Acute Otitis Media: A simple diagnosis, a simple treatment

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ABSTRACT

To assess the symptoms and signs of acute otitis media and efficiency of simple antibiotics like amoxicillin in its treatment in the primary health care setup.This is a prospective longitudinal study including 204 patients from different institutions. Patients were diagnosed as suffering from acute otitis media when presented with earache, fever, fullness and or otorrhea. Patients were divided into two equal groups on basis of the treatment they received, Group A received only symptomatic treatment while Group B were given Amoxicillin (40mg/kg/day) for 7 days.

Acute otitis media was common in children under 15 years (64.7%). Patients presented with earache (100%), aural fullness (90.68%), fever (76.47%) associated with recent onset of upper respiratory tract infections (88.23%). In group A, improvement was noticed in 28.43% in 3 days while 35.29% in 7 days. In group B, improvement was noticed in 48.03% in day 3 while 86.27% in day 7.In countries where medical care is scarce, patients lost to follow up, it is wise to treat with simple antibiotics like amoxicillin in adequate dose than to treat only symptomatically. It prevents chronicity, early hearing impairments and reduces antibiotic resistance.

Keywords: Acute Otitis Media, Amoxicillin, Antibiotic resistance, Earache

INTRODUCTION

Acute Otitis Media is a pyogenic infection of the middle ear cleft that last for less than 3 weeks. It is more common in infants and children and affects 70% of children at least once during their lifetime.[1]

Initially the infection starts with a viral upper respiratory infection. Within 24-48 hours, it is followed by bacterial infection.[2]This is mainly due to obstruction of eustachian tube that follows an upper respiratory tract infection. Besides nasal allergy, chronic rhinosinusitis, exposure to cigarette smoke, tumours of the nose and nasopharynx, cleft palate and breast feeding in supine position may contribute to the development of acute otitis media. These all directly or indirectly leads to the immobility of the cilia or edema of the eustachian tube leading to defect in ventilation and drainage of the middle ear cleft. The main organisms responsible are Streptococcus pneumoniae, Haemophilus influenzae and Moraxella catarrhalis.[3]

The main symptoms included are earache, fever, fullness, decrease hearing and spontaneous aural discharge in association with nasal congestion or nasal discharge. The course of the disease depends on the virulence of the infecting organisms, the host immune response and inadequate treatment with antibiotics. Unless there is an evidence of discharge present for culture and sensitivity, investigations are not necessary. This shows simple and straightforward symptoms and signs that are helpful for the diagnosis of acute otitis media even in the primary health care setup without expensive or sophisticated instruments.

There have been various recommendations concerning the treatment of acute otitis media and various guidelines have been published. Some withhold the use of antibiotics.[4] Evidence suggesting that routine use of antibiotics improves the course and outcomes of acute otitis media is weak.[5]

In country like ours, where antibiotics are available without prescription, higher antibiotics can be bought over the counter. Even some medical professionals prescribe higher antibiotics (2^{nd}, 3^{rd} generation cephalosporin, fluoroquinolones, macrolides etc.) for immediate relief or as a means of empirical treatment. This contributes to the rising prevalence of multidrug resistance of organisms mainly streptococcus pneumonia.

Antibiotic resistance has been declared a crisis by the World Health Organization, the Centers of Disease Control and Prevention.[6]Use of higher generation antibiotics unnecessarily in view of treating the disease early contributes to the antibiotic resistance.

The indiscriminate use of broad-spectrum antibiotics is associated with increasing bacterial resistance.[7] It has also come into view that excessive antibiotics in childhood have even been strongly associated with

subsequent obesity and inflammatory bowel disease later in life.[8]

MATERIAL AND METHODS

This is a prospective longitudinal study. This study was carried out in the Department of ENT in two medical colleges and teaching hospitals for the duration of 15 months from Ashad 2069 to Bhadra 2070. Total number of patient diagnosed during this period was 204. History and complete ENT examination was done after taking consent from the patient.All patients were examined by the author and diagnosed to have suffered from acute otitis media when they presented with earache, fever, fullness and or otorrhea. On the basis of these symptoms and signs using a simple instrument like otoscope, diagnosis was made.

INCLUSION CRITERIA

1. Recent onset of earache without previous episodes of similar illness within 6 months
2. Age more than 2 years
3. Patient who had not received any medications for the illness
4. Consent given

EXCLUSION CRITERIA

5. Patients who had received antibiotics
6. Children aged less than 2 years
7. Patients with previous history of similar episodes within last 6 months
8. Consent not given

The patients were randomly selected into two equal groups on the basis of odd and even numbers and the treatmentswere commenced. Group A patients received only symptomatic treatment with analgesic and decongestant while Group B patients were also given Amoxicillin (40mg/kg/day) for the duration of 7 days.

Patients were followed up on the 3rd and 7th day. Treatment failure was said to have occurred when patient presented with severe earache and fever (temperature > 38°c) even after 72 hours of commencement of medications.

Investigating the patient in the acute phase is generally painful and unhelpful.

Statistical analysis was done with MS Excel and SPSS ver 17.0 software using Fisher's exact test. Significance level was assessed by calculating two tailed p value. P value was labeled significant if it was equal to or less than 0.05.

RESULTS

Total number of patient suffering from acute otitis media was found to be 204. The patients were divided into two groups, Group A and Group B each consisting of 102 patients on the basis of treatment they received. It was found to be more common in children of 15 years and under (N=132, 64.7%) as shown in figure 1.Male (59%) were affected more than females (41%). 65.69% presented without perforation while 34.31% (N=70) presented with spontaneous discharging ear.

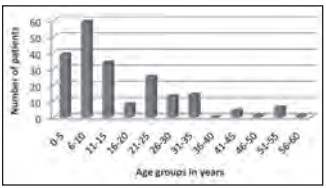

Figure 1. Age wise distribution

Figure 2 shows that most patients presented with unilateral earache (100%), aural fullness (90.68%, N=185), fever (76.47%,N=156) and aural discharge (34.31%) associated with recent onset of upper respiratory tract infections (88.23%, N=180). Diagnosis was based on red, hyperemic tympanic membrane (100%) with or without bulging and/or discharge on otoscopy.

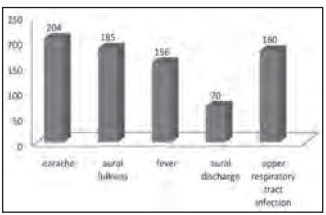

Figure 2. Presenting symptoms of Acute Otitis Media

Group A patient were treated only symptomatically without the use of antibiotics while group B patients were treated along with antibiotic (Amoxicillin:40mg/kg/day for children and 500 mg 8 hourly orally for adult for 7 days) upon diagnosis of acute otitis media.

As table 1 and 2 shows, in group A patients, improvement was noticed in only 28.43% (N=29) in 3 days while 35.29% (N=36) in 7 days. Similarly, in group B,

improvement was noticed in 48.03% (N=49) in day 3 while 86.27% (N=88) in day 7.

Table-1: Results at 3 days

	Group A	Group B	Total
Improved	29	49	78
Not improved	73	53	126
Total	102	102	204

p = 0.0060

Table-2: Results at 7 days

	Group A	Group B	Total
Improved	36	88	124
Not improved	66	14	80
Total	102	102	204

p = <0.0001

Of 70 patients with spontaneous perforation, 58 were of group A. Only 11.76% (N=12) had spontaneous perforation of tympanic membrane in group B. After spontaneous perforation of the tympanic membrane antibiotics were prescribed.Those not responding with adequate dose of amoxicillin until the 7th day, amoxicillin and clavulanic acid combination was given after which the patients improved. Not even a single patient had to undergo myringotomy in this study.

Number of patients improved with amoxicillin in the 3rd day compared to those with the symptomatically treated group was significant (p=0.006).

Moreover, significant improvement was seen within 7 days in patients who were prescribed with amoxicillin compared to those who were treated symptomatically. This was statistically significant (p=<0.0001).

No sequelae of the disease were noted. All tympanic membrane perforation healed within a month in both the groups. There was neither chronicity nor suppurative complications.

DISCUSSION

Acute otitis media is a relatively common disease of the middle ear. It affects all age group but more commonly children. Among the 204 patients in this study, 132 patients were 15 years and below. There was slight male preponderance (M=59%, F=41%).

Patients who presented with earache, aural fullness, fever with or without ear discharge with history of preceding upper respiratory tract infection and congested, hyperemic tympanic membrane on otoscopy was found to suffer from acute otitis media unless proven otherwise.

The bestpredictor of acute otitis media in otoscopic appearances typically is fullness or bulging of the tympanic membrane.[9]

The incidence of spontaneous eardrum perforation in acute otitis media varies from literature to literature. Ingvarsson[10] found it in 30% of patientswhereas; Pukander[11] at the other extreme reported it to occur in 4.6% of the patients with acute otitis media. In our study, spontaneous perforation occurred in 34.31% patients more in the group that did not receive the antibiotic.

Acute otitis media may be managed with antibiotics and analgesics or with observation alone depending on the severity. Clinicians should re-evaluate a child whose symptoms have worsened or not responded to the initial antibiotic treatment within 48 to 72 hours and change the treatment if indicated.[12]

Patients were divided into 2 groups on the basis of the treatment they received. Group A was treated symptomatically with analgesics and decongestants while group B patients were also given amoxicillin for the duration of 7 days.

Only 29 patients (28.43%) in group A showed improvement in 3 days while 73 patients had no improvement. By 7th day only 36 patients (35.29%) improved. Furthermore, of 70 patients with spontaneous perforation, 58 were of group A. In group B, improvement with respect to pain, fever was noticed in 49 patients (48.03%) in day 3 while 88 (86.27%) patients showed complete resolution in day 7. In this group, only 11.76% (N=12) had spontaneous perforation of the tympanic membrane. Compared to the treatment received in this group, patient showed significant improvement at day 7 than at day 3.

Mygind et al[13]found decreased pain in the penicillin group compared with the placebo group on day 2 but no difference for duration of fever, otorrhea or effusion up to 3 months.

In a study by Howie and Ploussard[14], all case patients receiving placebo were asymptomatic at early follow-up(2 to 7 days) but had more positive tympanocentesis cultures than those receiving antimicrobials.

Overall results, together with other earlier studies of acute otitis media, 3 days antimicrobial treatment appeared less effective than 7 days treatment. Various studies of acute otitis media and otitis media with effusion in which outcomes also were less favorable in younger than in older children, suggest that 3 days treatment will often prove inadequate for acute otitis media.[15,16] Amoxicillin is most effective against acute otitis media caused by Streptococcus pneumonia and Haemophilus influenzae. In high risk children (less than two years of age, in day care or received antibiotics recently)

who are likely to have drug resistant Streptococcus pneumonia (resulting from excessive antibiotic use) increasing the dose of amoxicillin to 80-90mg/kg/day increases the likelihood that drug concentration will exceed the MIC for more than 40 percent of the time. Failure to respond to amoxicillin suggests that the child is infected with beta-lactamase producing organisms and amoxicillin/clavulanate is the treatment of choice in this circumstances.[17]

This study concludes that patients with acute otitis mediainvariably presents with earache, aural fullness, fever, with or without ear discharge with history of preceding upper respiratory tract infection. Otoscopic examination reveals hyperemic tympanic membrane with retraction or bulge. In developing country like ours, where medical attention and care is scarce and patients are often lost to follow up, the treatment initially should be started with simple broad spectrum antibiotics like amoxicillin in adequate dose and duration. Either inadequate dose or duration is the cause for treatment failure. Such a measure reduces chronicity, prevents early hearing impairments and prevents unforeseen complications as in pre-antibiotic era. The use of higher generation antibiotics should be condemned. Patients, parents should be educated about the care of ear, antibiotic resistance and tackle the problem from community level by stopping inappropriate use of antibiotics.

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Clinical and Contact Allergological Observations on Hand Eczema: A Descriptive Study

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ABSTRACT

Hand eczema (HE) is a common and distressing condition that is perplexing to the patient and the physician alike. To study the frequency and clinical features of hand eczema and to correlate the frequency of atopy and contact sensitization with different clinical features A total of 61 clinically diagnosed patient of Hand eczema were included within a period of one year. Patch test was done in 47 patients and graded accordingly.The frequency of hand eczema was 0.57%. Morphologically pompholyx was the most common type while aetiologically endogenous hand eczema was the commonest. Contact allergy was observed in 55.3% of the cases of which nickel sulphate (18.5%) was found to be the commonest sensitiser followed by Gentamicin and Fragrance mix Though contact allergens with positive patch test in different morphological types of hand eczema have no apparent relevance but it still could contribute to the persistence or exacerbation of hand eczema.

Key words : Hand eczema; classification; patch test positivity; nickel; atopy

INTRODUCTION

The term hand eczema (HE) implies that the dermatitis is largely confined to the hands with only minor involvement of the other areas. 1 The reported prevalence of HE in the general population is estimated to be about 2-10% 1,2 and it accounts for 21-34% of all types of eczema.3It is difficult to subclassify HE as it is a multifactorial disease in which both exogenous and endogenous factors play a role1

Although most cases of hand eczema are of a patchy vesiculo -squamous nature without any special characteristics, about one third of cases present particular patterns that deserve special recognition. Clinically Li and Wang have divided HE into 5 groups: (1) vesicular form, (2) fissured form, (3) hyperkeratotic form, (4) hand and foot dermatitis and (5) pompholyx. 4

Atopy and especially atopic eczema are well known endogenous factors influencing the course and prognosis of HE 1 Contact allergens are the commonest exogenous cause of HE and 17 % of the HE may be precipitated by contact with chemicals that elicit an allergic reaction. 5

Patients with HE are well known to have impaired quality of life and it often leads to frequent dermatological consultations. Lack of study from Nepal had prompted us to undertake this study with the aims to know the frequency and clinical features of hand eczema. The frequency of atopy and contact sensitization in hand eczema with different clinical features was also correlated.

MATERIALS AND METHODS

Patients

This was a hospital based descriptive study in which all clinically diagnosed cases of hand eczema attending the Dermatology Out Patient Department of B. P. Koirala yInstitute of Health Sciences, Dharan over March 2010-Feb 2011, constituted the study population.

Other skin diseases involving the hand, such as infective dermatitis, dermatophytide, eczematous drug reactions, psoriasis and cummulative insult dermatitis were excluded by history and clinical examination.

A detailed history of each patient was recorded in the proforma designed for the study. A complete clinical examination was done in all patients about sites involved, morphology and a tentative clinical diagnosis was made and classified according to the criteria laid down by Li and Wang. [4] The study was approved by the institutional review board and the ethical committee.

Patch test

Patch test was done in all patients of hand eczema using the Indian Standard Series of Allergens including plant allergens as approved by the Contact and Occupational Dermatoses Forum of India. (CODFI).

Finn chambers were used and allergens, usually incorporated in petrolatum, were applied in round chambers of inert material (aluminum, polyethylene), which were mounted on adhesive tapes free from colophony. For volatile solution a drop of test material or aqueous solution (0.05 ml) on filter paper was applied immediately before patch testing.

For plant antigens others than the ones approved by the CODFI, 1 cm2 of leaf or 1 cm length of stem or root was mounted on the Finn chamber. The vegetable antigens were also crushed and applied similarly. Substances, which were likely to produce irritant reactions under occlusion in the standard patch test, were tested by the open patch test technique. Chemicals or cosmetics were painted in a 2 cm2 of the skin in the same concentration as present in the original product.

