in this study, 167 (63.3%) were positive for bacterial growth. The isolation rate of the pathogens was higher than previous study carried out by Acharya et. al. (50.7%)[12] but comparable with the previous studies of Rijal et al. (64.9%)[4] and Rai et al, (59.0%).[7] However, it was lower than the study done in Ethiopia (83.9%).[3] The observed variations might be due to the quality of specimens and prompt specimen processing facility in the laboratories.

Our study found *S. aureus* (60.4%) to be the predominant organism followed by *E. coli* (18.3%). This is in agreement with many previous studies on wound infection in different parts of the world including Nepal.[3,4,12,13] The high prevalence of *S. aureus* and *E. coli* could be attributed to acquisition from the patients' own endogenous flora.

Table 2. Antibiotic susceptibility pattern of bacterial isolates in this study.

Organisms Isolated	Total Isolates	*Ampicillin/ ** Amoxycillin S	R	Cloxacillin S	R	Ofloxacin S	R	Doxycycline S	R	Amikacin S	R	Gentamicin S	R	Ceftriaxone S	R	* Erythromycin/ **Imipenem S	R	Cotrimoxazole S	R
Gram Positive Isolates																			
S. aureus	102	4.9%	95.1%	34.3%	65.7%	35.3%	64.7%	64.7%	35.3%	73.5%	26.5%	79.4%	20.6%	60.8%	39.2%	73.5%	26.5%	43.1%	56.9%
CoNS	10	20%	80%	30%	70%	40%	60%	80%	20%	70%	30%	70%	30%	50%	50%	60%	40%	40%	60%
S. pyogenes	05	80%	20%	20%	80%	40%	60%	80%	20%	60%	40%	80%	20%	60%	40%	80%	20%	40%	60%
E. faecalis	02	-	100%	-	100%	50%	50%	100%	-	100%	-	100%	-	50%	50%	100%	-	50%	50%
Gram Negative Isolates																			
E. coli	31	29%	71%	35.5%	64.5%	41.9%	58.1%	67.8%	32.3%	77.4%	22.6%	80.6%	19.4%	67.7%	32.3%	90.3%	9.7%	48.1%	51.9%
P. aeurogenosa	10	30%	70%	30%	70%	30%	70%	70%	30%	60%	40%	80%	20%	70%	30%	80%	20%	50%	50%
Klebsiella spp.	4	25%	75%	25%	75%	75%	25%	75%	25%	50%	50%	75%	25%	75%	25%	100%	-	50%	50%
Acinetobacter spp.	2	-	100%	-	100%	-	100%	100%	-	100%	-	100%	-	50%	50%	100%	-	-	100%
Proteus mirabilis	2	-	100%	-	100%	100%	-	100%	-	100%	-	100%	-	100%	-	100%	-	50%	50%
Enterobacter spp.	1	-	100%	-	100%	100%	-	100%	-	100%	-	100%	-	100%	-	100%	-	100%	-
Total	169	*AST performed to Gram positive isolates only. ** AST Performed to Gram negative isolates only.																	

In our study the predominant bacterial isolates showed high rate of resistance to drugs such as Amoxycillin, Cloxacillin, Ofloxacin and Cotrimoxazole, which are commonly used for treatment of pyogenic infections. The prevalence of MRSA was found to be 30.4%. Moreover, the study also observed a high rate of emergence of multidrug resistance among the isolates associated in pyogenic infection. Although the prevalence of MRSA and other drug resistance pathogens is higher than that of isolates from developed countries,[14,15] it is comparable to the previous studies performed in Nepal,[4,12] and other developing countries like Ethiopia.[3,13] Our findings revealed the existence of high rate of drug resistance pathogens in our setting which could be due to massive use of antimicrobials as a prophylactic purpose in surgical cases, longer duration and irrational use of antimicrobials in hospitals and use of antimicrobials without prescription in our country.[6,16] Importantly, drug resistance pathogens in pyogenic wound infection may pose a risk of spread within hospital as well as community emphasizing the rational use of antibiotics.

CONCLUSION

The study revealed high frequency of drug resistance among isolates from pyogenic wound infections. There was an alarmingly high rate of resistance to the antibiotics commonly used for treatment of pyogenic infections. Continuous monitoring and surveillance are required to guide most appropriate therapy for wound infections and to prevent the emergence of drug resistance pathogens. This study could be helpful to formulate the proper guideline of antibiotics to be used for empiric treatment of pyogenic infection in our setting.

LIMITATIONS: Our study is based on characterization and evaluation of drug resistance among the isolates from wound infection. The risk factors associated with development of pyogenic infection and the development of drug resistance among the isolates were not evaluated. Moreover, anaerobic pathogens that could cause wound infections were not explored.

CONFLICTS OF INTEREST: None declared

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Prescription patterns of Antibiotics in Acute tonsillitis: Hospital-based study

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ABSTRACT

Introduction: Acute tonsillitis is caused by either viruses or bacteria and is characterized by a sore throat and painful swallowing. About 49% of cases are thought to be caused by Group A beta-hemolytic streptococci. Penicillin should be given empirically after the diagnosis of acute tonsillitis. This study was carried out to find out the prescription pattern of antibiotics among the admitted cases of acute tonsillitis. **Materials and Methods:** This study was conducted in the Department of Otorhinolaryngology and Head and Neck Surgery, Gandaki Medical College Teaching Hospital and Research Centre (GMCTH), Pokhara. All the patients who were admitted for acute tonsillitis in the ENT ward were enrolled in this study. Age, gender, the average number of drugs per prescription, and the most commonly prescribed drug was recorded. **Results:** There were 320 patients with acute tonsillitis and the mean age was 27.44 ± 11.1 years. Most of the cases were in their twenties. The average number of drugs per prescription was four while the total number of antibiotics used was 520. The single antibiotic was used in 170 (53%) cases and a two-drug combination was used in 91(28.4%) cases. Ceftriaxone was the most commonly prescribed drug. There were 78 (24.3%) cases under monotherapy and 69 (21%) in multitherapy. **Conclusion:** Among the various effective antimicrobials, ceftriaxone was the most commonly prescribed antimicrobial. The injectable route of antibiotics administration was a widely used method in hospitalized patients in our setting.

Keywords: Acute tonsillitis, Antibiotics, Ceftriaxone.

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INTRODUCTION

The antibiotic prescription pattern differs from one country to another which depends on common infecting organisms, antibiotic susceptibility, physician preference, and cost. It should be evaluated and monitored periodically to increase therapeutic benefit, decrease the adverse effect, and prevent drug resistance.[1, 2] Such practice will help the clinician to develop a protocol to treat the patients. The majority of serious cases of acute tonsillitis are found to be of bacterial origin. Group A beta-hemolytic streptococci are the most common causative agent that includes 5 to 80% of tonsillar infections.[3] Other bacterial agents are Hemophilus influenza, Mycoplasma pneumonia, Neisseria meningitis, etc., which are less common.[4]

In the Nepalese scenario, it is found that many patients either don't follow the prescribed drugs or they don't complete the course prescribed by a medical practitioner which finally leads to drug resistance. Penicillin is the drug of choice in bacterial acute tonsillitis. It may be ineffective or partially effective as most of the patients come to the hospital after taking multiple courses of antibiotics from the pharmacy

without prescriptions from medical practitioners which may lead to a serious problem.[5] Most of the patients would be under oral antibiotics when they visit the hospital. Therefore, it is difficult to treat them with oral medication. Such patients are admitted to the hospital for intravenous (IV) medications and fluid replacement after consultation with consultants.

The present study was aimed to determine the antimicrobial prescription pattern in a hospitalized patient with acute tonsillitis in GMCTH.