Patch tests were applied on the upper half of the back after cleaning the area with spirit and the results were recorded at 48 hours and 96 hours.

Statistical analysis

Data was tabulated and interpreted in terms of percentage, mean and standard deviation in the computer using SPSS version 10.0. To test the significance of association Chi square test was applied.

RESULTS

Patients demographic and baseline characters are shown in the Table 1.

Table 1: Demographic and baseline characteristics of Hand Eczema patients

Variables	No of patients (%)
➢ **Sex**	
• Male	20 (32.8)
• Female	41 (67.2)
➢ **Age**	
• Mean	33.9 ± 14.42
• Range	13-70
➢ **Duration (months)**	
• Mean	34.2 ± 37.64
• Range	1-120
➢ **Occupation**	
• Student	11 (18.0)
• Farmer	9 (14.8)
• Businessman	5 (8.2)
• Shopkeeper	4 (6.6)
• Teacher	2 (3.3)
• Doctor	2 (3.3)
• Press-worker	1 (1.6)

History suggestive of bronchial asthma, allergic rhinitis, atopic dermatitis and allergic conjunctivitis was specifically sought for each patient. A personal history of atopy was present in 15 (24.5%) cases, 5 (8.1%) of them had bronchial asthma, 11 (18.0%) allergic rhinitis, 5 (8.1%) atopic dermatitis and 4 (6.5%) allergic conjunctivitis.

Clinically on evaluating the sites of lesions, sides of fingers were involved in 51 (83.6%). (Fig 1)

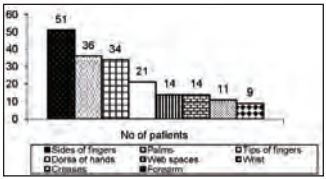

Fig 1: Site of lesions in patients with hand eczema

Regarding the morphology of lesions, scaling and vesiculation were seen in more than half of the patients i.e. 49 (80.3%) and 47 (77%) respectively followed by erythema and fissuring in 38 (62.3%) and 36 (59.0%) patients respectively (Table 2).

Table 2: Morphology of the lesion in patients with hand eczema

Morphology of lesion	No of patients (%)
Scaling	49 (80.3%)
Vesicles	47 (77%)
Erythema	38 (62.3%)
Fissuring	36 (59.0%)
Papulovesicles	30 (49.2%)
Dryness	29 (47.5%)
Plaques	21 (34.4%)
Hyperlinearity	14 (23.0%)
Papules	10 (16.4%)
Hyperkeratosis	5 (8.2%)
Swelling	3 (4.9%)

Most of the lesions were bilaterally distributed in 58 (95.1%) cases followed by unilateral distribution in 3 (4.9%) patients. The lesions were asymmetrical in 46 (76.4%) patients and symmetrically distributed in 15 (24.6%) patients. Nail involvement was seen in 8 (13.1%) patients of which chronic paronychia was seen in 7 (11.5%) and irregular pitting in 1 (1.6%) patient.

Morphological Classification

On morphological classification of hand eczema, most of the patients 31 (50.8%) were diagnosed as having pompholyx. (Fig 2)

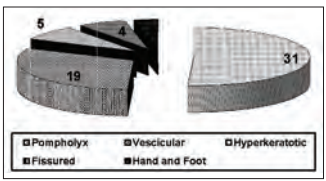

Fig 2: Morphological classification of hand eczema

Morphological Classification and sex

Morphologically, pompholyx was more in the females 21 (51.2%) as compared to 10 (50.0%) in males. (Fig 3)

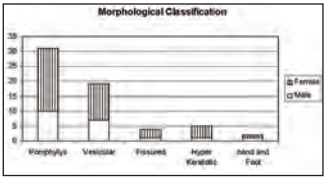

Fig 3: morphological classification and sex Aetiological Classification

Aetiologically, of all the 61 cases of hand eczema, 38 (62.3%) cases were diagnosed as having endogenous eczema while exogenous hand eczema was diagnosed in 23 (37.7%) cases with a ratio of 1.6:1. (Table 3)

Table 3: Aetiological classification and sex

Sex	Endogenous	Exogenous
Male	9 (45.0%)	11(55.0%)
Female	29 (70.7%)	12(29.3%)

Aetiological Classification and sex

Patch Testing

Patch testing was done in only 47 out of the 61 patients included in this study. The remaining patients either were in the acute dermatitis stage at the time of inclusion and were asked to return after 6 weeks or those who did not give consent for the patch test.

Out of the 47 patients who were patch tested, 26 (55.3%) were patch test positive at 48 as well as 96 hours. Out of the 47 patients, 17 (65.3%) had patch test positivity for more than 1 allergen. Nickel sulphate was the most common sensitizer, positive in 5 patients (18.5%). (Table 4)

Table 4: Common allergens in patients with Hand eczema

Allergen	No of Patients (%)
Nickel sulphate	5(18.5%)
Gentamicin	4(14.8%)
Fragrance mix	4(14.8%)
Epoxy resin	3(11.1%)
Potassium dichromate	3(11.1%)
Cobalt chloride	2 (7.4%)
Neomoycin sulphate	1 (3.7%)
Parabens	1 (3.7%)
Formaldehyde	1 (3.7%)
Mercapto mix	1 (3.7%)
Balsam of peru	1 (3.7%)
Nitrofurazone	1 (3.7%)

Patch Test with sex

By correlating patch test positivity with sex, 14 females (53.80%) showed PTP as compared to 12 (46.20%) males (Table 5)

Table 5: Patch test positivity (PTP) and sex correlation

Allergen	Sex	
	Male	Female
Nickel sulphate	2(15.4%)	3(21.4%)
Neomoycin sulphate	0(0.0%)	1(7.1%)
Cobalt chloride	0(0.0%)	2(14.3%)
Parabens	0(0.0%)	1(7.1%)
Formaldehyde	0(0.0%)	1(7.1%)
Gentamicin	2(15.4%)	2(14.3%)
Mercapto mix	0(0.0%)	2(14.3%)
Epoxy resin	3(23.1%)	0(0.0%)
Potassium dichromate	3(23.1%)	0(0.0%)
Fragrance mix	3(23.1%)	1(7.1%)
Balsam of peru	0(0.0%)	1(7.1%)
Nitrofurazone	0(0.0%)	1(7.1%)

PTP and Etiological and Morphological Diagnosis

PTP was more common in the exogenous HE 20 (76.90%) as compared to the endogenous HE 6 (23.10%).

Twelve (46.2%) patients with pompholyx, 10 (38.5%) patients with vesicular, 2 (7.7%) in the fissured group, 1 (3.8%) patient with hyperkeratotic and 1 (3.8%) in patients with Hand and foot dermatitis showed PTP

PTP and Occupation

A total of 5 (19.2%) students showed PTP as compared to 4 (15.4%) each in farmers and businessmen. Table 6

Table 6: Patch test positivity and occupation

Occupation	Patch test positivity (PTP)	
	Positive	Negative
Housewife	3(11.5%)	9(42.9%)
Farmer	4(15.4%)	3(14.3%)
Businessman	4(15.4%)	1(4.8%)
Staff nurse	2(7.7%)	4(19.0%)
Student	5(19.2%)	2(9.5%)
Shopkeeper	2(7.7%)	-
Labourer	2(7.7%)	1(4.8%)
Teacher	2(7.7%)	-
Pressworker	1(3.8%)	-
Doctor	1(3.8%)	1(4.8%)

Chi square test was used to find the association with the morphological types of HE and PTP and it was not found to be statistically significant in the various groups. No significant association was seen between positive patch test and sex, age, atopy, and occupation.

Atopy was present in 10 patients with pompholyx, 5 patients with vesicular hand eczema and 1 patient with hyperkeratotic hand eczema.

Association of atopy with age, sex, occupation and the different morphological types of hand eczema was done using the chi-square test. Significant correlation was observed between pompholyx and atopy (p=0.001). However associations with sex, age, occupation and vesicular hand eczema were not statistically significant.

DISCUSSION

Hand eczema (HE) implies to the dermatitis that is largely confined to the hands with only minor involvement of the other areas. in which endogenous, exogenous and environmental factors are often interwoven.1

The reported prevalence of HE in the general population is estimated to be about 2-10%.1,2 and it accounts for 21-34% of all types of eczema 4 The low prevalence of 0.57% in this study could be explained due to the less health seeking nature of the patients, the non-occupational setup of the study and the strict inclusion criteria.

It is difficult to sub classify HE according to the morphological and etiological classification and no single classification of HE is satisfactory. 1

Pompholyx, accounting for 5 - 26.9% of all cases of hand eczemas; considered to be more symptomatic, recurrent and severe than the other hand ezcemas, could explain the greater number of patients seeking medical treatment in our study.

Endogenous hand eczema is reported to be twice as common as the exogenous type 5 and observed female preponderance.

Atopy and especially atopic eczema are well known factors influencing the course and prognosis of HE.1,6 We found that 32.2% of patients with pompholyx had history of atopy and was statistically significant.

Patch testing has proved a useful tool for the detection of allergic contact dermatitis and identification of contact allergens and more than half of the patients with the vesicular form of HE showed positive PT results, supporting the hypothesis that most of vesicular HE is allergic contact dermatitis.5 Previous studies have shown 28%-78.5% patients with pompholyx have positive patch test with the standard series.6,7,8,9,10 as compared 38.7% in our study.

Contact allergens are also important as 17% of the HE may be precipitated by contact with chemicals that elicit an allergic reaction.5 Nickel is the most common cause of ACD in women in almost all countries, affecting 20% of young women in some series.12 In our study too the commonest positive patch test reaction was also to nickel sulphate followed by fragrance mix and gentamicin.

The most common allergens implicated in pompholyx are nickel, cobalt, balsam of peru, fragrances, neomycin, colophony, and ethylenediamine. Nickel has been reported to be the most common (20-33%) allergen.10,12 In our study also nickel sulphate was more commonly positive in pompholyx followed by fragrance mix. It is usually suspected, as ACD being the causative factor as more than half of the patients with the vesicular form of HE showed positive patch test results.5 Similar observation has been made in our study where vesicular form of HE had shown PTP in 38.5%. As the fissured form can also be caused by occupation, irritant or allergens, the commonest allergen in our study were gentamicin and potassium dichromate.

Endogenous factors may play more of a role than contact hypersentivity in the hyperkeratotic HE 5. Patch test positivity in patients of hyperkeratotic eczema have been variably reported in different studies, with some showing high rates (up to 56%) and others very low rates.10,13,14 Only 20% of our patients with hyperkeratotic HE showed a positive patch test. Nickel sulphate was the only common allergen that was positive in the hyperkeratotic HE and hand and foot HE, while hand and foot HE also showed positivity to cobalt chloride.

However the patch test positivity with the different contact allergens showed no significant correlation between the various types of hand eczema. Personal or family history of atopy also failed to show any significant correlation with PTP.

It may be concluded from this study that contact allergen may play a role in the etiology of different types of hand eczema specially the vesicular type. A personal or family history of atopy has a positive correlation in the endogenous HE only. Contact allergens with positive patch test in different morphological types of hand eczema have no significant relevance but it still could contribute to the persistence or exacerbation of hand eczema Further studies in larger number of patients are therefore necessary to determine the relationship between atopy and contact sensitization among the different morphological and etiological types of hand eczema.

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Reduced susceptibilityto Vancomycin in methicillin resistant *Staphylococccusaureus*: a time for action

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ABSTRACT

Infections by Methicillin resistant *Staphylococcusaureus* (MRSA) is an often encountered therapeutic challenge. The problem is accentuated by the emergence of MRSA strains which are resistant to Vancomycin, the recommended agent for the treatment of MRSA infections. We therefore carried out this study to determine the MIC values of vancomycin for the MRSA isolated from different clinical specimens in Nepal Medical College. MICs were determined by agar dilution method. Out of the 82 MRSA isolates tested, 18 showed MIC of 2µg/ml and 29 isolates had MIC of 1µg/ml and 35 isolates had MIC of 0.5 µg/ml.Although none had a MIC in the intermediate or resistant zone, 18 (2.9%) had MIC in the upper limit of the sensitive zone which is a matter of concern and calls for prompt preventive actions.

Keywords: Methicillin Resistant *Staphylococcus aureus*, Vancomycin, Minimum Inhibitory Concentration

INTRODUCTION

Staphylococcus aureus is one of the important human pathogen. Infections by MRSA have a higher morbidity and mortality and are a treatment challenge. Vancomycin, a glycopeptideantimicrobial, is the first line treatment for infections caused by MRSA.[1] Breakpoints have been developed byClinical Laboratory Standards Institute (CLSI) to define vancomycin susceptibility for S. aureus. Until 2006, the Minimum Inhibitory Concentration (MIC) for vancomycinIntermediate Staphylococcus aureus (VISA) was defined as 8 to16 µg/ml by the CLSI.[2] However, when the vancomycin MIC was 4 to 8 µg/ml high rate of treatment failure occurred. As a result, in 2006, CLSI modified the breakpoint and currently vancomycin susceptibility is considered when the MIC is 2µg/ml.[3] MRSAisolates with MIC 16 µg/ml are considered vancomycin resistant and with MIC of 4 to 8 µg/ml are intermediate.[4] Even when the MIC value is at the limit of the susceptibility range there may be presence of heteroresistance which may result in clinical failure.[3] High vancomycin MIC also correlates with resistance to several other classes of antimicrobial agents. This evolutionary trend may cause potential failure of treatment of S. aureus infections which necessitate further research and regulation of health policies.[5]

MATERIALS AND METHODS

This study was carried out from September 2012 to April 2013. Eighty two clinical isolates of MRSA as confirmed by cefoxitin (30 µg) disc diffusion test were tested for the MIC for vancomycin by agar dilution method as recommended by the CLSI. The test organisms were inoculated in nutrient broth and incubated for 4 hours at 37 ºC. The turbidity was adjusted to 0.5 McFarland unit. It was then inoculated intoMuller Hinton agar plates supplemented with 2% NaCl and containing different concentrations of vancomycin (0.25 µg/ml , 0.5 µg/ml, 1 µg/ml, 2 µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml and 32 µg/ml).After overnight incubation at 37ºC the MIC for each isolate was noted.

RESULTS

Among the82 MRSA isolates tested, all had MIC for vancomycin within the susceptible range. However, MIC towards the upper limit of the susceptible range (2µg/ml) was found for 21.9% of the isolates (Table-1).

Table-1: MIC values of vancomycin for MRSA

MIC (µg/ml)	Number of isolates (%)
0.25	0 (0)
0.5	35 (42.68)
1	29 (35.3)
2	18 (21.9)

According to the source of origin, inpatients accounted for higher number of MRSA and also higher number of strains with higher MIC of vancomycin (Table-2).

Table-2: MIC of vancomycin among MRSA isolates from inpatients and outpatients

MIC of vancomycin (µg/ml)	Inpatient	Outpatient
0.5	17	10
1	20	11
2	13	11
Total	50	32

DISCUSSION

Vancomycin wasintroduced clinically in 1958 for the treatment of gram positive bacteria. Its use has increased dramatically due to the increase in the prevalence of methicillin resistance in both coagulase negative staphylococci and Staphylococcus aureus.[6] The first report of decreased susceptibility to vancomycin in Staphylococcus aureus (VISA) came in 1997 from Japan.[7] Since then reports from around the world are emerging. No vancomycin intermediate or resistant strains were found in the current study. Nevertheless, it is worrisome that 22% of the strains had the MIC in the higher limits of microbial susceptibility. Clinical failure due to hetero resistant strains are likely in infections caused by strains with elevated MIC. In a study 66 patients with vancomycin MICs of ≥1.5 mg/liter had a 2.4-fold increase in failure compared to patients with MICs of ≤1.0 mg/liter.[8] Although some studies from abroad have reported the MIC for vancomycin for MRSA similar to our study (2µg/ml),[9] others have found different prevalence of VISA and VRSA among their clinical isolates. Two strains of VRSA and six strains of intermediate (VISA) were reported from Northern India.[10] Song et al[11] reported 6.3% VISA among the MRSA and Thati et al[12] reported 1.9% VRSA among their clinical isolates. No VISA or VRSA have been reported as yet from Nepal. However, this study was prompted by the author's experience in tertiary care hospital in Lalitpur, Nepal where 9.5% prevalence of VISA among the MRSA was recorded.

The possible mechanism behind the vancomycin resistance in staphylococcal isolates could be the thickening of cell wall in resistant isolates.[13] Recent exposure to vancomycin within one month of the current infection, prior recent hospitalization, surgery within last 6 months and those with blood stream infections prior to admission in intensive care unit may result in MRSA infection with higher vancomycin MIC.[14]

VRSA are resistant to large number of currently used antimicrobial agents compromising the treatment options and increasing morbidity and mortality.[12] In our study all the MRSA were multidrug resistant and among them a large number of isolates with MIC of vancomycin 2 µg/ml were resistant to higher number of drugs.

The emergence of VRSA/VISA may be due to selection pressure. The huge scale development and subsequent spread of resistance to vancomycin is a fearsome threat to the already challenging therapy of MRSA.[15] Strong organizational support and multiple strategies are required for the containment and prevention of MRSA and thus of VISA/VRSA. Infection control practices that have been documented to reduce the MRSA spread include: adherence to hand hygiene, contact precautions for patients with MRSA,active surveillance cultures, education, effective environmental cleaning and communication between healthcare workers and patients with MRSA.[16]

Thus this study is an early alarm to all stakeholders to take adequate and timely measures to stop the emergence of VISA/VRSA. Strict infection control practices must be religiously followed. Regular education of the staff and monitoring of compliance are must. Since the 30mg vancomycin disc diffusion test often misclassifies the intermediately sensitive isolates as fully susceptible,[17] microbiology laboratories must determine MICs for vancomycin and communicate the results to the treating doctors. "A stitch in time saves nine"- now is the time for appropriate action.

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Effectivity of Nd yag pi in treatment of acute primary angle closure glaucoma

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ABSTRACT

A Prospective hospital based study to note the efficacy of Nd Yag Peripheral Iridotomy (PI) in the treatment of acute primary angle closure glaucoma was carried out in Nepal Eye Hospital from Jan 2007 to Jan 2008. All the Patients(n=50) with acute primary angle closure glaucoma admitted to our hospital were selected for the study. Patients with secondary angle closure glaucoma were excluded. It is more common in age of 56-65 years (20%), in females (70%), and in tibetoburman ethnic group (56%). Mean duration of presentation to hospital was 5 days (22%) (Range 4-7days).Grade 1 Angle closure was present in 74%. All 50 patients (100%) with AACG had undergone Yag PI. Out of 50 patients, 11 patients (22%) were surgically operated i.e. trabeculectomy .Among 11,1 patient (9%)who had undergone trabeculectomy had presented with acute on chronic angle closure glaucoma .Majority of cases(66%) presented with visual acuity(VA) 1/60-PL at the time of presentation and the Intraocular pressure(IOP) in affected eye was 31-40mmHg (42%).After performing Yag PI the mean visual acuity in the affected eye at the time of discharge was 6/60 (20%) and the IOP was 12 mmHg (40%). Prolonged duration of attack, elderly age, acute on chronic angle closure glaucoma, very high IOP at presentation, patients needing repeat Yag PI were found to have failure Yag PI. In this study 78% eyes had controlled IOP following Yag PI .

Keywords: Acute primary angle closure glaucoma, Nd Yag PI, IOP control.

INTRODUCTION

Glaucoma is one of the leading causes of irreversible blindness in the world. It affects approximately 65 million people around the world and an expected 7.5million are blind due to this disease. It is the second most common cause of blindness worldwide.[1] It is estimated that half the blindness from glaucoma in the world is caused by angle closure and it is one of the causes of bilateral blindness.[2] Although it affects less than 10 percent of patients with glaucoma, acute narrow angle glaucoma is the most serious form of the disease. In the United States, fewer than 10% of glaucoma cases are due to angle-closure glaucoma. In Asia, angle-closure glaucoma is more common than open-angle glaucoma. In Acute angle closure glaucoma (AACG) the iris quickly covers the entire or almost the entire trabecular meshwork leading to sudden symptomatic elevation of intraocular pressure. AACG predominately affects females because of their shallower anterior chamber. As people age, the lens of the eye enlarges and pushes the iris forward, thus increasing the risk for angle-closure glaucoma. Acute angle-closure glaucoma is an emergency because optic nerve damage and vision loss can occur within hours of the onset of the problem. There is irreversible damage to optic nerve head and visual field loss in cases of glaucoma leading to irreversible blindness. So more emphasis has to be given for early diagnosis. Acute angle closure glaucoma is ocular emergency and receives distinction due to its acute presentation, need for immediate treatment. Rapid diagnosis, immediate intervention have profound effects on patient outcome and morbidity. In Nepalese population the effectivity of Yag PI has not been studied till yet .Therefore this study would provide baseline suggestion in the management of AACG .This study was to assess the demography, presenting signs and symptoms, IOP control, Improvement of visual acuity, effectivity of Yag PI and various causes of ineffectiveness of Yag PI.

MATERIALS AND METHODS

Fifty patients presenting to the glaucoma department of Nepal Eye hospital with uniocular AACG during a 24 month period were included in the study. With the verbal informed as well as written consent from the patients with AACG were included in the study. Patients with secondary angle closure glaucoma like phacomorphic glaucoma, malignant glaucoma were excluded. Initial examination included assessment of Snellen corrected visual acuity , intraocular pressure by Applanation tonometry, gonioscopy by Goldmann single mirror goniolens, Anterior segment was examined by Haag Striet 900 slit lamp and fundus examination by 90D lens.

Following a diagnosis of uniocular AACG the following treatment was administered: Mannitol 1gm/kg body weight intravenously over 45 minutes followed by oral acetazolamide 250 mg 6 hourly by mouth,pilocarpine drops 2% 6hourly to the affected and fellow eye, timolol eyedrops 0.5% 12 hourly to both eyes.Corticosteroid mixed antibiotic eye drop was installed in the affected eye 6 hourly. The intraocular pressures were measured every 2 hours until they fell below 21 mm Hg.Following initial medical control of IOP, Nd Yag PI was performed by single surgeon. The person sits in a special chair with his chin resting on a frame or support to prevent head from moving. After the anaesthetic has taken effect, laser beam (3-5mJ) was exposed into affected eye through the Ahamed iridotomy contact lens which is placed on the cornea. A treatment site was chosen in the superior iris, in a crypt where present and repeated until patency was achieved. Patency was assessed by direct visualisation of the posterior chamber.And the same procedure was repeated in the fellow eye. All patients had an intraocular pressure measurement performed 24 hours after treatment and slit lamp examination to assess patency of yag PI. After the procedure corticosteroids mixed antibiotics were given in both eyes 6 hrly for 7days, pilocarpine eye drops was stopped and oral acetazomalide 6hrly was given to control post laser IOP rise for 2 days. After stoppage of antiglaucoma drugs IOP and best corrected visual acuity were recorded at the time of discharge (5-7days of admission). PI was considered effective if IOP was controlled (≤21 mm Hg) by iridotomy alone. In contrast, when trabeculectomy was needed to control IOP (≤21 mm Hg) PI was considered to have failed.

STATISTICAL ANALYSIS

All the data were collected and entered in Microsoft Excel. Statistical Analysis was performed using SPSS version 11.5 software. Significance was set as P<0.05.

Table-1: Age distribution of patients with acute primary angle closure glaucoma

Age of Patients	n. (%)
35-45 years	7 (14)
46 -55 years	15 (30)
56-65 years	20 (40)
66-75 years	6 (12)
76-85 years	2 (4)
Total	**50 (100)**

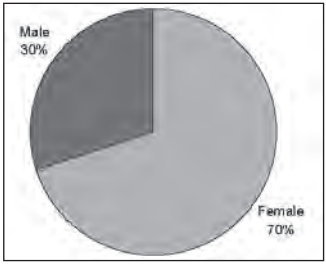

Fig. 2. Sex Distribution of Patients with Acute primary angle closure glaucoma

RESULTS

Fig 2. shows the sex distribution of patients (n=50) .Among them female were 35(70%) and male were 15(30%). The age of patients ranged from 46 years to 85 years. Table-1 shows age wise distribution of patients admitted in hospital. The maximum number of patients was within 56-65 years age group (40%). Out of 50 patients, 28 were Tibetoburman (56%) and 22 (44%) were Indoaryan. Table-3 shows the symptoms of the patients .All presented with Diminish vision, 49(98%) presented with headache, 39 patients (78%) with vomiting and 18 patients (36%) presented with coloured haloes. Twenty nine patients (58%) were presented on 4-7 days of attack of angle closure, followed by 10 patients (20%) on 1-3 days and 1 patient (2%) presented on 20-23 days. Only 5 patients (10%) had previous attack of glaucoma whereas 45 patients (90%) had no previous attack of glaucoma. Majority 49 patients (98%) had no positive family history. Only 1 patient (2%) had positive family history. Out of 50 patients, 13 patients (26%) had hypermetropia.And no patient had myopia. Out of 50 patients, 33 patients (66%) had cup disc ratio of 0.3:1 followed by 14 patients (28%)had cup disc ratio of 0.4:1.One patient (2%) had cup disc ratio of 0.5:1, another had 0.6:1 and other had 0.8:1. On gonioscopic examination 37 patients (74%) had Grade I angle closure.

Table-3: Presenting symptoms of acute angle closure glaucoma

Presenting Symptoms	n. (%)
Headache	49 (98)
Vomiting	39 (78)
Diminish Vision	50 (100)
Coloured haloes	18 (36)

Eight patients (16%) had Grade 0 angle closure and 5 patients (10%) had Grade II angle closure. Eleven cases (22%) had Peripheral Anterior Synechiae (PAS)<180 degree. Table-4 shows the visual acuity of affected eye at the time of admission. Nine cases (18%)had the VA of >6/60 on the affected eye at the time of presentation. Eight cases (16%)had VA in the range of 5/60-2/60. Majority 33 cases(66%) had VA in the range of 1/60-PL. Out of them 14 cases(42%) had a VA of HM,followed by 11 cases (33%)having counting finger close to face,4 cases(12%) had 1/60 and 4 cases (12%) having perception of light at the time of presentation. Fig. 5 shows the visual acuity of affected eye at the time of discharge. Forty six patients (92%) had VA > 6\60 in affected eye at the time of discharge followed by 4 patients (8%) had VA <6/60.Table-6 shows the IOP of affected eye at the time of presentation. Majority of patients 21(42%) presented with IOP in the range of 31-40 mmHg at the time of presentation. Fourteen cases(28%) with IOP 41-50mmHg,12(24%) with IOP of 51-60mmhg,2(4%) in the range of 21-30 mmhg and 1(2%) presented with IOP of 61-70mmhg.At the time of discharge all 50 patients(100%) had IOP in the range of 10-20mmhg on the affected eye. Table-7 shows the treatment received by the patients with APACG.Out of 50 cases, 10(20%) needed repeat PI,11 (22%) had undergone trabeculectomy .PI was effective in 39 patients(78%) and was failed in 11patients (22%).

Table-4: Visual acuity of affected eye at admission with acute primary angle closure glaucoma

VA at admission affected Eye	n. (%)
6/12	1 (2)
6/24	2 (4)
6/36	3 (6)
6/60	3 (6)
5/60	1 (2)
4/60	1 (2)
3/60	1 (2)
2/60	5 (10)
1/60	4 (8)
Finger counting at 1 m	11 (22)
Hand Movement	14 (28)
Perception of light	4 (8)
Total	**50 (100)**

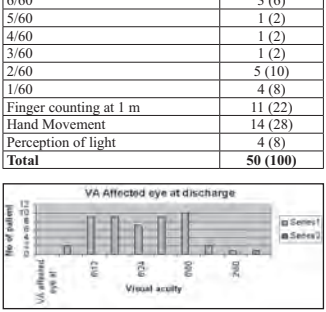

Fig. 5. Visual acuity of affected eye at the time of discharge

DISCUSSION

Out of 50 patients enrolled, the age was grouped from 35-85 years**.** It was seen that the youngest patient was 37 years and oldest was 84 years. Most of the patients reported were in 56-65 years (n=20) (40%) which is similar to the sudy done by Lim [the] average age affected by ACG was 61.5 +/-10.6 years.[3] Similar result was shown by Mazhar Ammar where ACG was more common in 5th decade of life. [4] In a study conducted by S Senthil the incidence of ACG was more in above 40 years.[5] Hence in view of these studies it is concluded that ACG is more common in older age. In this study 20 patients were within 56-65years, out of which 16 patients had effective PI and 4 patients had failed PI.7 Patients with age of 35-45years out of which, 4 patients had effective PI and 3 had failed PI.46-55 years of 15 patients, 13 had effective PI, 2 had failed PI. Of 66-76 years out of 6, 5 had patent PI and 1 had failed. At age range of 76-85 1 patient had effective PI and 1 had failed PI. In S a Buckley study one of the factor for failure of Yang PI was elderly age group[6] .But in this study there was no statistically significant correlation between age of presentation and the effectivity of Yag PI (p=0.47).

In this study out of 50 patients, Female were 35 (70%) and male were 15 (30%). Female were 2.1 times more affected than the male.

Similar result was found in Bojic L study where 122 were women and 54 were men. The relative risk of developing ACG was 2.1 times higher for women as compared to men.[7]Various other study also showed female preponderance. Ivanisevic study showed that ACG affects female twice more than male.[8] Lai study found that ACG is 3.8 times more common in female than in male.[9] Hence Female is regarded as a risk factor for Acute Angle Closure Glaucoma.

Table-6: Intra ocular pressure of affected eye at admission with acute primary angle closure glaucoma.

IOP affected eye at admission	n. (%)
21-30	2 (4)
31-40	21 (42)
41-50	14 (28)
51-60	12 (24)
61-70	1 (2)
Total	50 (100)

Table-7: Treatment Received by patients with acute primary angle closure glaucoma

Treatment	n. (%)
Nd Yag PI	50 (100)
Repeat PI	10 (20)
Trabeculectomy	11 (22)

Regarding the ethnic distribution out of 50 patients, 28 (56%) were Tibetoburman and 22 (44%) were Indoaryan. Incidence and ethnic distribution of glaucoma in patients attending Kedia eye hospital, Birgunj was studied by Amar Deuja. However his study showed no clear ethnic preponderance of any type of glaucoma [10]. Similar study was done by Ramesh S et al about Ethnic aspect of ACG at Bolton hospital which showed that Chinese ethnicity were at risk for ACG.[11] In Tin Aung study out of 90 patients all were Asian and 78 patients were Chinese (86.7%).[12]Hence this study concluded that ACG is more common in patients with small eye.