MATERIALS AND METHODS

This is a Prospective cross-sectional hospital-based study conducted in the Department of Otorhinolaryngology and Head & Neck Surgery, GMCTH, Pokhara from January 2016 to June 2018. Ethical clearance was obtained from the Institutional Ethical Review Committee. All the patients who were admitted for acute tonsillitis in the ENT ward were enrolled. The inclusion criteria for admission of such patients were fever more than 100.4 degrees Fahrenheit, severe odynophagia and pharyngotonsillar exudate, uvular edema, and painful axillary lymph node. Those patients who had a recurrent episode of acute tonsillitis, peritonsillar abscess, cough, rhinorrhea, and pyrexia of unknown origin were excluded from the study.

Information about age, gender, the average number of drugs per prescription, and the name of the drug was obtained. All the data were entered into a customized data collection sheet. Data were presented in appropriate tables and figures. Appropriate statistics were applied whenever applicable by using SPSS 23.0 software.

RESULTS

The total number of patients included in this study was 320 with the mean age of 27.44 ± 11.1 years and range from 5 to 73 years. Majority of the patients visiting the hospital with signs and symptoms of acute tonsillitis were found to be in the age group of 21 to 30 years with 138 (43.1%) patients followed by 11 to 20 years. (Table.1) The prevalence of drug use is shown in Table 2. Ceftriaxone was the most commonly prescribed drug. There were 78 (24.3%) cases under monotherapy among which 65 (20.3%) were given Amoxicillin-clavulanic acid. Similarly, 69 (21%) cases were given multitherapy. (Table 3)

Table 1. Distribution of patients receiving antibiotics in acute tonsillitis by age group

S.N.	Age group in years	No of patients (%)
1.	<10	9 (2.8%)
2.	11-20	77 (24.1%)
3	21-30	138 (43.1%)
4	31-40	60 (18.1 %)
5	41-50	20 (6.3%)
6	51-60	14 (4.4%)
7.	> 61	2 (0.6%)
	Total	320 (100%)

Table 2. Prevalence of antibiotics uses.

	No (%)
The average number of drugs per prescription	3.5
Percentage of prescriptions with injections	85.6 %
Total number of prescriptions with antimicrobials	320
Total number of antimicrobials prescribed	510
The number of prescriptions with:	320
Antibacterial drugs	
Single antibacterial agent	170
Two drug combination	91
Three drug combination	40

Table 3.   Different antibiotics used in Mono and combination   therapy.

Antibiotics used	Monotherapy	Combination therapy
Ampicillin-cloxacillin	22	17
flucloxacillin	28	9
Amoxicillin- clavulanic acid	65	18
Ceftriaxone	78	42
Ceftriaxone -tazobactam	24	3
Piperacillin- tazobactam	12	2
Ornidazole	-	60
Metronidazole	-	64
clindamycin	-	12

DISCUSSION

The present study shows the general trend of prescribing IV antibiotics among hospitalized patients with acute tonsillitis. The most common age group who needed IV antibiotics were within 21 to 30 years, 138 (43.1%); it also shows an acute episode of tonsillitis decrease with increasing age. It is better to make national guidelines to prescribe antibiotics. A study by Murphy et al shows 78.5% prescriptions were not according to national guidelines.[6] The number of drugs per prescription is 3.5 in our study, which is higher than the study by Rehan et al who found 3.2%. Polypharmacy needs to be controlled to reduce adverse drug reaction, drug interaction, and cost.

In our study, prescription of the drug was empirically

chosen on the day of admission, however, it is better to tailor antibiotics according to Gram stain, culture, and sensitivity report. Ceftriaxone was found to be the most prescribed antimicrobial which is a bit costly. Therefore, it was not advised to our patients to reduce total cost as most of the patients were of low socioeconomic status. Bird et al reported in their study that, patients presenting with an inability to swallow with systemic features, the use of an IV antibiotic initially, and then converting to oral therapy later is likely to be sound clinical practice.[7] Therefore, 85.6% of patients were given IV antibiotics as they were unable to take oral antibiotics or had worsening symptoms with oral medications.

Most commonly prescribed categories of antibacterial drugs were found to be from β-lactam antimicrobials (Penicillin and Cephalosporins).[8, 9] However, Das et al. in his study mentioned that ciprofloxacin is most commonly prescribed drug followed by Amoxycillin.[11] In our study, the antibiotics prescribed in the majority of patients were ceftriaxone followed by Amoxicillin with Clavulanic acid as these drugs are standard regimes recommended by the World Health Organization (WHO 2013) in the management of upper respiratory tract infections.[10] Combination therapy is also used for the treatment of tonsillitis. In our study most commonly prescribed drug in combination therapy is either metronidazole or ornidazole with Amoxycillin/clavulanic acid or ceftriaxone. Metronidazole has an antibacterial activity for non-beta-hemolytic streptococcus tonsillitis.[12] In this study, antibiotics were prescribed empirically. It would be better if the culture and sensitivity tests were done to tailor the prescription of drugs.

CONCLUSION

This study concludes that Beta-lactam antimicrobials were the most commonly prescribed antimicrobials. The injection was a widely used route of administration among hospitalized patients with acute tonsillitis.

Conflict of Interest: none

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Exploring the potential of human adipocytes in periodontal regeneration: A review

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ABSTRACT

Stem cells, initially identified in embryonic tissues and later in numerous adult tissues, tend to possess the potentiality to differentiate into various cell types. Though most flexible of all stem cell lines, ethical issues restrict the use of embryonic cells. Furthermore, induced pluripotent stem cells (iPS) and adult stem cells (e.g: bone marrow stroma) can also be used. However, procurement of autologous bone marrow has its potential limitations. An alternate source of autologous adult stem cells which can be procured in large quantities, under local anesthesia, with minimal discomfort would be of keen interest. In the present context, human adult adipose tissue may be the best appropriate alternative source of mesenchymal stem cells. Studies have shown that adipose stem cells (ASCs) extracted from subcutaneous human adult adipose tissue tend to contain heterogeneous cell population called stromal vascular fraction (SVF). It may be used directly or cultured in for selection and expansion of an adherent population, and hence, they are called ASCs. The adipose tissue, obtained by suction-assisted lipectomy (i.e., liposuction), are processed to obtain a fibroblast-like population of cells, also called processed lipoaspirate (PLA). PLA cells has the potentiality to differentiate in vitro into adipogenic, chondrogenic, myogenic, and osteogenic cells in the presence of lineage-specific induction factors. This attributable feature of ASCs may be of significant importance in future clinical cell-based therapy for periodontal disease as well. This review describes current knowledge & recent advances in ASCs & their application. This review describes current knowledge and recent advances in ASCs and their application in periodontal regeneration.

KEYWORDS: Adipose-derived stem cells, Periodontal disease, Processed lipoaspirate, Stem cells, Stromal vascular fraction, Tissue engineering.

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INTRODUCTION

Periodontal disease is a chronic inflammatory condition of the supporting structures characterized by persistent inflammation, connective tissue breakdown, and alveolar bone destruction. If left untreated, it continues with progressive alveolar bone destruction, leading to increased tooth mobility & subsequent tooth loss. One of the major goals of periodontal therapy is to obtain regeneration of the affected tissues to their original architecture & function.

Several procedures have been developed to achieve periodontal regeneration, including root surface conditioning,[1-3] bone graft placement,[3-5] guided tissue regeneration,[1,3,5,-7] enamel matrix derivatives[8-10] & growth factors[11] application. However, these procedures used alone or in combination have limitations in obtaining a predictable outcome, especially in cases of advanced periodontal breakdown. So, recent advances in regenerative medicine &stem cell biology have provided the opportunities for tissue engineering & gene-based approaches in periodontal therapy.