Regarding the association of Angle closure glaucoma patients with respect to family history showed that out of the total 50 patients 49 patients (98%) did not have family history of glaucoma. Only 1 patient (2%)

had positive family history. Ronald F studied about Positive Family history in Angle closure glaucoma. Over 300 patients of ACG were questioned carefully about the disease in their families. Positive Family histories are very uncommon which was similar to this study.[13]

Most of the patients were presented within 4-7 days of the onset of attack, n=29 (58%). The median time of presentation to hospital in this study was 5 days. In DC Saunder study the mean duration of symptoms was 4-7 days[14] . In Bojic L et al study the median time from the onset of symptoms to presentation at the hospital was 2 days (range 1-15 days)[7]. S A Buckley study showed that prolonged duration of attack was factor for failure of Yag PI.[6] But In this study association between duration of presentation and effectivity of PI was statistically insignificant (p=0081).

In this study out of 50 patients there were 45 patients (90%) who did not have previous attack of Angle closure glaucoma and 5 patients (10%) had one episode of previous attack of angle closure. Patients with Previous attack of ACG, out of 5 patients 2 patients had effective Yag PI and 3 had failed PI. There was no statistically significant (p=0.064) association between previous attack of ACG and effectivity of PI.

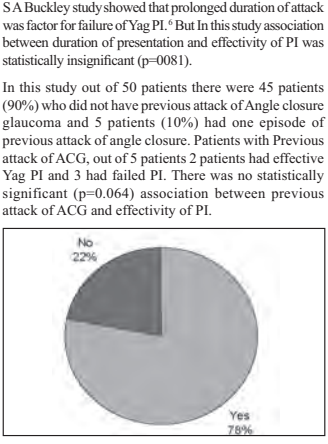

Fig. 8. Effectivity of Yag PI

Out of 50 patients, 41patients (82%) presented with VA <6/60 at the time of presentation and 9 Patients (18%) with VA >6/60.And at the time of discharge 46 patients (92%) had VA>6/60 and 4 patients (8%) had VA <6/60. In this study visual acuity at the time of presentation and at the time of discharge after performing Yag PI in the affected eye was (p=0.0004) which was statistically significant. In this study the lowest IOP in the affected eye was 30 mm hg and the highest recorded was 64mmhg at the time of presentation.2 patients (4%) presented with IOP of 21-30mmhg, 21 patients (42%) with 31-40mmhg, 14(28%) patients with 41-50mmhg, 12 (24%) patients with 51-60mmhg and 1(2%) patient with 61-70mmhg. All 50 patients (100%) had IOP of 10-20mmhg in the affected eye at the time of discharge which was statistically significant (p=0.0004).

Out of the total 50 patients Cup disc ratio was examined where 32(64%) had cup disc ratio of 0.3:1 In Mazhar Ammar study out of total 84 eyes 27 eyes were classified as Yag PI failure .Out of 27 failures eye 17 eyes had CDR >0.8 which was the cause for failure Yag PI [4] .In this study also one patient (2%) with 0.8:1 CDR who presented with acute on chronic angle closure glaucoma had failed Yag PI and undergone Trabeculectomy.

On Gonioscopic examination 11 patients (22%) were found to have Peripheral Anterior Synechiae <180

degree .Out of the 11 patients (22%) with PAS< 180 degree, 4 patients (8 %) needed Trabeculectomy.

Nolan Winifred study showed that once the glaucomatous optic neuropathy is associated with synechial

angle closure, iridotomy alone is less effective at controlling IOP [15]. In this study there was no statistically significant (p=0.53) corelation between PAS< 180 and effectivity of Yag PI.

In this study 39 patients (78%) had effective Yag PI. Total 11 patients (22%) had undergone trabeculectomy. In P L Blaxter et al study 39 cases of acute congestive glaucoma was treated with peripheral iridotomy where it has control the disease in all but not in 3 cases.[16] In Gray *et al* study efficacy of Yag PI was studied in 150 patients. Forty of these patients (27%) were treated for acute angle closure glaucoma and three of them (7%) suffered recurrent AACG at a maximum post-YAG interval of six weeks, and all had previously undergone more than one YAG iridotomy. Following a peripheral iridectomy the IOP became normal without any medications.[17] In this study 78% eyes had controlled IOP following laser PI without medication and only 11% required trabeculectomy. Similarly B.W Fleck found that 70.4% of operative iridectomy patients and 71.8%

of laser iridotomy patients had an intraocular pressure less than 21 mm Hg without medication 3 years after treatment.[18] Playfair and Watson reported 72% of patients had an intraocular pressure less than 21 mm Hg without medication after 6–12 months of follow up.[19] Buckley *et al* reported that 75% of operative iridectomy patients and 65% of laser iridotomy patients had an intraocular pressure less than 21 mm Hg without medication after a minimum follow up period of 1 year.[6] A Chinese study found 82.4% of patients had a 'successful' outcome 3 years following Nd: YAG laser iridotomy for acute angle closure glaucoma.[20] However, in this study as the number of eyes studied was small and long term follow up was not taken, further studies with greater number of eyes and long term follow up are required to confirm these findings.

ACKNOWLEDGEMENTS

I am grateful to my honorable Prof. Dr. D.B Karki, Prof. Dr. Suman Thapa for their constructive guidance and encouragement, Dr. Satish Deo and Dr. Pawan Chalise for assisting in data and statistical analysis and my family for help and support.

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Prevalence of intestinal parasitic infections among public school children in a rural village of Kathmandu Valley

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ABSTRACT

Intestinal parasitic infections (IPI) are one of the most prevalent infections in humans residing in developing countries and its burden is high among school aged children. This cross-sectional study was conducted to determine the prevalence of intestinal parasitic infection and types of intestinal parasites in rural public school children of Nepal. It included students from Nursery to Class X of a rural public school located in the northeast part of the Kathmandu Valley, Nepal. Among the 194 participating children, prevalence of intestinal parasitic infection was found as 23.7%; (28.2% for boys; 20.2% for girls). Amongst the infected children, single and mixed parasitic infection was detected in 43 (93.5%) and 3 (6.5%) children respectively. Among protozoan parasites, *Giardia lamblia* was the most common (58.6%) whereas *Hymenolepis nana* was the most common (21.7%) among the helminths. Statistically different prevalence of intestinal parasitic infection was observed among children aged above 10 years and children aged below 6 years as well as 6 to 10 years. Gender-wise, there was no statistical difference in prevalence of intestinal parasitic infection. This study suggests the need of health education program in schools along with regular screening of intestinal parasites and treatment for effective management of the intestinal parasites among school children in Nepal.

Keywords: Prevalence, Intestinal Parasitic Infection, School children, Nepal

INTRODUCTION

Intestinal parasitic infections (IPI) caused by intestinal helminths and protozoan parasites, are one of the most prevalent infections in humans residing in developing countries.[1] WHO has estimated about 3.5 billion people to be affected with these parasites worldwide, and 450 million people fall ill as a result of these infections, with the majority being children.[2] IPI is a major public health problem in Nepal.[3-5] School-aged children are particularly susceptible to IPI, often carrying higher loads of parasites.[6] The reported prevalence of intestinal parasites varies from place to place and time to time. A study conducted on the prevalence of intestinal parasites among school children in two rural villages of Chitwan district[4] in 1999has shown a prevalence of 44.0% whereassimilar studies conducted at Pokhara[3] in 2004and Dharan[7] in 2008 have shown the prevalence to be 21.3% and 22.5% respectively. Similar studies conducted on school going children at different places and period of time in Kathmandu valley have shown the prevalence ranging from 17.6%to 82%.[8-12]Mostcommon intestinal parasites reported from school going children in Nepal are *Ascarislumbricoides*, *Hymenolepis nana*, Hookworm, *Trichuristrichiura* and *Giardia lamblia*. These parasites are associated with diverseclinical manifestations such as malnutrition, irondeficiency anemia, malabsorption syndrome, intestinal obstruction, and mental and physical growth retardation.[7] This study was conducted to determine the prevalence of IPI and types of intestinal parasites in a rural public school children of Kathmandu valley.

MATERIALS AND METHODS

This cross-sectional study was conducted on September 9, 2012 at a rural public school located in the northeast part of the Kathmandu Valley, Nepal. Students from Nursery to Class X were included in the study. Consent was taken from the school authority prior to the commencement of the study. One day prior to the study,school children were distributed with a properly labeled clean, dry, screw capped plastic container and a plastic spatula. The method of stool collection was explained to the students from Class IV to Class X. Parents of students from Nursery to Class III, were given an information leaflet mentioning the purpose of the study and instruction on method of collection of stool sample. On the day ofthe study, a team of faculty and volunteer students from Patan Academy of Health Sciences (PAHS) went to the school with all the necessary equipments (e.g. microscopes, glass slides, reagents) to carry out this study. Demographic data collected during the study were verified from the school records.

Stool samples were examined for the presenceof

parasites both macroscopically and microscopically. Direct microscopic examination was done by normal saline and Iodine wet mount. Students detected with intestinal parasites were treated with anti-protozoal and anti-helmintic drugs by an accompanying physician. The study was approved by the research committee of PAHS. Data entry and analysis were done in the Epi-Info V3.5.3 and SPSS V15.000 software respectively. Chi-square test was used to analyze the differences of proportions.

RESULTS

Out of 296 enrolled students, only 194 (65.5%) school children chose to participate in this study. The overall prevalence of intestinal parasites was found to be 23.7% (either protozoa, helminths or mixed). Among all infected children (n=46), single parasitic infection was detected in 43 (93.5%) children, while 3 (6.5%) children had mixed infection. The prevalence of protozoal infection and helminthic infestation including the mixed infection were found to be 18.5% and 6.1% respectively.

Six different species of parasites; 2 protozoan and 4 helminthic species were detected. Among protozoan parasites, *Giardia lamblia* was the most common (58.6%) followed by *Entamoebahistolytica/dispar*(19.6%). Among helminths, *Hymenolepis nana*was the most common (21.7%). Only one Hookworm, *Trichuristrichiura* and *Ascarislumbricoides* was detected. Mixed infections of *Hymenolepis nana* with protozoan parasite (*Giardia lamblia*) were detected in two children and with helminth (*Ascarislumbricoides*) were detected in one child (Table-1).

Table-1: Prevalence and distribution of intestinal parasites

Intestinal parasites	Number (n)	Prevalence %	Positive %
Protozoa (single infection)	34	17.5	73.9
Giardia lamblia	25	12.9	54.3
Entamoeba histolytica/dispar	9	4.6	19.6
Helminths (single infection)	9	4.6	19.6
Hymenolepis nana	7	3.6	15.2
Hookworm	1	0.5	2.2
Trichuristrichiura	1	0.5	2.2
Mixed infections	3	1.5	6.5
Giardia lamblia and Hymenolepis nana	2	1.0	4.3
Ascaris lumbricoidesand Hymenolepis nana	1	0.5	2.2
Total (N)	**46**	**194**	**46**
Total (%)	**–**	**23.7**	**100**

Note: Total may not add to 100% due to rounding.

The prevalence of IPI among boys was 28.2% which was higher compared to that of girls (20.2%), but this difference was not statistically significant (p value = 0.191) (Table-2).

Table-2: Prevalence of IPI by gender

Gender	Total (N)	Positive (n)	Positive (%)	p-value
Male	85	24	28.2	0.191 (>0.05)
Female	109	22	20.2	
Total	194	46	23.7	

IPI was highest among children aged less than 6 years (37.5%) followed by children aged 6-10 years (31.6%) and children aged more than 10 years (16.8%). The prevalence of intestinal parasite was found to be statistically different among the age groups. Further, Post Hoc (pair wise) comparison of prevalence of intestinal parasites in different age groups showed statistically different prevalence among children aged less than 6 years and greater than 10 years as well as among children aged 6-10 years and greater than 10 years. However, prevalence of intestinal parasites among children aged less than 6 years and 6-10 years was not found to be statistically significant (Table-3).

Table-3: Prevalence of IPI by age group and pair wise comparison between different age group

Age group	Total (n)	Positive (n)	Positive (%)	p-value	Age group	p-value
< 6	24	9	37.5	0.024 (< 0.05)	< 6 vs. 6 – 10	0.615
6 – 10	57	18	31.6		6 – 10 vs. > 10	0.032*
> 10	113	19	16.8		< 6 vs. > 10	0.047*
Total	**194**	**46**				

*This shows that the two proportions are significantly different. ($p<0.05$)

IPI was highest among children studying below primary level (42.3%) followed by primary (38.4%) and above primary (16.7%). The prevalence of intestinal parasites was found to be statistically different among the different grades. Further, Post Hoc (pair wise) comparison of prevalence of intestinal parasites in different grades showed statistically different prevalence among children in below primary and above primary level. On contrary, prevalence of intestinal parasites among children in below primary and primary level as well as between primary and above primary level was not found to be statistically significant (Table-4).

Table-4: Prevalence of IPI by grades and pair wise comparison of different grades

Grade	Total (n)	Positive (n)	Positive (%)	p-value	Grade	p-value
Below Primary	26	11	42.3	0.019 (< 0.05)	Below primary vs. Primary	0.131
Primary	72	19	38.4		Primary vs. Above Primary	0.125
Above Primary	96	16	16.7		Below Primary vs. Above Primary	0.005*
Total	194	46				

Note: Below primary = Nursery, KG; Primary = Grade 1-5, Above Primary = Grade 6 and above.

*This shows that the two proportions are significantly different ($p<0.05$)

DISCUSSION

In this study, nearly one fourth (23.71%) of the rural public school children were found to be harboring one or more intestinal parasites. This was close to the findings of different studies conducted in school going children of Nepal.[3,7,13] Yet, lower prevalence of intestinal parasites has been reported from studies done in school children of urban cities of Nepal.[9, 14] On the other hand, prevalence of intestinal parasites up to 82% has been reported from a study done among school children in Lalitpur district of Nepal.[12]

The prevalence of protozoal infection was found to be higher than that of the helminths in this study. This finding is in accordance with the results of similar studies conducted in school children of Nepal,[7,14] and elsewhere.[15] On the contrary, higher prevalence of helminths has been reported in other studies from Nepal.[8,9,11] Among the protozoan parasites, *Giardia lamblia* was the most common intestinal parasite followed by *Entamoebahistolytica*. Likewise, *Giardia lamblia* was reported as the commonest protozoan parasite in school going children in other studies conducted in Nepal[7,14] and elsewhere.[15]

Among the helminths, *Hymenolepis nana* was the most common intestinal parasite. This is in contrast with the finding of other studies conducted on school going children of Nepal.[7,9,11,13,14] Various studies have found *Ascaris lumbricoides*[7,14,16] and *Trichuris trichiura*[9,11,13] as the commonest intestinal helminth in school children of Nepal. Low prevalence of helminths in this study can be attributed to the nationwide bi-annual integrated deworming as well as Vitamin A supplementation programme[17] and implementation of mass drug administration in which single dose of albendazole is given.[18] But, single dose of albendazole is not sufficient to clear *Hymenolepis nana* infestation[19] as well as protozoal infections. These findings point out the need for regular screening of intestinal parasites among school children for effective management of these infections.

Prevalence of intestinal parasites was found slightly higher among boys but the difference was not statistically significant. Similar findings have been reported by various studies conducted in Nepal[11,13] and elsewhere.[15] However, other study has shown significantly lower prevalence of parasitic infection in girls attributing it to their good hygiene compared to boys.[9] On the contrary, various studies have reported higher prevalence of intestinal parasites among girls.[14,20]

Age wise prevalence of IPI was found to be highest among children of age below 6 years followed by children of age ranging from 6-10 years, which were significantly higher than that of children of aged 10 years and above. Further, pair wise comparison revealed statistically significant lower prevalence of IPI among children above 10 years than children aged less than 6 years and 6-10 years. The decrease in prevalence of IPI with the increase in age of children have been reported in other studies as well, in which they have attributed this finding to rise of awareness regarding hygienic practices[9,14] and environmental sanitation.[14] On the contrary, higher prevalence of IPI among children aged 10-14 years have been reported by different studies done in Nepal attributing it to lack of parental control regarding dietary habits[13] and increased outdoor activities.[11]

Grade wise prevalence of IPI was found to be highest among children of below primary level, followed by children of primary level and children of beyond primary level. However, pair wise comparison revealed significantly different prevalence of IPI between children of below primary level and children of above primary level only. On contrary, a study found higher intestinal parasitic prevalence among lower secondary students than primary and secondary students.[13]

In conclusion, the prevalence of intestinal parasites was found to be 23.7%. No significant difference in prevalence of intestinal parasites between boys and girls were observed. However, significant difference in prevalence of parasitic infection was found between students aged less than 10 years and more than 10 years as well as between students of below primary and above primary.

Although the existing mass deworming programs of Government of Nepal is found to be effective in clearing helminths like *Ascaris lumbricoides* and Hookworm, it is not effective to reduce helminths like *Hymenolepis nana* and protozoan parasites. Therefore, this study emphasizes the need of targeted health education programs for students, teachers and parents along with regular screening and specific treatment for effective management of the intestinal parasites among school children in Nepal.

ACKNOWLEDGEMENTS

We would like to express our sincere gratitude to the school teachers of Okhreni Secondary School, Sundarijal and all the volunteer PAHS medical students. We are thankful to all the school children and their parents/guardians who participated in this study. We would also like to thank Ms. Sylvi Prajapati for helping in data entry and Mr. Arun Kumar Yadav and Mr. Indra Sapkota for providing their technical support in the study. We are indebted to Dean, Dr. Rajesh Gongal, School of Medicine, PAHS for providing funding and other logistics for this study.

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Status of iron, oxidant and antioxidants in chronic type 2 Diabetes mellitus patients

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ABSTRACT

Diabetes mellitus is a common health problem of the world. Iron may be a part of the cause of the disease and its complications. Iron is a trace element which produces reactive oxygen species (ROS) participating through Fenton reaction and that ROS may be a cause to produce oxidative stress and further diabetic complications. The study aims to access the iron and its effect in producing oxidative stress in type 2 diabetic patients. Serum iron, total iron binding capacity (TIBC) and percentage transferrin saturation are calculated as the index of iron. Malondialdehyde (MDA) is estimated as index of oxidant and vitamin C, vitamin E are measured as index of antioxidants. This is a case control study conducted in the department of Biochemistry in collaboration with department of Medicine at B P Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal. 52 chronic type 2 diabetes mellitus patients and 52 age and sex matched normal healthy controls were included in the study. Plasma iron, TIBC, percentage transferrin saturation were found (89.14±30.50 µg/dL), (266.78±48.80 µg/dL), (36.61±14.31 %) in diabetic cases as compared to (83.98±24.19 µg/dL), (279.08±40.23 µg/dL), (31.05±10.98 %) of healthy controls. A significant increase in MDA level (6.35±1.52 nmol/ml in cases and 4.18±1.12 nmol/ml in controls, p<0.001) and significant decrease in vitamin C (0.85±0.19 mg/dL in cases and 1.28±0.21mg/dL in controls, p< 0.001) and vitamin E (0.85±0.25 mg/dL in cases and 1.34±0.38mg/dL in controls, p<0.001) were observed.

Keywords: Antioxidant, Iron, MDA, Oxidant, Oxidative stress, Type 2 DM, TIBC, Vitamin C, Vitamin E.

INTRODUCTION

Type 2 diabetes mellitus is a clinical condition characterized by hyperglycemia due to the absolute or relative deficiency of insulin. It is also followed by pathological abnormalities like impaired insulin secretion, peripheral insulin resistance, and excessive hepatic glucose production. Although type 2 diabetes mellitus is a multiple etiological disease, emerging scientific evidences show there is somewhat relationship of the disease with iron metabolism. In recent years development of diabetes has been predicted with increased iron stores which is protective with iron depletion.[1] Although plasma concentration of iron is low total body iron content is approximately 4 gm in which a significant amount of iron is stored as ferritin and hemosiderin.[2] Iron is a transition element capable of reduction and oxidation activity and a potential harm is circumvented to body by binding iron with transport or storage proteins.[3] In recent years the role of iron has been investigated as a prooxidant which contributes to lipid peroxidation[4] causing oxidative stress. Ferric form of iron released from binding proteins can participates in production of free radicals by Heber-Weiss or Fenton reaction and cause oxidative damage. Role of iron is positively associated with the development of glucose intolerance and type 2 diabetes[5] as well as gestational diabetes mellitus. In fact iron level in serum is manifestation of storage iron, ferritin and there is increasing evidence that glucose metabolism is influenced by high ferritin level in the body. It has been observed frequent blood donation improves insulin sensitivity[6] and constitute protective factor for the development of diabetes mellitus[7]. Serum ferritin, the storage form of iron is well correlated with baseline serum glucose[8] and beta cell function[9]. Iron in serum in ferrous form is the culprit for generation of free radicals. In our body iron is present usually in ferritin as well as in transferrin. For iron to act as a prooxidant agent it must be in free form and released by ferritin by the action of prooxidant that convert Fe^{++} to Fe^{+++}. Glycation of transferrin decreases its ability to bind ferrous iron, hence increases free iron pool which in turn facilitates ferritin synthesis. A continuous production of free radicals causes increased lipid peroxidation and decreased antioxidant status. As a result Malondialdehyde (MDA), the end product and marker of lipid peroxidation is released to the plasma. Under physiological conditions damage due to free radicals is countered by antioxidants. When the excessive free radical formation takes place in the body antioxidant system can't cope up with the situation i.e. proxidants overwhelm antioxidants. This improper balance between free radical production and antioxidant defense system tends to produce oxidative stress. This study aims to find out the level of iron and TIBC as iron status, MDA as oxidant status and Vitamin C and Vitamin E as antioxidant status.

MATERIALS AND METHODS

This study was hospital based and case control study which was carried out in department of Biochemistry in collaboration with department of Medicine at B P Koirala Institute of Health Sciences, Dharan, Nepal. 52 chronic patients of type 2 diabetes mellitus and 52 age and sex matched healthy subjects were enrolled for the study (Fig. 1 & 2). Informed consent was taken from all the participants. Those diabetic patients who were also suffering from rheumatic heart disease, arthritis, infectious diseases, pulmonary tuberculosis, were excluded for the study.

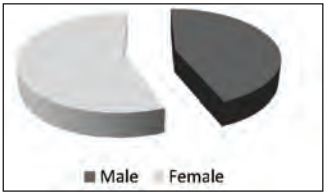

Fig. 1. Gender wise distribution of Case

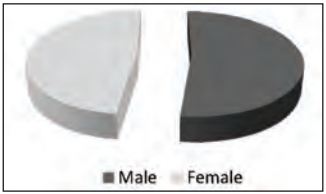

Fig. 2. Gender wise distribution of Control

Blood samples were collected in the fasting stage through vein puncture in two different vials, one EDTA and the other plain vial. Plasma and serum were separated by centrifugation. Plasma was utilized to estimate vitamin C and vitamin E as antioxidant parameters and serum was utilized for the estimation of MDA, Iron and TIBC. MDA was estimated by method of Yagi. This method is based on the formation of red pigment as a result of condensation lipid peroxidation breakdown products like MDA with thiobarbituric acid.[10] α-tocoferol was estimated by Biery *et al* method in which α-tocoferol is oxidized to tocopheryl quinine by ferric chloride and resultant ferrous ion forms complex with ethanolic α-α' dipyridyl complex in aqueous medium.[11] Plasma Ascorbic acid was estimated by Sulivan *et al* method which depends on the reduction of ferric ion to ferrous ion by ascorbic acid forming a red-orange α-α' dipyridyl complex.[12] Iron and TIBC were estimated by using commercial kit produced by Ranbaxy Company. In which ferric ions are first reduced to ferrous ions by releasing in acid pH which ultimately react with ferocine to form a violet colored complex. UIBC was estimated by the kit method. A known amount of ferrous ions are added to serum at an alkaline pH. The ferrous ions bind with transferrin at unsaturated iron binding sites. The additional unbound ferrous ions are measured using the ferrocine reactions. The difference in amount of ferrous ions added and the unbound ions are measured in the unsaturated iron binding capacity. TIBC is calculated by adding iron level and UIBC.

RESULTS

In our study we found increased level of serum iron and percentage transferrin saturation, and decreased level of TIBC in diabetic patients (Table-1). Serum iron shared 89.14±30.50 µg/dL in diabetic patients as compared to 83.98±24.19 µg/dL in control (p=0.34). TIBC was found 266.78±48.80 µg/dL in diabetic cases as compared to 279.08±40.23 µg/dL of healthy controls (p<0.16). Transferrin saturation was found 36.61±14.31 % in diabetic cases as compared to 31.05±10.98 % of healthy controls (p< 0.15). When serum iron was stratified as male and female value shared 94.72±36.92 µg/dL and 92.35±24.16 µg/dL in males in cases and controls respectively (p=0.78). Among females serum Iron level was found 84.72±24.78 µg/dL and 72.93±21.14 µg/dL in cases and controls respectively (p=0.13). Serum TIBC and percentage transferrin saturation were also stratified as male and female. Serum iron was found increased, TIBC decreased, and percentage transferrin saturation increased in diabetes patients in both groups of males and females (Table-2). Likewise, MDA level was found to be 6.35±1.52 nmol/ml in cases and 4.18±1.12 nmol/ml in controls, p< 0.001 (Table-3). Vitamin C was found to be 0.85±0.19 mg/dL in cases and 1.28±0.21mg/dL in controls, p< 0.001 and vitamin E was found to be 0.85±0.25 mg/dL in cases and 1.34±0.38mg/dL in controls, p<0.001which were significantly decreased in diabetes patients as compared to healthy controls (Table-3).

Table-1: Distribution of serum iron, TIBC, and transferring saturation in case and control

Groups	Iron (µg/dL) (mean±SD)	TIBC(µg/dL) (mean±SD)	Transferrin saturation (%) (mean±SD)
Case (n=52)	89.14±30.50	266.78±48.80	36.61±14.31
Control (n=52)	83.98±24.19	279.08±40.23	31.05±10.98
p-value	0.34	0.16	0.15

Table-2: Distribution of serum iron, TIBC and transferrin saturation in males and females

Groups	Iron (µg/dL) (mean±SD)		TIBC(µg/dL) (mean±SD)		Transferrin saturation (%) (mean±SD)	
	Male	Female	Male	Female	Male	Female
Case	94.72±36.92	84.72±24.78	256.26±40.51	275.12±53.69	38.23±16.61	31.74±11.69
Control	92.35±24.16	74.93±21.14	276.42±42.03	281.95±38.84	34.50±11.37	27.33±9.41
p-value	0.78	0.13	0.09	0.60	0.35	0.13

DISCUSSION

Serum iron was found slightly high, TIBC slightly low and percentage transferrin saturation slightly high in diabetes patients (Table 1) but there was no significant difference in iron, TIBC and percentage transferrin saturation. MDA level was found significantly increased and vitamin C and Vitamin E were found significantly decreased in diabetes patients as compared to healthy controls (Table 3). There was high serum iron level in diabetic females as compared to control females (Table 2). The low level of iron in females may be due to the loss of iron through menstruation since age group ranges from premenopausal cases. Sheu *et al*[13] have also found the relationship between serum ferritin levels and insulin resistance, which existed only in diabetic females but not in males. Serum ferritin has been studied in detail and very few studies have conducted to see the association between serum iron and diabetes mellitus. Toumainen *et al*[8] have found that approximately 10% of type 2 diabetes patients with high ferritin levels have transferrin saturation greater than normal. Thomas *et al*[14] stated that the prevalence of elevated transferrin saturation was 3-4 folds higher in patients with diabetes, compared with historical prevalence described in the general population.

Table-3: Distribution of MDA and vitamin C and vitamin E in case and control

Groups	MDA (nmol/ml) (mean±SD)	Vitamin C (mg/dL) (mean±SD)	Vitamin E (mg/dL) (mean±SD)
Case (n=52)	6.35±1.52	0.85±0.19	0.85±0.25
Control (n=52)	4.18±1.12	1.28±0.21	1.34±0.38
p-value	0.0001	0.0001	0.0001

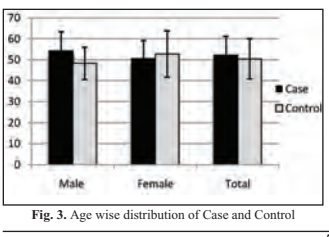

Fig. 3. Age wise distribution of Case and Control

The different kind of result has been observed by Dinneen *et al*[15] who found no role of iron in diabetes mellitus. They determined distribution of iron histochemically by evaluating hepatic iron stores in autopsy specimens. No significant difference was observed. Similar type of result was also obtained by the study of Elis *et al*[16] whose study population comprised three subject groups with severe diabetic retinopathy, without retinopathy and non diabetic non retinopathy subjects. Serum iron and ferritin levels did not differ significantly between the three groups and there was no correlation between $HbA1_c$ level and serum iron and ferritin levels between the diabetic patients groups.

Malondialdehyde (MDA), the end product of lipid peroxidation was also studied in cases and controls. Significant increase in MDA was observed in cases as compared to controls (6.35±1.52 Vs 4.18±1.12 nmol/ml). MDA is produced as a result of lipid peroxidation which act as a marker of balance between prooxidant and antioxidant. In diabetes mellitus there is imbalance between prooxidant and antioxidant and prooxidant is actively predominant. As a result there is a high level of MDA present in sera of diabetic patients. This result is favored by other studies.[17,18] MDA level may be increased due to the action of iron by producing free radicals through Fenton reaction ultimately initiating chain reaction to cause lipid peroxidation.[19] Many studies have found increased level of lipid peroxidation even with small increment of iron concentration.