Multilineage stem cells have substantial therapeutic potential

for applications in tissue engineering & gene therapy. Potent stem cells widely useful for these applications are: embryonic stem cells[12] (ESCs), induced pluripotent stem cells (iPS)[13,14] & autologous stem cells.[15] Although they seem to be appealing due to their pluripotentiality, their practical use is limited due to the potential problems of cell regulation & ethical considerations. The autologous stem cells obtained from bone marrow have proven experimentally promising but its procurement is a major issue. Studies have shown that a population of stem cells can be isolated from human adipose tissue.[16] Subpopulation of fibroblasts present within the fat tissues can be processed to obtain the processed lipoaspirates (PLA). These PLA cells, in vitro, can potentially differentiate into adipogenic,[17,18]chondrogenic,[18-21]myogenic[22,23]&osteogenic cells[18,19,24-26]in the presence of lineage-specific induction factors. The evidences have suggested that the periodontal microenvironment may induce ADMPC (Adipose-derived multilineage progenitor cells) to differentiate into periodontal tissues and that the ADMPC themselves might secrete various factors that stimulate resident progenitor cells.[27] These unique properties make ADMPC an important cell source for stem cell-based therapeutic approaches in the field of periodontology. The effect of autologous mesenchymal stem cells transplantation has been investigated in clinical trials for periodontal regeneration of healthy patients.[28] This review study discusses the potential use of adipose-derived stem cells in the field of periodontal regeneration.

Isolation of adipocytes-derived stem cells: Rodbell & colleagues in the 1960s pioneered the methods to isolate cells from adipose tissue using a rat.[29-31]A study was done by Tobita et al, 2007 in Wistar rat in which control group was passed platelet rich plasma (PRP) only & cases group were passed with adipocyte stem cells & PRP.[32] This study showed that after two to four weeks of implantation, a small amount of alveolar bone regeneration was observed & eight weeks after implantation, periodontal ligament like structure was observed along with the alveolar bone. Human studies were also done by several groups.[33-34] Different progenitor cells were successfully isolated and characterized from human orbital adipose tissue.[35]

Obtaining the adipose tissues: Adipose tissue is harvested by liposuction procedure. Under local anesthesia, around 1cm incision is given, through which a hollow blunt-tipped cannula is inserted into subcutaneous space. The cannula is connected externally to a gentle suction which mechanically disrupts fat tissue &draws them out from the adipose compartment. In order to minimize the blood loss & contamination of tissue by peripheral blood cells,

a solution of saline & epinephrine is infused into adipose compartment.[36] In this way, raw lipoaspirate (∼300 cc) is obtained.[16]

Processing of raw lipoaspirate: When preserved at room temperature, the aspirated fat should be processed as quickly as possible. It can be stored or transported overnight without adipose-derived stem cell yield loss or changes in biological properties if it is preserved at 4[0]C.[37] The obtained raw lipoaspirate is extensively washed with equal volume of phosphate buffered saline(PBS). The extracellular matrix is then enzymatically digested at 37°C for 30 min with 0.0075 % collagenase[16] or 40 min with 0.1% collagenase.[33] This enzymatic activity is neutralized with Dulbecco's modified Eagle's medium (DMEM), containing 10% FBS(Fetal Bovine Serum) & the cell suspension is centrifuged at 1200 xg for 10 minutes to isolate stromal vascular cells (SVC)[38,39]pellets from primary adipocytes.

Obtaining processed lipoaspirates: SVF pellet is resuspended in basal medium containing 10% fetal bovine serum[40] or in 160 mM NH_4Cl & incubated at room temperature for 10 minutes to lyse the contaminating red blood cells.[16] The cell suspension is then filtered through 100μm cell strainer / nylon mesh to remove cellular debris. The cells are plated & incubated at 37°C / 5% CO_2 in control medium. The plates are then washed extensively with PBS to remove residual non-adherent RBCs. The medium should be changed in every second day till the cells reach 80-90% confluence. The cells were maintained at subconfluent levels in order to prevent their spontaneous differentiation. In this way, a large number of ASCs can be harvested approximately 2,50,000 cells per gram of tissue.[41,42] Furthermore, the use of PRP augments the proliferation of human ASCs.[32]ASCs can be successfully stored for more than six months which ensures the availability of autologous banked ASCs for clinical applications in the future.[43,44]

Characterisation of adipocytes stem cells:

1) **Indirect immunoflourescence of PLA cells:**

   It uses two antibodies; the unlabeled first (primary) antibody specifically binds with the target molecule, and the secondary antibody, that carries the fluorophore, recognizes the primary antibody and binds to it. PLA cells are then processed by using monoclonal antibodies to specific CD markers and lineage-specific protein. The cells are plated onto glass chamber slides, fixed in 4% paraformaldehyde in100 mM Na_3PO_4buffer (pH 7.0) for 15 min.[16] The cells are then extensively washed for 10 min in 100 mM glycine in PBS (PBS/glycine) & blocked for one hour in

immunofluorescent blocking buffer (IBB) that contains 5% bovine serum albumin (BSA), 10% FBS, 1× PBS, 0.1% Triton X-100) followed by incubation for one hour in IBB containing the following cell-specific monoclonal antibodies:

(1) Anti-smooth muscle actin: -To identify smooth muscle cells & pericytes.

(2) Anti-Factor VIII:- To identify endothelial cells.

(3) ASO2:- To identify fibroblasts & cells of mesenchymal origin.

The cells are again washed with PBS/glycine & incubated for one hour in IBB containing a fluoroisothiocyanate (FITC)-conjugated secondary antibody mounted with a solution containing DAPI(diamidino-2-phenylindole) to detect the nuclei.

2) Spectrophotometric assays:

a) Alkaline Phosphatase (AP): Samples of PLA cells are placed in osteogenic medium (OM) for aroundsix weeks followed by washing with PBS. AP enzyme activity is then assayed using a commercial AP enzyme kit according to the method of Beresford et al(1986).[45] AP activity is expressed as nanomoles of p-nitrophenol produced per minute per microgram of protein.

b) Total Calcium: PLA cells are placed in osteogenic medium for six weeks, then washed with PBS (no Ca^{++} or Mg^{++}), harvested & extracted in 0.1 N HCl at 4°C for around four hours & lastly centrifuged for five minutes at 10,000 ×g. A commercial kit is used to determine total calcium (millimolar Ca^{++} per microgram of protein) in the supernatant.[46]

c) Dimethyldimethylene Blue: High-density micromass protocol is employed to differentiate PLA cells inchondrogenic medium (CM) for three weeks.[47] With the help of an established method,[48] PLA nodules are harvested & assayed for sulfated proteoglycans.

3) **Cell Senescence Assay:** The existence of senescence-associated β-galactosidase (SA-β-gal or SABG) was proposed in 1995 by Dimri et al[49] following the observation that when β-galactosidase assays are carried out at pH 6.0, only cells in senescence state develop staining & not the proliferating cells. It is a hypothetical hydrolase enzyme which helps in catalyzingthe hydrolysis of β-galactosides into monosaccharides in senescent cells. Cells from each culture passage (passage 1 to passage 15) are fixed for five minutes in 2% formaldehyde/glutaraldehyde and incubated in a β-Gal reaction buffer (containing 1 mg/ml X-Gal, 40 mM citric acid/sodium phosphate buffer (pH 6.0), 5 mM each of potassium ferrocyanide and potassium ferricyanide, 150 mM NaCl, and 2 mM ($MgCl_2$).Senescent cells (blue) are identified by light microscopy.

4) **Flow Cytometry:** Flow cytometry helps in analyzing light scatter which distinguishes different cells based on their size, shape, & internal complexity. The antibodies or ligands conjugated with fluorescent probes are used to measure the presence and amount of specific intracellular and cell surface molecules. Similarly, fluorescent indicators help in measuring the transport of ions across the cellular membranes, as well as assess the mitochondrial activity & other metabolic parameters. PLA samples are cultured in control medium 72 hours before analysis & then flow cytometry with a FAC scan argon laser cytometer is performed. Briefly, cells are harvested in 0.25% trypsin/EDTA & are fixed in ice-cold 2% formaldehyde for 30 min. The fixed cells are then washed in the buffer solution (PBS, 2% FBS, 0.2%Tween-20) & then incubated for 30 min inflow cytometry buffer containing fluorescein isothiocyanate-conjugated monoclonal antibodies to SH_3, STRO-1, and the CD antigens: 13, 14, 16,31, 34, 44, 45, 49d, 56, 62e, 71, 90, 104, 105, and 106. PLA cells are stained with a phycoerythrin-conjugated nonspecific IgG to assess background fluorescence.