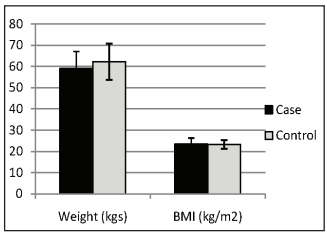

Fig. 4: Distribution of weight and BMI in Case and Control

There was significant decrease of vitamin C and vitamin E in cases as compared to controls (0.85±0.19 Vs 1.28±0.21 mg/dL of Vitamin C and 0.85±0.25 Vs 1.34±0.38 of Vitamin E). Free radicals are formed disproportionately in diabetes by glucose oxidation, non-enzymatic glycation of proteins and the subsequent oxidative degradation of glycated proteins.[20] The reduction in vitamin C and vitamin E could be because of action of these vitamins as antioxidant in which both of them are used up to neutralize reactive oxygen species. Different studies have supported the above findings[21,22] and have shown significance decrease in antioxidant vitamins. In some studies it is also found the positive effects of vitamin C and vitamin E therapies in diabetic patients.[23]

ACKNOWLEDGEMENTS

I am thankful to the authority of BPKIHS for providing me an opportunity as well as financial support to carry out this study. I am grateful to my teachers Prof. Shankhar Majhi, Prof. Madhab Lamsal, Prof. Sanjib Kumar Sharma and Prof. Nirmal Baral as well as seniors, colleagues and other departmental staffs at BPKIHS for their for their guidance and constant help throughout the work. I thank Dr. R M Pandey for his valuable help in statistical analysis. I also like to thank those people who allow their participation in my study being diabetic and healthy subjects without whom this study would not have been possible.

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Outcome of Treatment of Nonunion Tibial Shaft Fracture by Intramedullary Interlocking Nail augmentated with Autogenous Cancellous Bone Graft.

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ABSTRACT

To assess results of operative treatment of non union fracture shaft of Tibia by intramedullary interlocking nail augmented with autogenous cancellous bone graft in our setup.

A total of 25nonunion tibial shaft fractures were evaluated among which20 cases were male and 5 female with the mean age 31.84 years. Hypertrophic non- union were 14 and atrophic non union were 11. Upper one third of tibial diaphysis was involved in 4 cases, middle one third in 14 cases and lower one third in 7 cases. In all cases open reduction, interlocking nailing and autogenous cancellous bone graft was applied.

The mean follow up was one year. Mean time for healing was 8.08 months. Mean operation time was 110 minutes (range 70 to 160minutes). Satisfactory results (excellent and good) were achieved in 88% cases and unsatisfactory (fair and poor) results in 12 %cases.

This operative treatment option appears to have a high success rate and should be considered in nonunion of tibial diaphysis.

INTRODUCTION

Fracture of tibial shaft is important for two reasons, first is that they are more common; the second is that they are controversial and anything that is both common and controversial must be important[1]. Because of subcutaneous position, the tibia is more commonly fractured and more commonly sustain open fracture than any other long bone[2]. The incidence of tibial non-union is estimated to range from 2 to 10 % of all tibial fracture and is greater with high energy injuries and open fractures[3].

Several factors may predispose to non- union. Many of these are related to the fracture characteristics such as degree of fracture communition and bone loss, whether the fracture is open or closed and degree of soft tissue injury. Subsequent complication such as infection or compartment syndrome may also play a role.

The patient related factors such as cigarette smoking, use of NSAIDS, steroid, poor nutritional status, systemic diseases like uraemia, jaundice etc and non-compliance to post operative regimes also contributes to the incidence of non- unions. Iatrogenic injury to soft tissue envelope, distraction across fracture site, inadequate immobilization or fixation and splinting effect of an intact fibula may contribute to the development of a non-union[3].

Historically, the definition of nonunion has been based on time frame from the onset of injury. More recently the exact time frame is considered to be less important. Fracture healing is a dynamic progressive process and intervention is warranted by 3 to 5 months following injury if monthly radiographic studies do not show progression of fracture healing[3].

Several non- operative options have been described for the treatment of this complication such as immobilization in a cast, use of functional brace, electrical stimulation and pulsed ultrasound. Surgical treatment includes fibular osteotomy, Posterolateral, subcortical or open cancellous bone grafting and a variety of methods of stabilization like external fixation, plate and screws, intramedullary nailing and intramedullary nailing supplemented with plate and screws. Every treatment option mentioned above had its own advantage and disadvantages.

In this series, the effectiveness of interlocking nailing augmented with autologus cancellous bone graft in the treatment of tibial diaphyseal fracture non-union has been evaluated.

MATERIALS AND METHODS

All 25 patients who underwent open reduction and intramedullary interlocking nailing with autogenous cancellous bone graft during 2009 to 2011 and all were followed for one Year. The inclusion criteria were non-union tibial shaft fracture, age >18 years, both male and female were taken. Exclusion criteria were infected non-union tibial shaft fracture, gap and delayed non- union, pathological and children's fracture.

Fracture site was opened through antero-lateral approach and nail was introduced through patellar tendon

splitting approach which was followed by placement of autogenous cancellous bone graft at the fracture site. In all cases reaming was done.

Postoperatively both active and passive toe movements started immediately after anesthesia. Static quadriceps exercise began as soon as pain allowed, followed by movement of knee and ankle. Patients were allowed partial to full weight bearing as pain allowed; with the help of crutch. Patients were discharged on 4th to 12th postoperative day. In cases of wound infection, patients needed longer stay.

The first follow up was on three weeks interval and thereafter at monthly intervals till the fracture united. The last follow up was at one year.

The criteria for assessing the outcome after intramedullary nailing have been set by different workers .In this series, Tucker criteria was used for evaluation of the results. The results were expressed as excellent, good, fair, and poor according to the criteria followed by Tucker et al 4.

Grading

Excellent: - the results were graded as excellent when the following criteria were fulfilled.

Fracture union

Full knee extension and 125 degree flexion

Ankle motion 75% of normal side (in bilateral fracture, ankle motion should be above neutral and have 30 degree flexion)

No leg length discrepancy of more than 1 cm

No angulations greater than 7 degree in any plane

No infection

No pain on weight bearing

Good: - Fracture union and one criterion above missing

Fair: - Fracture union and two of the above criterion missing

Poor: - Fracture union with three criteria missing

Results

Table 1. Age distribution of patients

Age (Years)	No of patients
11-20	2
21-30	10
31-40	8
41-50	5

Table 2. Sex distribution of patients

Sex	No. of patients
Male	20
female	5

The total number of patients was 25 with 80% male. All patients had one year follow up. The mean operative time was 110 minutes (range 70-160 mins). Full weight bearing was achieved within 2 days of operation. Bony union was achieved in all nonunions (100%).The mean time ofunion was8.08months. Fifteen cases required fibular osteotomy that allowed transfer of stress from intact fibula back to tibia and aided in realignment of tibia.

Regarding time to union; Hypertrophic non union required an average of 6.79 months and atrophic non union at 9.73 months. In this study, 4 cases (16%) had superficial wound infection,5 cases (20%) had limb shortening (three cases-1.5 cm ; two cases- 2cm) and 2 cases had knee pain(8%),4 cases had ankle stiffness(16%), significant restriction of knee movement more than 15° were found in 4 (16%) cases, one case each (4%) had ankle pain and valgus deformity. Functional range of motion of both the knee and ankle were maintained. In this series, final result was considered on the basis of tucker's criteria. According to which, 9 cases (36%) had excellent result, 13 cases (52%) had good result, 2 cases had (08%) had fair result and 1 case (04%) had poor result.

Table. 3: Complications

Complications	Number of cases	Percentage
Superficial wound infection	4	16
Shortening	5	20
Knee pain	2	8
Valgus deformity	1	4
Ankle stiffness	4	16
Knee joint movement deficit >15°	4	16
Ankle pain	1	4

DISCUSSION

Many factors have to be considered to select optimal treatment for a patient with tibial

non union to achieve speedy recovery and return to function. Bone quality, bony defects, soft tissue coverage, potential occult infections, insufficient primary stabilization and mal-union are essential determinants 5.

It must be taken in consideration that numerous investigators have reported result of treatment of same type of non union by using different operative techniques and implants used in many recent series6,7,8,9. Comparison among those studies i

s often difficult and wide variation frequently exists. Some of these discrepancies may be

attributed to factors including patient selection, various methods of rehabilitation, and difference in length of follow- up. Patient in various clinical series also differ not only with respect to type of non union, but with regard to age, sex, lifestyle and level of activity.

The tibial diaphyseal fracture non union is the commonest among all long bone fracture nonunion. An orthopaedist must consider many factors in choosing the best treatment for patient who has non union of the tibia .Insipite of vast experience gained over the last few decades, at present, no single method is universally accepted, and many methods appear to result in an acceptable rate of union. One of the most important differences among those methods is the duration of disability to be expected. Most patient who have non union of tibia have been incapacitated for six to twelve months and any methods that encourages early return to function is appreciated.

With these shortcomings in mind, the present study has been undertaken in NMCTH, Kathmandu to evaluate the result of treatment of the tibial diaphyseal fracture non-union by interlocking nail augmented with autogenous cancellous bone graft.

A prospective study was carried out from 2009 to 2011 at NMCTH.A total of 28 patients satisfying the inclusion and exclusion criteria were selected for this study and three patients were lost for follow up. So this study comprised of 25 patients.

In this series, the age range of patient was from 18- 50 years, with mean of 31.84 years. Similar findings were also noted by Johnson and Marder (1987) in the study where the average age was 43.4 years10. Majority of the patients in this series were in the age group of 21-30(40%) years.

Male population in the series constituted 80% of cases, while the female made up the remaining 20%. Male being the major working force of a society and are thus more consistently exposed to external environment, which probably accounts for this discrepancy.

The major cause of initial injury was road traffic accident (RTA). Thirteen (52%) patients developed fracture following RTA. RTA was the initial cause of fracture in other previous studies too10.

Most non union occurred in middle third of the diaphysis of tibia (56%), followed by distal third(28%) and then proximal third(16%) in this series. This was consistent with the observation of Johnson and Marder(1987)10.

In this series, hypertrophic non union were 14 cases(56%), atrophic type were 11 cases(44%).Similar result observed by Johnson and Marder (1987), hypertrophic type were 55% cases, atrophic type were 45% cases. Whereas Rosson et al. (1992) reported hypertrophic type 54.16% cases, atrophic type 45.84% cases 10, 11.

In this series, 15(60%) cases were initially treated by plaster immobilization after initial injury, 8(32%) cases were treated by external fixation, and 2(8%) cases was internally fixed by plate and screw. All were without evidence of infection for at least six months before the intramedullary nailing.

The time elapsed from injury to treatment for non union varied from 6 months to 19 months with mean of 10.08 months. Johnson and Marder (1987) reported time from injury to nailing from 9 months to 36 months with mean of 17.43 months. Rosson et al (1992) reported time from injury to nailing varied from 10 months to 54 months with mean of 21.37 months 10, 11. This variation in time is due to the change in definition of non union itself. Previously when 9 months had elapsed after the initial injury and no progressive signs of healing were visible, then only it was called non union 12.But according to the newer definition a fracture can be called non united when it shows no signs of union after 6 months of initial injury 3.

Fibular osteotomy was done in 15 cases, because this osteotomy allows transfer of stress from the intact fibula back to the tibia and aids in realignment of tibia. Osteotomy was performed 6 to 8 cm away from non union. An osteotomy at this distance allowed correction of alignment but left some interosseous membrane intact and prevented excessive shortening and instability6. Static interlocking was done in all cases. Autogenous cancellous bone graft taken from iliac crest was given in all cases. No external immobilization was used post operatively.

Post operative hospital stay ranged from3 to 12 days with mean of 5.08 days. Hospital stay was comparatively much longer in cases where external fixators were used.

Assessment of union at follow up was made radiographically and clinically. The follow up period was 12 months. A non union was considered to be healed clinically when the patients could walk with full weight bearing without assistance and had no pain even with provocation test. A non union was considered to be healed radiographically when the radiolucent fracture line was obliterated or when callus bridged the site of non union.

Mean union time in this study was 8.08 ± 2.53 months (range4 to 12 months).In the study of Waren et al. (1992) mean time of union was 8 months (range- 2 months to 15 months), Rosson et al (1992) showed a mean union time of 9 months (range- 2 to 15 months) 11,13.

In this series, union occurred in all (100%) cases. Johnson and Marder (1987) achieved union 100% cases. In the study by Meargo et al (2007, union occurred in 99% of cases within 5.2 months with the use of intramedullary nail 10, 14.

Hypertrophic non union showed union at an average of 6.79 months (ranging from 4 to 10 months) and atrophic non union had union at an average of 9.73 months (ranging from 6 to 12 months).This difference is statistically significant (P< 0.05). In Johnson and Marder study (1987) also showed statistically significant difference between union time of hypertrophic and atrophic non union. Similar findings were also found in the study of Rosson et al (1992) 10, 11.

In this series, the mean union time taken for union at proximal third was 6.75 months, at the middle third it was 7.5 months and lower third it was 10 months. This finding is similar to the study of Rosson et al (1992), which showed longer time for union of distal third but contrary to the study of Johnson and Marder (1987) which showed no difference in union time according to the site involved 10,11.

In this series, non united fracture that were open initially needed multiple surgical debridement and took longer time for union (mean- 9.09 months) and non united fracture that were initially closed achieved union at a mean of 7.28 months. This difference is statistically significant (P< .001).In Johnson and Marder (1987) also found that non united closed fracture united earlier than non united open fracture which was statistically significant. In the critical analysis of 705 cases, Nicoll (1964) also found that there was statistically significant difference between mean union time of closed and open fracture. This supports the theory that severe damage to soft tissues at the time of fracture or from repeated striping of soft tissues is detrimental to the healing fracture 10,15.

In this series, 4 patients (16%) had a post operative superficial infection which healed with regular dressing and antibiotics .Rosson et al (1992) reported infection rate in 16 % cases in their series. Johnso and Marder (1987) found infection rate in 10% cases and Waren et al (1982) reported infection rate 0% 10, 11, 13.

In the present series significant restriction of knee movement more than 15° were found in 4 (16%) cases but mild degree of knee stiffness (< 15°) was present in 14 (56%) cases. Restriction of ankle movement was found in 4 (16%) cases. These were probably due to inadequate physiotherapy.

Anatomical alignment of the fragments to a neutral position in all cases except 1 who developed valgus deformity of 10°.

Limb length discrepancy is an important problem of treatment. Two patients in this series had shortening of 2 cms and another 3 had shortening of 1.5 cm. No patients in this series had an unacceptable shortening. Two patients had knee pain and 1 had incidence of ankle pain on weight bearing. The final outcome was in excellent in 09 (36%) cases, good in 13 (52) cases, fair in 2 (08%) cases and poor in 1 (04%) cases, as shown in figure 1.In final follow up the satisfactory result (excellent and good) was obtained in 22 (88%) cases. In the study of Johnson and Marder, satisfactory result was in 100% cases 10.

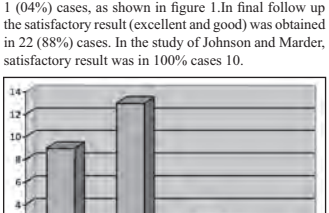

Fig.1: Functional outcome

Intramedullary interlocking nail augmented with autogenous cancellous bone graft is a good option for the patients of tibial diaphyseal fracture non union with high rate of union and early mobilization with a low complication rate and early return to function.

In conclusion, this technique should be considered a viable option for refractory non union of tibial diaphysis.

This study was conducted on small sample size (25 patients), sampling was not done randomly and follow up period was 12 months. So, further prospective study with larger sample size is required to delineate outcomes.