Multilineage differentiation potential of PLA cells: PLA cells possess adipogenic, chondrogenic, osteogenic, myogenic & neurogenic lineages depending upon their cultured in specific induction media (Table 1).[16] DMEM is used for differentiation into all the cell types.

Table 1. Lineage-specific differentiation

Medium	Serum	Supplementation
Control	10% FBS	None
Adipogenic (AM)	10% FBS	0.5 mM isobutyl-methylxanthine (IBMX), 1 µM dexamethasone, 10 µM insulin, 200 µM indomethacin, 1% antibiotic/ antimycotic
Myogenic (MM)	10% FBS, 5% HS	0.1 µM dexamethasone, 50 µM hydrocortisone, 1% antibiotic/ antimycotic
Chondrogenic (CM)	1% FBS	6.25 mg/ml insulin, 10 ng/ ml TGF- ß₁, 50 nM ascorbate-2-phosphate, 1% antibiotic/ antimycotic
Osteogenic (OM)	10% FBS	0.1 µM dexamethasone, 50 µM ascorbate-2-phosphate, 10 mM ß-glycerophosphate, 1% antibiotic/ antimycotic

Table 2. Markers and assays of lineage -specific differentiation

Lineage	Serum	Lineage-specific determinant
Adipogenic	10% FBS	Lipid accumulation
Myogenic	1. Phase contrast microscopy	1. Multinucleation
	2. Myosin- and MyoD1-specific and MyoD1 expression mono-clonal antibodies	2. Skeletal muscle myosin heavy-chain
Chondro-genic	1. Alcian Blue (pH 1.0) stain	1. Sulfated proteoglycan- rich matrix
	2. Collagen II-specific mono-clonal antibody	2. Collagen II synthesis
Osteogenic	1. AP stain	1. AP activity
	2. Von Kossa stain	2. Calcified matrix production

Adipogenesis:

Adipogenic inductive compounds (Table 1):

a) Glucocorticoid receptor ligands (dexamethasone),

b) Insulin,

c) Biotin,

d) d-pantothenate,

e) Cyclic AMP agonist (forskolin) &

f) Equivalent Peroxisome proliferator activated receptor gamma agonist (PPARγ)

In response to these adipogenic induction medium, ASCs tend to differentiate into adipocytes.[16,50-52] Within two weeks after placing in adipogenic medium, the ASC contain vacuoles filled with neutral lipid cells. The cells are fixed at room temperature in 4% formaldehyde/ 1% calcium for 60 minutes, washed with 70% ethanol, incubated in 2% (wt/vol) Oil Red O reagent for five minutes at room temperature. Oil Red O stain (Table 2) is used to stain intracellular lipid droplets accumulation.[53]Excessive stain are removed by washing with 70% ethanol & counterstaining is done with hematoxylin for two minutes. ASCs tend to undergo morphological changes & reduce their proliferation rate during the differentiation. During differentiation, parameters including leptin, adipogenic mRNA like fatty acid binding protein, aP2 mRNA levels are increased by several hundred-fold.[51,54,55]

Clinical implication:

i. Replacement of adipose tissue

ii. Replacement in large soft tissue defects formed due to trauma, burns & oncological resections.

Chondrogenesis:

Chondrogenic induction medium (Table 1):[16, 21, 56]

a) Ascorbate,

b) Dexamethasone

c) Transforming growth factor-β

ASCs cells are incubated for three weeks in DMEM containing 1% FBS, 40mg/ml proline, 100 mg/ml pyruvate, 50 mM L-ascorbic acid-2-phosphate, 10 ng/mltransforming growth factor-b3.[57] The cells are then fixed for one hour with 4% paraformaldehyde & rinsed with PBS. After induction, cell condensation occurs followed by ridge spheroid/nodule formation by two days. Chondrogenesis is confirmed using the histologic stain Alcian Blue (Table 2) at acidic pH (one& below) (pH 2.5, Wako) which stains sulfated proteoglycans present in cartilagenous matrices. Quantitation of sulfated proteoglycan levels revealed a time-dependent increase in cartilage-induced PLA cells upto two weeks of induction, followed by a slight decrease at three weeks. Expression of aggrecan, chondroitin sulphate, cartilagenous collagen type II & IV isoformsare suggestive of chondrogenesis. Standard hematoxylin and eosin staining are used to assess cellular morphological features of the processed lipoaspirate cell nodules (paraffin sections), & the presence of collagens was confirmed with Goldner's trichrome stain.

Clinical implication:

i. Envisioning the therapies that facilitate cartilage repair

ii. Substantial cartilage remodeling and repair

Osteogenesis: Osteogenic induction medium (Table 1): a) Ascorbate, b) β-glycerophosphate, c) Dexamethasone d) Vitamin D3.

ASCs cells are incubated for three weeks in DMEM containing 10% FBS, 100 nM dexamethasone, 10 mM β-glycerophosphate and 50 μM L-ascorbic acid-2-phosphate.After fixation cells are incubated with 0.16% naphthol AS-TR phosphate and 0.8% Fast Blue BB dissolved in 0.1 M Tris buffer (pH 9.0) for one hour at 37°C. For osteogenic differentiation, cells were also incubated in 1% alizarin red S for three minutes to detect calcium deposition.[58]In order to assess osteogenesis, levels of AP enzyme activity is determined by alkaline phosphatase stain &extracellular matrix calcification is quantitated by Von Kossa staining. To detect AP activity, cells are incubated in osteogenic medium for two weeks, rinsed with PBS, & stained with a 1% AP solution (1% naphthol ABSI phosphate, 1 mg/mL Fast Red TR) at 37°C for 30 min. For Von Kossa staining (Table 2), the cells are incubated in OM for four weeks, fixed with 4% paraformaldehyde for 60 min at room temperature, rinsed with distilled water &then overlaid with a 1% (wt/ vol) silver nitrate solution in the absence of light for 30 minutes. The cells are developed under UV light for 60 minutes & finally counter-stained with 0.1% eosin in ethanol. Expression of genes

& proteins associated with osteoblasts phenotypes such as osteopontin, osteonectin, osteocalcin, collagen type I, BMP-2 and BMP-4, CBFA-1 are suggestive of osteogenic potential of adipocytes stem cells.[16,54] The first case of autologous ASC use for osseous repair has been reported in the treatment of a calvarial defect in a seven-year-old girl.[59] A study involved treatment of four patients possessing large cranial defects with a combination of ADSCs and b-tricalcium phosphate granules. Computed tomography scanning subsequently revealed improved ossification in all of the cases.[60]

Myogenesis:

A. Skeletal muscle: Skeletal muscle myogenic medium (MM) (Table 1):-

   a) 0.1 mM Dexamethasone,

   b) 50 mM Hydrocortisone,

   c) 10% FBS

   d) 5% Horse serum

PLA cells, when placed in myogenic medium (MM) for upto six weeks, result in the expression of the multiple myogenic transcription factor myod1, myosin, myf6, myf5 & myogenin[16,18] followed by fusion & the formation of multinucleated cells that expressed the myosin heavy chain.[61] The expression of myogenic transcription factors can be confirmed by immunohistochemical staining &real time-PCR. Thus, skeletal myogenesis is peculiarized by a period of myoblast proliferation, followed by the expression of muscle-specific proteins and fusion to form multinucleated myotubules.

B. Smooth muscles: Smooth muscle inductive medium (Table 1):-

   a) MCDB131 supplemented with 1% FBS &

   b) 100u/ml of heparin(for upto six weeks at 37°C with 5% CO_2)

The expression of smooth muscle specific proteins like: - smooth muscle actin (SMA), calponin, SM22, smoothelin, h-caldesmon &smooth muscle myosin heavy chain (SMMHC)[62,63] are suggestive of myogenic potential of adipocytes stem cells. The smooth muscle differentiation has effect on cell size, shape, membrane potential, metabolic activity & responsiveness to external signals.

Differentiated SMCs exhibit two specific phenotypes[64] namely,

   i.   Synthetic &proliferative

   ii.   Contractile and quiescent phenotype

Contractility is an important characteristic of SMCs which plays role in angiogenesis, blood vessel maintenance, & mechanical regulation of hollow organs such as bladder. This property can be of value in the repair of smooth muscle defects in the gastrointestinal and urinary tracts..

Neurogenesis: Human ASCs also exhibit neuronal and/or oligodendrocytic markers. The neurogenic induction medium include (Table 1):-DMEM enriched with 500 mM IBMX, 200 mM INDO, & 5 mg/ml insulin for 1 hour. PLAs in neurogenic medium, when express neuronal markers like:-S100, NF70, NSE,NeuN, MAP-2, nestin in addition to GFAP and GalC as markers of astrocytes and oligodendrocytes respectively, are suggestive of neurogenic potential of ASCs. It is still unclear that whether the transplanted cells replace the lost neurons or provide a supporting scaffold for existing stem cells and injured neurons. Specifically, these PLA cells undergo retraction,forming compact cell bodies with multiple extensions. Western blotting test confirms an increase in NSE protein upon induction while real time-PCR analysis confirms the expression of nestin. This sums up that the expression of nestin, NSE, & NeuN, along with absence of choline acetyltransferase, myelin-basic protein, or GFAP expression, suggests that PLA cells may be capable of assuming an early neuronal or neural precursor phenotype.

DISCUSSION

The ultimate goal of the treatment of periodontal disease is the reorganization of functional tissues, predictable regeneration of the lost periodontal tissues and eventually achieving the periodontal health. The regeneration of periodontal tissue requires the restoration of cementum, periodontal ligament, and alveolar bone.[65] A number of surgical regenerative techniques including osseous grafts,[3-5]alloplast grafts,[3]guided tissue regeneration (GTR) technique,[1,3,5,6]enamel matrix proteins,[8-10]chemical mediators,[11] have been developed to regenerate the lost periodontal tissues. Although these treatment modalities have been widely accepted, the amount of tissue regenerated could not be well-predicted in cases of advanced periodontal defects. So, periodontal tissue regeneration with autologous stem cells (embryonic stem cells, iPS,bone marrow mesenchyme stem cells) was introduced for cell-based therapy in periodontal diseases. However, the procurement of such stem cells has its own potential limitations.

Till date, human adult adipose tissue may be the best suitable alternative source of mesenchyme stem cells which is obtainable in large quantities, under local anesthesia, with minimal site morbidity & patient discomfort. This alternative source of autologous stem

cells contain multilineage potential to differentiate towards the adipogenic, osteogenic, chondrogenic, neurogenic & myogenic lineages with appropriate medium supplementation. Following induction, the differentiation to specific lineage can be assessed using histology & immunohistochemistry.

Tissue-specific scaffolds (stable supporting structures), signalling systems & vascularization are the major pre-requisites for the differentiation of stem cells into the desired cells & use them effectively to construct the three-dimensional (3D) tissues. Various scaffolds that can be used are: - collagen I (excellent cellular compatibility), porous collagen bead (injectable cell delivery vehicles), placental dermal matrix, XLHA (hyaluronan sulphate) incorporated in placental dermal matrix, hyaluronic acid gel, HFIP silk fibroin chitosan scaffold, gelatin sponges, monofilament polypropylene, polyglycolic acid meshes etc. The autologous transplantation of MSCs in combination with tissue engineering, such as cell sheet technology, has been shown to be effective for regeneration of the periodontium.[66,67]

A histological analysis after injection of ASCs+PRP admixture into periodontal defect showed a small amount of alveolar bone regeneration at 2nd & 4th weeks of implantation and a periodontal ligament-like structure at 8th weeks.[32] The effect of autologous MSC transplantation has been investigated in clinical trials for periodontal regeneration of healthy patients.[28,68] Some studies have shown that the periodontal microenvironment induces ADMPC to secrete various factors that stimulate resident progenitor cells to differentiate into periodontal tissues.[27]

Although many clinical trials have demonstrated the efficacy of autologous MSC transplantation, their clinical application is limited by age restrictions, tissue quality and systemic diseases (such as diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus), which alters the intrinsic properties of MSCs and also increase the difficulty of isolating MSCs.[69] Efficient use of allogeneic MSCs may be an alternative strategy that overcomes the limitations of autologous MSC transplantation procedures for the regeneration of large periodontal defects.

In recent years, evidence has supported periodontal regeneration by allogeneic MSC transplantation in periodontal defects using animal models.[70] Bone-morphometric analysis following the allogeneic transplantation of ADMPC in a micro-mini pig periodontal defect model showed a significant amount of bone regeneration ability. The histologic analysis showed a comparable regeneration potentiality of ADMPC by allogenicc transplantation as compared to those of autologous transplantation. These results indicate that ADMPC have immune-modulation capability that can induce periodontal tissue regeneration by allogeneic transplantation.[65]

CONCLUSION

The future of engineering the mesodermally derived tissues from stem cells is very promising & it definitely requires a readily available source of donor cells. Although further characterization of the PLA cells within adipose tissue is necessary, the results obtained from large number of studies suggest that adipose tissue may be another potent source of pluripotent stem cells with multiple germline potential.

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Intramuscular Hemangioma of the sternocleidomastoid muscle: A Rare Unusual Neck Mass.

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ABSTRACT

Hemangiomas of the head and neck region comprise about 60 to 70% of all benign tumors. Intramuscular hemangioma is a rare, slow-growing, angiomatous tumor. We report a rare case of an Intramuscular Hemangioma of Right sternocleidomastoid muscle in a six years old girl presenting for four years and with extensive involvement necessitating excision. Microscopic excision reduces the risk of recurrence.

Keywords: Intramuscular Hemangioma, Microscopic excision, Recurrence.

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INTRODUCTION

Hemangiomas are benign vascular tumors consisting of 60 to 70% of benign head and neck tumors[1] Hemangiomas are a tumor of infancy and most commonly occurring in cutaneous and mucosal surface.[2] Intramuscular hemangiomas (IMH) are rare tumors with the masseter muscle being the most common site, followed by the trapezius and sternocleidomastoid muscles respectively. Intramuscular hemangioma occurs during the first three decades of life. Pain is one of the presenting symptoms, and it is exacerbated by the exercise of the involved muscle as a blood vessel of the muscle dilates. Rarely there are bruits, thrills, compressibility like in other vascular malformations. Because of the rarity of intramuscular hemangioma, deep location, and unusual presentation, the inaccurate preoperative diagnosis had led to inappropriate diagnosis and incomplete excision.[3]

CASE

A six years old female child presented with progressively increasing swelling in the right lateral neck for four years in the Department of Otorhinolaryngology and Head and Neck Surgery (ORL-HNS) of Gandaki Medical College, a tertiary care hospital in western Nepal. There was no pain, no discharge, and no history of trauma to the neck. On examination, there was a 4x3cm firm globular mass at the apex of the right posterior triangle of the neck, no signs of inflammation overlying skin, no pulsation, and non-tender, soft consistency, irregular margin, non-compressible, and

fixed to underlying tissue but overlying skin is mobile. (Fig 1) No pulsation or bruit was noted and there was no lymphadenopathy.