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Value of Conventional cervical cytology as a screening test for cervical cancer

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ABSTRACT

This is a prospective study conducted in the department of Obstetrics and Gynecology of Nepal Medical College Teaching Hospital, Kathmandu, Nepal. The main Objective of the study is to see the value of opportunistic screening program for cervical pre-cancerous lesion for prevention of cervical cancer. It also aims to see how many cases can be picked up by such screening test and is it worth doing this tset? We analysed 1751 cases of pap smear taken during almost 2 years period and found inflammatory smear being the predominant finding and it was found in reproductive age group. We had 1.14% cases of abnormal smear and 0.74% cases were proved by biopsy.

Keywords: Pap smear, pre-cancerous lesion, carcinoma cervix

INTRODUCTION

Cervical cancer is the second most common cancer in women worldwide.[1] it is the most common cancer of women in developing countries, where it is estimated that only about 5% of women have been screened for the disease with pap smear, compared to 40-50% in developed countries.[2] Unlike most other canc ers, cervical cancer is readily preventable when effective programs are conducted to detect and treat its precursor lesions. The easy accessibility of the cervix and the propensity of the cancer cells to exfoliate from its surface have enabled us to study the process of malignant transformation in the cervix in very early stage.[4] Because of poor access to screening and treatment services, the vast majority of deaths still occur in women living in low- and middle-income countries. Cervical cytology screening is helping to reduce cervical cancer rate dramatically since its implementation from 1950s.[4,5] Effective methods for early detection of precancerous lesions using cytology (Pap smear) exist and have been shown to be successful in high income countries. However, competing health care priorities, insufficient financial resources, weak health systems, and limited numbers of trained providers have made high coverage for cervical cancer screening in most low- and middle-income countries difficult to achieve. Every year more than 270 000 women die from cervical cancer, more than 85% of these deaths are in low and middle income countries.[2]

Cervical cancer is caused by sexually-acquired infection with Human papillomavirus (HPV). Most people are infected with HPV shortly after onset of sexual activity, which is the most common viral infection of the reproductive tract. Almost all sexually active will be infected with HPV at some point in their lives and some may be repeatedly infected. The peak time for infection is shortly after becoming sexually active. The majority of HPV infections resolve spontaneously and do not cause symptoms or disease. However, persistent infection with specific types of HPV (most frequently, types 16 and 18) may lead to precancerous lesions. If untreated, these lesions may progress to cervical cancer.[5]

The increasing availability of an alternative screening technology called VIA (visual inspection with acetic acid), VILLI (visual inspection with lugol iodine) and new vaccines against the Human papillomavirus (HPV) may help to prevent cervical cancer further. Moreover, because HPV vaccination targets 9–13 year old girls, there is the opportunity to catalyse a life course approach to cervical cancer prevention and control from childhood and through adulthood.[6] Cervical cancer screening is the systematic application of a test to identify cervical cellular abnormalities in an asymptomatic population. Women targeted for screening may actually feel perfectly healthy and see no reason to visit health facilities.[1-4] Screening services may be provided either as an opportunistic (i.e. taking advantage of a woman's visit to the health facility for another purpose), organized services or a combination of both. It is generally accepted that organized screening is more cost-effective than opportunistic screening, making better use of available resources and ensuring that the greatest number of women will benefit.

Three different types of tests are currently available:1

- Conventional (Pap) and liquid based cytology (LBC)
- Visual inspection with Acetic Acid (VIA) and VILLI (visual inspection with lugol's iodine)

- HPV testing for high risk HPV types (e.g. types 16 and 18).

HPV vaccination does not replace cervical cancer screening. In countries where HPV vaccine is introduced, screening program may need to be developed or strengthened.

Conventional pap smear is simply examination of exfoliated cells in cervical scraping taken with ayer's spatula and endocervical brush, Slide are prepared and examined under microscope for it's cellular architecture, nuclear pattern and cytoplasm nuclear ratio. Degree of cellular differentiation is classified according to Bethesda system.

Visual inspection with acetic acid (VIA) involves swabbing the cervix with a three- to five-percent acetic acid (vinegar) solution before visual examination with a strong light source. The application of acetic acid causes precancerous lesions to temporarily turn white, allowing the health care provider to determine whether precancerous lesions are present.

Visual inspection with Lugol's iodine (VILI)- also known as "Schiller's test," uses Lugol's iodine instead of acetic acid to identify potential precancerous lesions. The screening test is similar in approach to the Schiller's iodine test advocated in the 1930s and widely used early in the twentieth century, before the development of cytology. The application of iodine to the cervix results in a brown or black stain on areas that contain glycogen. Because precancerous lesions and invasive cancer do not contain glycogen, they do not take up the iodine, and therefore appear as well-defined, thick, mustard-colored or saffron-yellow areas. VILI may have better test performance characteristics than VIIA.

There is growing interest among cervical cancer prevention researchers in HPV DNA testing as a primary screening test. HPV DNA testing identifies high-risk HPV DNA subtypes. The presence of high-risk HPV strains indicates that a woman has an increased risk of developing cervical cancer. When used in women in their thirties and forties, HPV DNA testing is objective and generally has a higher sensitivity and specificity for detecting high-grade cervical lesions than does visual screening.

Sensitivity levels for HSIL have been found to range from 84 to 97 percent; specificity has been found to be approximately 90 percent. Screening programs based on HPV DNA testing could give women the option of using self-collected sampling techniques, although this approach has not yet been broadly tested. Self-collection of vaginal samples could significantly reduce the number of trained medical personnel needed to implement the screening program, because vaginal examinations only would be required for the fraction of screened women who are HPV positive.

women younger than 30 years of age need not undergo HPV DNA screening except for women known to be HIV-infected or living in a high HIV prevalence area.

OBJECTIVE

The objective of the study is to analyse the results of pap smear taken in NMCTH.

To see the ability of opportunistic pap smear to pick up abnormal smear.

MATERIALS AND METHODS

The present study is a prospective study conducted in the department of Obstetrics and Gynecology of Nepal Medical College Teaching Hospital. Kathmandu, Nepal. Basically it was an opportunistic screening where patients visiting hospital for one or other reason were included in the study. It was started from 1st of January 2010 and was continued till December 2012. All consecutive female were included in the study if they were agreed for the test. All women were explained about the importance of pap smear and it's role for preventing cervical cancer.

Detail history and clinical findings were recorded and Pap smear was taken. All of them were told to bring the report of smear to the department and according to the reports treatment was provided. Inadequate sample, Severe infection or bloody sample which makes difficult to comment on cellular pattern were excluded from study. As our pathologists follow the Bethesda system to write the reports of pap smear, we tabulated accordingly. The reports were generally classified as normal, inflammatory, ASCUS, LSIL, HSIL and in situ. Data were collected from pathology department as well as from operation theatre to tally the reports and to confirm the diagnosis in cases of abnormal reports. Data were tabulated and analysed simply.

DISCUSSION

Screening for any disease aims to detect the disease in it's earlist possible stage and treat it so that it prevents the morbidity and mortality due to it's advanced stage which also decreases the economic burden to the person, family as well as to the country. Cervical cancer is the most commonly screened disease in female and if it is carried out effectively, it significantly decreases the death due to it.

There are a lot of research on screening of cervical precancerous lesion world wide with the aim to

reduce the death of women due to cervical cancer. In Nepal also many papers are published but overall prevalence is not exactly known. Various studies are carried out in different region, some of them are organized screening program me and some are opportunistic screening program. The incidence depends on the type of screening program. The result of organized screening (where everyone will be screened) can not be compared with opportunistic screening where women come for check up with some gynecological problem. Although one-third of the world cervical cancer burden is endured in India, Bangladesh, Nepal and Sri Lanka, there are important gaps in our knowledge of the distribution and determinants of the disease in addition to inadequate investments in screening, diagnosis and treatment in these countries.[7]

Though the prevalence of cervical cancer in Nepal is not well documented, it is the most commonly reported malignancy among women in Nepal with approximately 2150 invasive cervical cancers reported and 1100 deaths annually.[8]

A large study conducted in only one full fledge cancer hospital in Nepal over 10 years period from 1999 till 2008 showed total of 11469 cases of all cancer with 3372 cases(29%) of cervical cancer . Total number of cases showed a rising pattern over the ten year period. This is in sharp contrast to the US and other western countries where there has been decreasing trend over the past few years .It demands for dare need of organized screening program for cervical carcinoma.[9] Data from studies in developing countries such as Western Cape, South Africa suggest that even limited Pap smear screening reduces the risk of cervical cancer.[10]

Comparison of studies done in the same Hospital (NMCTH)

Year	no.of cases	Abnormal smear	References
2003	100	3%	11
2008	800	4.8%	P. Pradhan12
2010	1751	1.14%	present study

This variation is probably due to the fact that during initial days after establishment of the Hospital, we used to take the smear only in selected cases (the cervix looks abnormal and need to be screened). For the last few years we take pap smear routinely in all female visiting Gynecological out patient departments if they have no smear in the last one year. Incidence also depends on sample size.

COMPARISON OF DIFFERENT STUDIES DONE IN DIFFERENT PARTS OF NEPAL

References	Year	no.of cases	Abnormal smear
Sherpa et al13	2009	n=932	3.6% abnormal pap smear
Dharbhadel et al14	2004	n=350	0.57% positive pap smear
Sankaranarayanan et al[15]	2003	n=4444	3.4% Abnormal sample
K Vadehra16	2006	n=500	5.5% abnormal smear
Tamrakar S[8]	2012	n=1506	1.7% abnormal smear
Vaidya A17	2003	n= 200	9% - high risk,3%- non high risk
Ranabhat SK18	2011	n= 880	abnormal smear 1.7%
Present study	2010	n= 1751	1.14% abnormal smear

While comparing the results of some of the studies carried out in south Asial countries, we get a lot of variation in the results, it all depend on the type of screening and trained personnel involved in the procedure.

A study done in India by Tiwari et al in 100 cases, 5% were abnormal smear with equal number of CIN I and CIN II.[19] Similar result was found by Neelima and colleague with 80% inflammatory and 9.5% epithelial abnormality.[20] Similar study done in Maharastra, India by Dhiraj et al showed 5.8% of study population to be abnormal or precursor of cervical malignancy.[21] As cervical cancer is more common in women with multiple sex patrnes and may be associated with other STDs including AIDS. A study done in Panjab, India in STD clinic with 300 cases of pap smear ,the incidence of abnormal epithelial smear was in 3.6%.[22] All of these results showed higher incidence of abnormal smear than our study.

In Pakistan-Rubina et al studied pap smear of 500 cases, 55.6% the smear was reported negative for malignancy, 36.4% had an inflammatory smear, 6% had CIN and in 2% the smear was inadequate for cytological examination.[23] Another study in Pakistan by Sonia et al in 300 cases found abnormal smear in 2.6% cases.[24] A large study conducted in Karachi,Pakistan over 7 years period in 20995 smear in a muslim population showed 0.55 abnormal smear ,34% inflammatory and 3.8% normal smear and remaining others.[25] This is too low in comparison with our study.

A study done in a Medical college of Bangladesh in 550

cases of pap smear and positive results were confirmed with biopsy, they had 15% abnormal smear of various degrees of epithelial abnormalities .[26] Another study carried out in a large group in the same country by Banik and colegues with 1699 pap smear, they found 8.18 % abnormal epithelial lesion.[27] Another small study done by jahanara B in 100 cases showed 1% abnormal epithelial abnormality.[28] So collectively the incidence of abnormal smear in Bangladesh is comparatively high. Incidence found to be as low as 1% and as high as 15%.

A study done in middle east in a private clinic about 12% of the study sample had abnormal (precursors of cervical cancer) results and the majority (88%) had normal and benign changes so the place of study also influence the result because in private clinics usually symptomatic patients are presented.[29]

A large study done in Teheran with an organized screening program including 13315 pap smear showed abnormal smear 1.18%.[30]

In our study we also tried to find out the relationship between gross look of cervix and result of pap smear. We had incidence of abnormal smear of less than 1% among healthy cervix and 1.2% among unhealthy cervix. Similar study was carried out by Pradhan B and found the malignancy as high as 15% among unhealthy looking cervix and it was confirmed by cervical biopsy.[31]

As we had 20 cases of different grades of abnormal epithelial abnormalities. For LSIL, we treated them with antibiotics and took smear after 3 months. Those who had same result on follow up smear were subjected for cervical biopsy. All five cases of smear with HSIL and 9 cases of LSIL had cervical biopsy (6 cases of LSIL were negative on repeat smear). Out of 5 cases of HSIL, 2 had biopsy proven HSIL, 2 had LSIL and 1 had chronic cervicitis. Out of 9 cases of LSIL, 3 had chronic cervicitis and 6 had LSIL. Total cases of abnormal finding by smear were 20.

Americal College of Obstetrician and Gynecolology (ACOG) recommended guideline for cervical cancer screening- 2012.[32]

A. when to start screening

Age 21 regardless of the age of sexual activity. Women aged <21 years should not be screened regardless of age at sex rega of sexual and other behavior-related risk factors

About annual screening

In women aged 30–65 years, annual cervical cancer screening should not be performed. Patients should be counseled that annual well-woman visits are recommended even if cervical cancer screening is not performed at each visit.

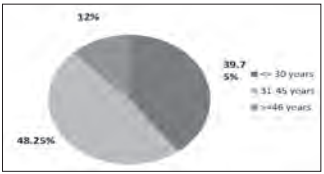

Fig. 1. Age distribution

Fig. 2. Gross look of cervix
(*Unhealthy = Erosion, hypertrophied, congested, growth)

	Pap reports				
Gross Cx	Normal*	Percentage	Abnormal*	Percentage	Total
Healthy	935	99.1%	8	0.8%	943
Unhealthy	796	98.5%	12	1.4%	808
total	1731		20		1751

Normal smear* = Normal, Inflammatory and ASCUS; Abnormal smear*= LSIL,HSIL,CIS; None of them were carcinoma in situ

Reports of Smear

S.N	Reports	Number	Percentage
1.	Normal	571	32.60%
2.	Inflammatory	1152	65.79%
3.	ASCUS	8	0.45%
4.	LSIL	15	0.85%
5.	HSIL	5	0.28%
6.	CIS	0	0%
	TOTAL	1751	100

ASCUS is taken as normal since this is not a premalignant condition Therefore, it shows that majority of the smear were with inflammatory pattern accounting for 65.8% of the total smear and it was mainly prevalent in the age group 30-60 years. Out of 20 cases of abnormal smear 14 had biopsy and 10 had biopsy proven ab normal smear.

Frequency of screening

Every 3 years for conventional cytology and liquid base cytology

Every 5 years in case of conventional and HPV co-testing

HPV co-testing should not be performed in women aged < 30 years.

B. When to stop

Aged >65 years with adequate screening history(having hysterectomy done for some other reason beside precancerous and cancerous lesion)

Women who have received the HPV vaccine should be screened according to the same guidelines as women without vaccination.