Ultrasonography of the neck showed a 3.7x2.3 cm heteroerotic intramuscular lesion with thin septations. Color Doppler showed well defined hypoechoic mass with heterogeneity. Contrast-enhanced computed tomography reported hypodense lesion with peripheral nodular to central filling contrast enhancement, located within Sternocleidomastoid muscle. (Fig 2) With the above clinical features and radiological findings, the case was diagnosed as intramuscular hemangioma of the right Sternocleidomastoid muscles Due to an increase in the size of the swelling and extensive involvement of the tissue, she was planned for excision under general anesthesia.

Surgical exploration was done via a single horizontal transcervical incision from the posterior triangle. Tumor mass was exposed. Further dissection was performed under the vision of the microscope. The tumor was extensive involving SCM and part of to trapezius and scalenus anterior. Involved part of muscle resected. The spinal accessory nerve was identified and preserved. (Fig3). The post-operative period was uneventful Histopathological diagnosis was cavernous hemangioma. (Fig 4) No recurrence was observed during one year follow up period.

DISCUSSION

Intramuscular hemangiomas are benign vascular neoplasm which occurs in less than 1% of all hemangioma.[4, 5] It frequently involves the following muscle such as masseter, trapezius, and sternocleidomastoid Diagnosis of this type of hemangioma is difficult.[6] The differential diagnosis for intramuscular hemangioma includes schwannoma and rhabdomyosarcoma. FNAC does not help in diagnosis as aspirate is blood.[7]

Unlike superficial hemangioma, IMH does not demonstrate spontaneous regression.[3] In addition to that, according to the literature, radiotherapy therapy is contraindicated in the treatment of IMH because of the possibility of malignant transformation and low success rate. [8]

Based on our knowledge, tumor mass was resected along with the adjacent involved part of muscle tissue while taking care to preserve the spinal accessory nerve.[9] Careful microscopic dissection was done under a microscope so that whole of the tumor mass along with that involved muscles can be removed properly. The tumor was adherent to spinal accessory nerve and separated

carefully under microscope No recurrence was observed in one year follow up period. in our study, The reported rate of recurrence varies from 9 to 28%.[10]

CONCLUSION

Hemangioma involving sternocleidomastoid muscle is a rare unusual neck mass. Surgical en-bloc excision is the main modality in the treatment of IMH. The microscopic excision of adjacent muscle tissue reduces the risk of recurrence.

Conflict of interest: None

Informed consent Written informed consent was obtained from the patients for this case report and any accompanying image

Financial Disclosure: No financial support

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Figure 1 Swelling in the right

Swelling in Right upper part of Neck

Figure 2 CECT Neck showing lesion in Right sternocleidomastoid muscle
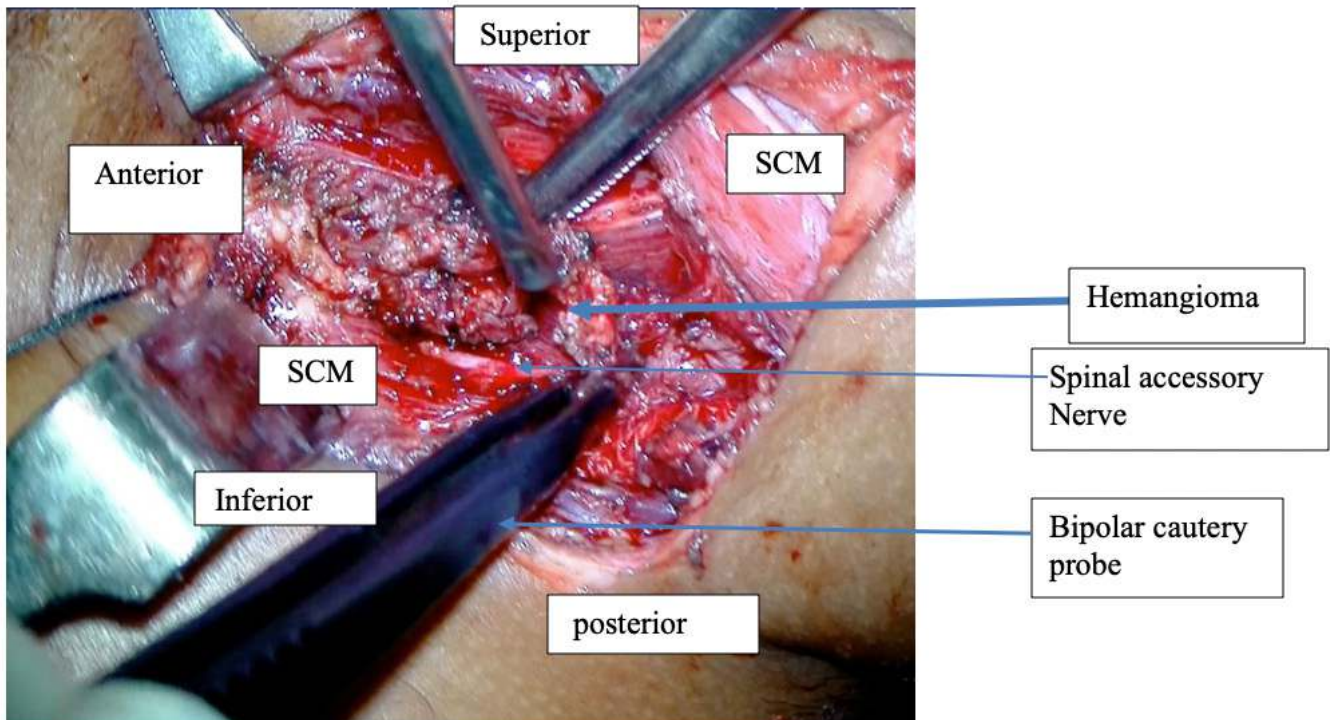
A hypointense lesion with hyperintense area

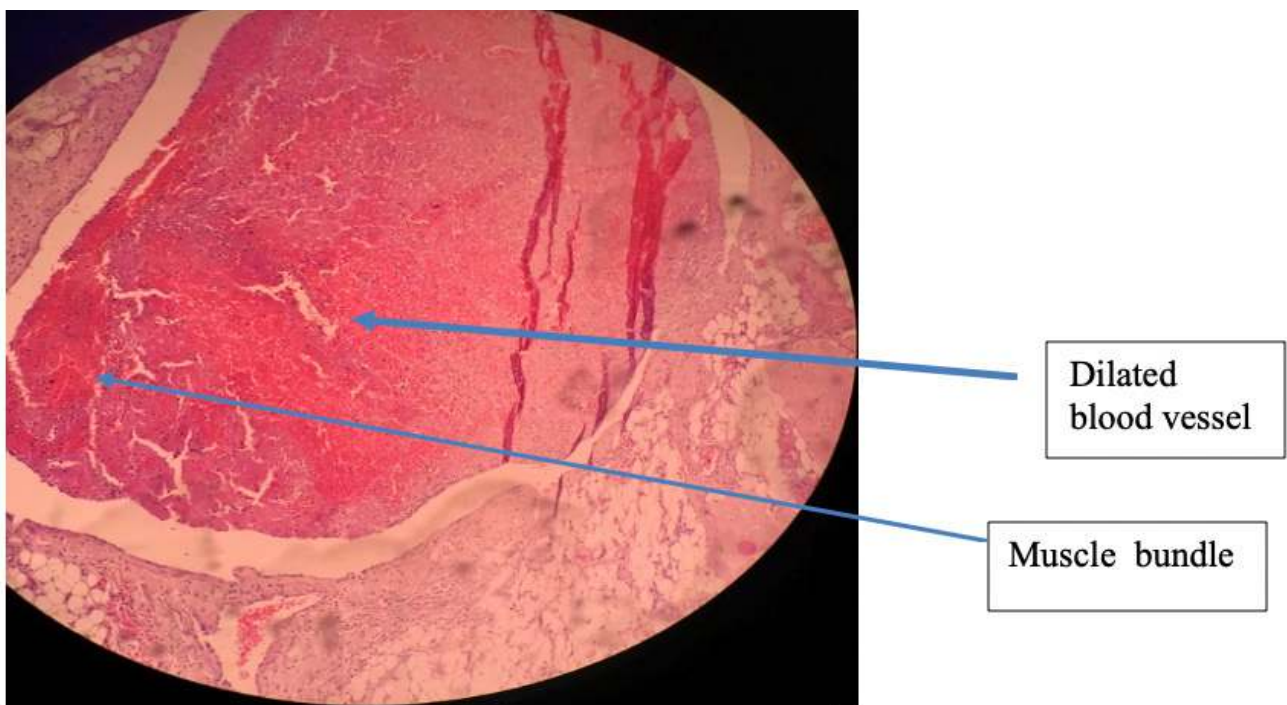Fig.3 Intraoperative microscopic view of haemangioma in Right sternocleidomastoid muscle.

Fig 4. Histopathology of tissue specimen showing

thin, ulcerated, and dilated blood vessel wall.and.

muscle bundles in the periphery

All about COVID-19 what do we know?

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1. INTRODUCTION

In December 31st 2019, China alerted WHO several flu like cases. As we know the flu has been occurring since decades but the cases were exceptional at those times. January 5th 2020 WHO put travel restrictions so that the flu would not transfer from one country to another. Amid the fear lockdown was made. In January 1st 2020 first death was reported form the same flu that had started in December 2019. On January 7th the virus was identified as novel-corona virus. The number morbidity and mortality was increasing day by day. In January 31st it was public health emergency of international concern. Seeing the epidemic and possible pandemic WHO strategic preparedness and response plan was released in February 3rd. And on February 4th there was request activation of UN crisis management policy to establish a crisis management team. On February 6th WHO briefed on strategic preparedness and response plan. On February 11th the new name was given COVID-19. And on February 12th strategic preparedness and response plan operational planning guide was released.

The main aim of the plan was to support the UN country team in providing guidance to national governments and partners in scaling up response to COVID-19. And three core response strategies are to rapidly establish international coordinates and operational support. Scaling up country readiness and response operation and accelerating priority research and innovation.

2. ABOUT THE DISEASE COVID-19

It is an acute viral illness caused by severe acute respiratory syndrome CoV-2(SARS-CoV-2). It is related to group of coronaviruses that cause disease in mammals and birds. Similar to the recent pandemic there was severe acute respiratory syndrome (SARS) in 2003 and Middle east respiratory syndrome (MERS) in 2012. They are enveloped viruses with a positive-sense single-stranded RNA genome. The name coronavirus is derived from the Latin corona, meaning crown, which refers to the characteristic appearance reminiscent of a solar corona around the virions.

3. TRANSMISSION OF THE VIRUS

It is transmitted by droplet infection by sneezing, coughing and contamination of fomites and surface articles. It infects human cells by attaching to the angiotensin-converting enzyme 2 (ACE2) receptor. The incubation period of the virus ranges from two to fourteen days and main risks to the viruses are health care personnel, 60+ age groups, immunocompromised patients, children and those with malignancy. The symptoms can range from mild viral prodrome like dry cough, fever, malaise to severe lethal consequences like pneumonia, kidney failure and death.
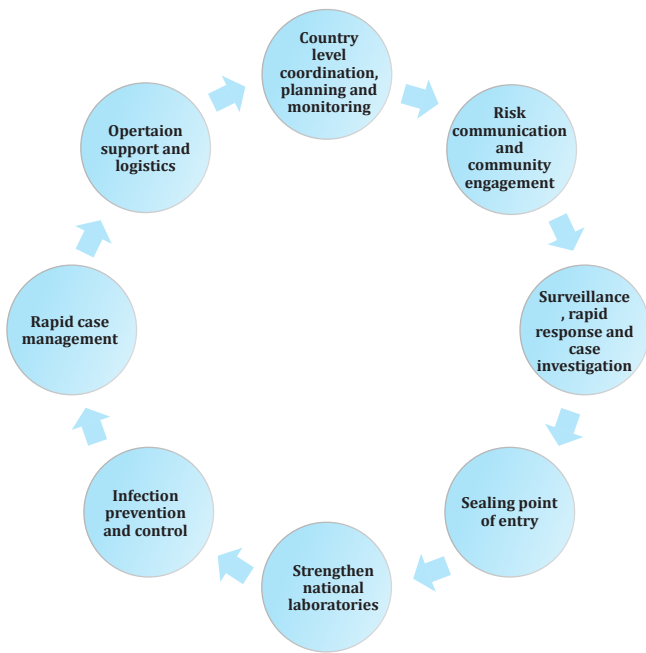
4. DIAGNOSIS

The diagnosis is done by two methods RT-PCR and detection of the antibodies in the serum. In clinical settings RT-PCR is best method and antibodies detection should be limited to surveillance. No any cure is made till now nor vaccine is made and the treatment is symptomatic.

5. PREVENTION OF THE DISEASE

- Cover mouth and nose while sneezing and coughing
- Avoid close contacts
- Use PPE while in health care facility
- Cook the foods thoroughly
- Stay at home during epidemic
- Seek early medical advice amid symptoms

6. EIGHT PILLARS TO STOP OUTBREAK, EPIDEMIC AND PANDEMIC

The eight pillars to stop any possible outbreak, epidemic and pandemic are given as below:

7. THE PROTOCOL FOR THE INVESTIGATION OF THE CASES ACCORDING TO WHO

Objectives of the investigation	The objectives of any investigation of a suspected COVID-19 case include: • Rapidly detect COVID-19 and any evidence of human-to-human transmission among contacts • Reduce human-to-human transmission, prevent outbreaks, and delay the spread of disease
Composition, protection of and tools for the investigative team	**Composition** Personnel with the capacity, knowledge, and authority to: • Interview persons with suspected COVID-19 and conduct contact tracing • Triage suspected COVID-19 cases and contacts for health care depending on clinical condition • Collect respiratory specimens from suspected COVID-19 cases • Recommend and implement measures to prevent further transmission **Protection** The COVID-19 virus is spread through contact, droplet, and fomites. To minimize risk of infection of the investigation team: • Optimize size of team to minimize contact with a suspected COVID-19 case • Ensure all those in the investigation team are trained in IPC measures specific to COVID-19[1] • Interview suspected cases and contacts over the phone, if feasible, or at a distance of more than One metre. **Tools** • Provide sufficient and appropriate PPE[2] • Gather biological specimen collection material, transport containers, viral transport media, labels, bags, coolers, and cold packs • Gather copies of case investigation protocols, questionnaires, contact tracing and monitoring tools, and the national case definition

Investigation case definition	• Adjust national/WHO case definitions[3] for the purpose of investigation (define time, person, place) for additional case finding and contact tracing (Figure)
Contact Tracing	Identify all social, familial, work, and health care worker contacts who have had contact[8] with a confirmed case from two days before symptom onset of the case and up to fourteen days after their symptom onset. Create a line list, including demographic information, date of first and last common exposure or date of contact with the confirmed or probable case, and date of onset if fever or respiratory symptoms develop. The common exposures and type of contact with the confirmed or probable case should be thoroughly documented for any contacts that become infected with COVID-19. Instructions can be found WWW.WHO.COM
Management of case(s) and contacts	**COVID-19 case(s)** • All patients with suspected COVID-19 who have severe acute respiratory infection should be triaged and isolated at the first point of contact with the health care system. Emergency treatment should be started based on disease severity.[4] • For those presenting with mild illness, hospitalization may not be required unless there is concern about rapid deterioration. If there is only mild illness, providing care at home may be considered, with strict precautions regarding when to seek care.[5] **Contacts** • For contacts of a suspected COVID-19 case, at a minimum, health authorities need to encourage respiratory and hand hygiene and may encourage, depending on the epidemiological context and resources available, self-monitoring for symptoms, social distancing, or quarantine. • For contacts of a laboratory-confirmed COVID-19 case, WHO recommends that such persons be quarantined for 14 days from the last time they were exposed to a COVID-19 patient.[6]
Collection and testing of specimens	From all confirmed cases and their contacts, a respiratory sample should be collected and tested as soon as possible, particularly contacts with symptoms.[7] Respiratory samples from quarantined persons, irrespective of whether they have symptoms, should be sent for laboratory testing at the end of the quarantine period. Ensure that all those involved in collection and transportation of specimens should be trained in safe handling practices and spill decontamination procedures.[6] For laboratory-confirmed cases, two negative specimens at least one day apart indicate recovery from infection. Based on initial data, this is estimated to be 14 days after the end of illness for mild cases of infection.

Risk communication	Assess the initial perception of risk among affected and at-risk populations, manage expectations and communicate uncertainties Encourage people to adopt protective behaviors: frequent hand hygiene and good respiratory etiquette, avoiding people with respiratory symptoms Encourage people to call hotline (if available) or their health care provider if they have concerns/questions or develop symptoms Discourage use of medical masks, unless one of the following exists: • Individuals with respiratory symptoms • Health care workers • Individuals in close contact (within One metre) of a patient with a respiratory infection
Reporting	National authorities need to report probable and confirmed cases of COVID-19 to WHO within 48 hours of identification.[8] Report using the Individual Case Reporting Form and consider transitioning to the aggregate Daily/Weekly Reporting Form if the number of cases increases and resources are no longer available for individual case reporting.[8]
Further investigations	Standardized epidemiological protocols have been developed for COVID-19 and are available on the WHO website.9 These may be initiated in addition to the public health investigation, but should not replace the investigation: • First few cases and contacts transmission investigation protocol, which evaluates extent of infection among cases and their contacts • Household transmission investigation protocol, which evaluates extent of infection within the household setting • Assessment of risk factors for COVID-19 among health care workers working in a health care setting in which a confirmed COVID-19 patient is receiving care • Surface sampling of COVID-19 virus: A practical 'how to' protocol for health care and public health professionals to assess surface contamination and the role of environmental contamination in transmission • Global COVID-19 Clinical Characterization Case Record Form and data platform for anonymized COVID-19 clinical data to collect clinical data to better understand the natural history of disease and describe clinical phenotypes and treatment interventions

8.   CONTACT TRACING:

In the community: Once the case is identified we have to identify contacts and monitor them for 14 days. If no symptoms develop monitoring can stop. If symptoms appear then isolate, test and treat for COVID-19. The monitoring can only be stopped if two consecutive tests are negative.

In Hospital: If there is case in hospital is identified then we have to identify contacts and monitor them for 14 days. We should test all the health care workers and patients contacts regardless of the appearance of symptom. If positive then isolate the patient until two consecutive negative tests. And while monitoring if the symptoms develop then we should isolate the case until two consecutive negative tests.

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Journal of Gandaki Medical College-Nepal (J-GMC-N)

कर्मण्येवाधिकारस्ते
My right is to my work

AIMS & SCOPE OF THE JOURNAL

Journal of Gandaki Medical College-Nepal (J-GMC-N) is an official, open access, peer-reviewed, biannual, biomedical, scientific Journal published by Gandaki Medical College Teaching Hospital & Research Centre Pvt Ltd, Pokhara, Nepal.

The J-GMC-N publishes original scientific articles (not published or submitted for publication elsewhere) written in English from all over the world, related to research done in the field of biomedical sciences related to all the disciplines of the Medical Sciences, Public health, Medical education, Health care management, including ethical and social issues pertaining to health. The Journal publishes original articles, systematic reviews and meta-analyses, case reports, editorial articles, viewpoint, and letters to the editor.

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The editors review/screen all submitted manuscripts initially for format and style as per the guidelines and if not matched, the paper will be returned for resubmission as per the guidelines. Manuscripts with insufficient originality, serious scientific and technical flaws or lack of a significant message are rejected. If good articles are written poorly, then authors will be requested to revise and resubmit according to the J-GMC-N format.

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1. Manuscripts must be clearly typed double-spaced on one side only on A4 size white paper with Arial Font, size of 12 points, with a margin not less than 25 mm.

2. The pages should be numbered consecutively, beginning with the title page. Uniformity in the language is required, with preference to American English.

3. There should be no abbreviations in Title and abstract; however, universally popular abbreviations such as HIV, WHO may be used.

4. Abbreviations should be fully spelled out at its first use.

5. Do not use '&', '@' in the text.

6. SI units should be used in the manuscript. BP should be in mm Hg, the temperature in °C.

7. Always abbreviate units when reporting numerical information. Write in full in a non-numerical context. e.g. The mean height was 48.2 cm. The length was measured in meters.

8. Write a percentage as % without a space between the number and the sign. Write percentage to two decimal points if population size more than 100, one decimal if 10-100, and no percentage at all if the population is less than 10.

9. When starting a sentence with a number and unit, both must be spelled out as words e.g. Eighty-three milligrams of …………..

10. Put a space between number and unit e.g. 232.1 m.

11. Words, not numbers should begin a sentence.

12. Numbers less than 10 should be written in words.

13. Use 0 before the decimal point when writing numbers between -1 to 1.

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16. Do not insert a tab, indent, or extra spaces before the beginning of a paragraph.

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results, discussion, conclusions, acknowledgment, references, tables, and figures.

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The title page should be submitted as a separate attachment and it should carry

1. Type of manuscript (e.g. Original article, Review article, Case report, etc).

2. Title of the article: The simpler the title better; should be concise and informative, should reflect the content of the paper.

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8. Counts

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Abstract

1. The abstract should contain the essence of the whole paper. Be clear and concise without any cited references and avoid unnecessary detail.

2. The abstract must not exceed 250 words and should be presented in prescribed structured format: Introduction and Objectives, Methods, Results, and Conclusions.

3. Provide three to six keywords (will not be included in

the word count for the abstract) below the abstract arranged alphabetically.

4. The abstract need not be structured for a review article or case report. Universally accepted standard abbreviations used in standard textbooks can only be used.

Introduction

1. The introduction should be short and tell the reader why you undertook the study.

2. Divide the introduction into three paragraphs. The first paragraph should be a very short summary of the existing knowledge of your research area. This should lead directly into the second paragraph that summarizes what other people have done in this field, what limitations have been encountered, what questions still need to be answered? This, in turn, will lead to the last paragraph, which should clearly state what you did and why.

Objectives should be written in the last paragraph of the introduction.

Materials and Methods

This section should describe how and why a particular study was done in a particular way. Basically, it should include three questions: How was the study designed? How was the study carried out? And how was the data analyzed? Mention the following, in order of their appearance, and writing in past tense or passive verb.

1. Study type and study design e.g. randomized clinical trials, cross-sectional study, retrospective study, experimental study, cohort study, survey, etc. Investigators embarking on Randomized clinical trial reports should present information based on the CONSORT (Consolidated Standards of Reporting Trials) statement (http://www.consort-statement.org)

2. Place and duration of the study

3. The setting for the study

4. Sample size and sampling method

5. Inclusion and exclusion criteria

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7. Measurement of the outcomes/operational definition of variables

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procedures should be provided in detail to allow other workers to reproduce the results. Give references to established methods

9. Ethical approval and patient consent

10. Protocols are followed if any

11. Statistical analysis and computer software used

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1. Ethics committee approval (for both human as well as animal studies) from the respective institutions is obligatory for manuscript submission.

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State the conclusions that are linked with the objectives of the study, directly supported by the evidence and explore the implications of the findings for future research and for clinical practice.

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