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Evaluation of intradermal vaccination at the anti rabies vaccination OPD

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ABSTRACT

Rabies is a virtually 100% fatal acute viral encephalitis caused by an RNA virus belonging to family Rhabdoviridae and genus Lyssavirus. The virus can infect all warm blooded animals. The disease is transmitted to humans by the bite, lick or scratch of an infected animal. More than 99% of all human rabies deaths occur in the developing world. It is preventable with timely and proper usage of modern immunobiologicals (vaccines and immunoglobulins). Once exposure occurs, modern prophylaxis entails immediate wound care, local infiltration of rabies immune globulin and parenteral administration of modern cell culture vaccines in multiple doses. The annual medicinal (vaccines and other drugs) cost for animal bite treatment is Rs. 2 billion approximately (2004). The objective of the present study is to evaluate the performance of the Intradermal (ID) route vis a vis the Intramuscular (IM) route in our clinical setting the Antirabies Vaccination (ARV) OPD, Sir J.J. Hospital, Mumbai. A total of 1460 patients were administered the Antirabies vaccine by the Intradermal route over the 1 year period as compared to 1075 patients who were administered the Antirabies vaccine by the Intramuscular route in the previous year. 1230 (84.2) of the patients who were administered the vaccine by the ID route completed the schedule and 230 (15.8%) partially completed the schedule. Four hundred thirty two (40%) of the patients who were administered the vaccine by the Intramuscular route completed the schedule and 643 (59.8%) partially completed the schedule. The vaccine cost for ID was Rs. 2,80,600. The vaccine cost for the intramuscular (IM) assuming 84% compliance was estimated as Rs. 15, 64, 000. Assuming 40% compliance the cost was estimated as Rs. 7, 82, 230. Thus a saving of Rs. 5, 01, 630 to Rs. 12, 83, 400 was effected. In our setting, the Intradermal regime was cost effective and increased patient adherence and enrolment. It has now been routinely adopted at the clinic.

Keywords: Rabies, intradermal vaccination, cost effective

INTRODUCTION

Rabies is a virtually 100% fatal acute viral encephalitis caused by an RNA virus belonging to family Rhabdoviridae and genus Lyssavirus. The virus can infect all warm blooded animals. The disease is transmitted to humans by the bite, lick or scratch of an infected animal. More than 99% of all human rabies deaths occur in the developing world. The disease has not been brought under control in most of the affected countries.[1]

An estimated 55,000 persons die of rabies globally every year of which 31,000 are from the Asian continent. In India, the Annual Incidence of Human Rabies is 20,000 Cases. The frequency of human rabies deaths is 1 case every 30 minutes (1/2 hour) approximately. The principal animal reservoir is dog (96.3%). The Animal bite incidence rate (per 1000 population) is 17.4 and this translates to a whopping 17.4 million bites every year. The frequency of animal bites in India is 1 every 2 seconds and the annual man-days lost due to animal bite is 38 million. It is preventable with timely and proper usage of modern immunobiologicals (vaccines and immunoglobulins).[2] Once exposure occurs, modern prophylaxis entails immediate wound care, local infiltration of rabies immune globulin and parenteral administration of modern cell culture vaccines in multiple doses. Pre-exposure vaccination should occur in selected population groups at risk of occupational exposure.[3]

Government of India has banned the production and use of Nervous Tissue Vaccine (NTV) in December, 2004 which was the vaccine widely used in the public sector. With the stoppage of NTV, the availability and affordability of modern Cell Culture Vaccine became a major issue with many States.[4]

The annual medicinal (vaccines and other drugs) cost for animal bite treatment is Rs. 2 billion approximately.[2]

Intradermal regimens: The intradermal regimens are of particular interest in areas where rabies vaccines are in short supply or available but inaccessible, in view of their price, to people at risk of contracting rabies.[5] With the recommendations of WHO, National experts

and ICMR study on the use of intradermal vaccines, National Regulatory Authority has permitted the use of this economical and efficacious route in India in 2006.[2-7] Chhabra *et al* (NICD) demonstrated that PCECV is safe and highly immunogenic in Indian subjects when administered intradermally as 0.1 mL/site using the "2-2-2-0-1-1" post-exposure regimen.[8]

Sufficient clinical evidence was presented indicating that a single dose of vaccine given on day 90 of the original Thai Red Cross regimen ("2–2–2–0–1–1" regimen) can be replaced if two doses of vaccine are given on day 28 ("2–2–2–0–2" regimen).[9,10] The Thai Red Cross regimen considerably lowers the cost of vaccination as the total volume of vaccine required is much less than that needed for intramuscular regimens.[7]

The WHO recommends that this route of administration is one of the ways to ensure the provision of effective treatment to the large number of bite victims at an affordable cost.[8] The anti-rabies vaccines currently approved for use in India through ID route are Rabipur (PCECV), Verorab (PVRV), Abhayrab (PVRV) and Pasteur Institute of India, Coonoor (PVRV).[9]

Our clinical setting was the Antirabies Vaccination (ARV) clinic in a tertiary government hospital in Mumbai, Maharashtra. The Purified Chick Embryo Cell Vaccine (Rabipur TM) supplied by the hospital on rate contract costed Rs. 230 per vial. The cost was a major concern as a patient enrolled in the OPD would require 5 vials to complete the Essen's regime. After the DCGI clearance to Intradermal vaccination, it was initiated in the Antirabies Vaccination (ARV) OPD on the 1st of July 2008.[11] All the patients enrolled in the OPD from the 1st of July 2008 were administered the vaccine using the Intradermal route.

Objective of the study: To evaluate the performance of the Intradermal(ID) route vis a vis the Intramuscular (IM) route in our clinical setting in terms of adherence and cost

MATERIALS AND METHODS

Setting: Antirabies Vaccination OPD, Sir J.J. Hospital, Mumbai

Study Period: 1st July 2008 - 30th June 2009 for the Intradermal (ID) route

I year data (2007 - 2008): For the Intramuscular (Essen's) regime

Vaccine used: Purified Chick Embryo Cell Vaccine (RabipurTM) supplied by the hospital on rate contract

2 site Intradermal schedule (2-2-2-0-2) : As per Drugs Controller General of India (DCGI) (Annexure-2), the schedule recommended for IDRV is the updated Thai red cross schedule. [11] One dose of 0.1 ml vaccine at each of the two sites was given on days 0,3, 7 and 28. [12] The most common site was the deltoid.

Essen's regime: One dose 1 ml intramuscular (deltoid) on day 0.3, 7, 14 and 28.[10]

Data was analysed using the Stata SE 10.1 software. Pearson's Chi square test (2 sided p values), Odds Ratio's and 95% Confidence Intervals (Exact) were calculated.

RESULTS

A total of 1460 patients were administered the Antirabies vaccine by the Intradermal route over the 1 year period as compared to 1075 patients who were administered the Antirabies vaccine by the Intramuscular route in the previous year (Table-1). One thousand two hundred thirty (84.2) of the patients who were administered the vaccine by the ID route completed the schedule and 230 (15.8%) partially completed the schedule. 432 (40%) of the patients who were administered the vaccine by the Intramuscular route completed the schedule and 643 (59.8%) partially completed the schedule (Fig. 1). OR (95%CI) = 7.96 (6.61 - 9.59) X^2= 532.3, d.f.= 1, p<0.0001 (VHS)

Table-1: Patient enrolment ID vs IM regime

Route	Completed Schedule	Partially Completed Scheduled	Total
ID (2008 - 2009)	1230 (84.2)	230 (15.8)	1460
IM (2007 - 2008)	432 (40.2)	643 (59.8)	1075
Total	1662 (65.6)	873 (34.4)	2535

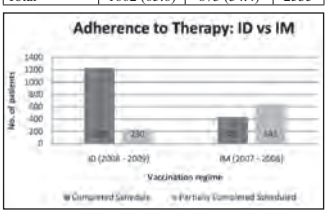

Fig. 1. Adherence to therapy

OR (95%CI) = 7.96 (6.61 - 9.59) X2= 532.3, d.f.= 1, p<0.0001 (VHS)

The PCECV Rate Contract at Sir J.J. Hospital was Rs.

230/ Vial. A total of 1220 vials were used. The vaccine cost for ID was Rs. 2,80,600. The vaccine cost for the intramuscular (IM) assuming 84% compliance was estimated as Rs. 15, 64, 000. Assuming 40% compliance the cost was estimated as Rs. 7, 82, 230. Thus a saving of Rs. 5, 01, 630 to Rs. 12, 83, 400 was effected (Fig. 2). 1230 patients received 4 doses each (Fig. 3). A total no. of 1220 vials were used during the study period and the total no. of doses administered were 5482. Thus per vial, 4.49 doses were administered. The vaccine wastage was 10.13%.

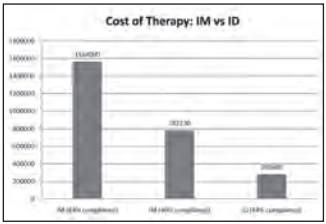

Fig. 2. Cost of therapy (in Indian Currency)

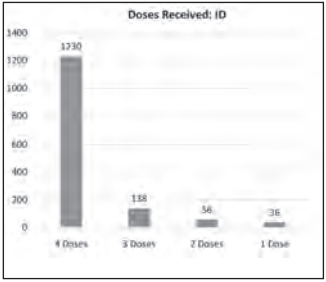

Fig. 3. Number of doses received during the study period

DISCUSSION

Patients attending the clinic and ARV used has increased over the years, adding financial burden to the institute. The main issue with the previous Essen (IM) regime was the cost of the vaccine resulting in supply issues. To address these issues, where vaccine and money are in short supply, ID route seemed ideal in terms of economic benefits, safety and efficacy. The introduction of Intradermal vaccination at the ARV Clinic was associated with a very significant rise in patient compliance. We were also able to enroll more patients after initiating the intradermal regime. The vaccine wastage was minimal (10.13%).

It has reduced the cost of vaccination by about 82% (assuming 84% compliance). This makes it an attractive option for middle and low level income countries like ours.[13] With commitment and effort, an ideal IDRV vaccine clinic can be set-up. ID administration requires some amount of technical skills which may be imparted by training interns and staff nurses.[14] We have trained 196 interns in this time period. In our setting, the Intradermal regime was cost effective and increased patient adherence and enrolment. It has now been routinely adopted at the clinic.

Sudarshan *et al*[15] carried out a comparative study of IDRV, Semple vaccination and modern vaccines by intramuscular route based on the available studies and prevailing cost factors and estimated that IDRV would cost nearly one third of the intramuscular Essen regimen.

Rahim *et al*[16] did a retrospective analysis of case records of a three-year period (2006-2008). All cases who have been treated with intramuscular ARV (both partial and complete) for a period of three years (2006-2008) in the preventive clinic of Calicut Medical College were included in the study. The cost of ARV for three years was calculated and compared with intradermal regimen (modified Thai schedule). The benefit in terms of expenditure to the Government was calculated if ID regimen had been used in all these cases. They estimated that the ID regimen reduces the cost of vaccination by about 70-80%. This concurs with our findings.

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Stapled Haemorrhoidectomy in the operative treatment of grade III and IV haemorrhoids

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ABSTRACT

Stapled haemorrhoidectomy (SH) is a minimally invasive intervention that uses a stapling device which avoids the need for wounds in the sensitive anal area and reduces the pain after surgery. This study was undertaken in Nepal Medical College Teaching Hospital from January 2010 to December 2012 to evaluate the efficacy of this modality of treatment among patients (32) who presented in the Surgery OPD with grade III and grade IV haemorrhoids. The results of SH were evaluated by the relief of symptoms, severity of post operative pain, and complications of SH. Twenty five (78.1%) patients had grade III and 7 (21.9%) presented with grade IV hemorrhoids. The most frequent presentation reported in our study was bleeding per rectum with perianal prolapse. Mean operating time was 40-60 minutes whereas mean hospital stay was 1.9 days. Urinary retention was the most common complication found in 12 (37.5%) patients in the immediate post operative period. SH is a safe, rapid, and convenient surgical remedy for grade III and grade IV hemorrhoids with low rate of complications, minimal postoperative pain, and shorter hospital stay.

Keywords: Stapled haemorrhoidectomy, haemorrhoids, prolapse

INTRODUCTION

Prolapse of anal cushion with or without bleeding per rectum is known as hemorrhoids. Around 5% of general population suffer from symptoms of hemorrhoids and one third seeks medical treatment.[1] Stapled haemorrhoidectomy (SH) is a minimally invasive intervention that uses a stapling device which avoids the need for wounds in the sensitive anal area and reduces the pain after surgery.

This technique was introduced in 1997 by Sir Antonio Longo.[2] Conventional haemorrhoidectomy provides symptomatic relief for most patients. However, the wounds created by this method of surgery are usually associated with considerable post operative pain which necessitates prolonged recovery period. SH a new approach to the treatment of grade III and IV hemorrhoids, removes a circumferential strip of mucosa and sub mucosa about 4 cm above the dentate line with a circular stapler, interrupting the haemorrhoidal vessels and stretching the prolapsed anal cushion.[3] Thus it is reported to be an effective treatment with low morbidity, high patient satisfaction and good long-term control of hemorrhoidal symptoms.[4] It is not routinely performed in our hospital. Hence this study was undertaken to evaluate the efficacy of this modality in our set-up.

MATERIALS AND METHODS

This study was performed in Nepal Medical College Teaching Hospital from January 2010 to December 2012. Patients who presented in the Surgery OPD with clinical history of per rectal bleeding, perianal prolapse, constipation associated with or without discharge and pain underwent proctoscopic evaluation. Those with grade III and grade IV hemorrhoids were treated by stapled haemorrhoidectomy using Ethicon Endosurgery PPH 30mm circular stapling device. The procedure was performed by a single surgeon (the first author) with assistance by other co authors.

An informed consent was taken from all the patients. Ezivac enema was administered at 6 am on the day of surgery. Preoperative antibiotics Ciprofloxacin 200 mg and Metronidazole 500 mg were administered intravenously at the time of induction of spinal anaesthesia. The procedure was performed in lithotomy position. After dilatation of anal canal, a purse string suture was placed over the mucosa and sub mucosa about 3-4 cm above the dentate line with 2(0) polypropylene. Subsequently a circular stapler was introduced transanally. The anvil of the device was positioned proximal to the purse string and the suture was tied down on to the anvil. Retraction of the suture pulled the attached rectal mucosa into the stapler. Closure of the anvil and firing of the stapler simultaneously excised a doughnut of mucosa and submucosa proximal to the haemorrhoides, thus interrupting the blood supply but

maintaining continuity of the rectal mucosa. The stapled line was inspected for any bleeding and if present, haemostatic sutures were placed with 3(0) polyglactin. Postoperatively, patients were allowed oral intake after 4-6 hours. Post operative pain was assessed as per the requirement of doses of parenteral analgesia in the first 24-48 hours. Sitz bath and stool softner were prescribed for 2 weeks post operatively. Almost all the patients were discharged after second post operative day. They were followed up in the surgery OPD after 2 weeks, then after 2 months, 6 months and 1 year after the surgery. The results of SH were evaluated by focusing on the relief of symptoms, severity of post operative pain, and complications of SH.

RESULTS

There were total 32 patients of which 24 (75.0%) were males and 8 (25.0%) were females. The age varied from 34 to 66 years with a mean age of 50 years. (Table 1)

Age distribution with category of patients:

SN	Age distribution(yrs)	Male (%)	Female (%)
1	0-20	nil	nil
2	21-40	14 (58.3)	6 (75.0)
3	41-60	7 (29.2)	2 (25.0)
4	60	3 (12.5)	
	Total	24 (75%)	8 (25%)

In this study, only patients with grade III and grade IV hemorrhoids were included. There were 25 (78.1%) grade 3 and 7 (21.9%) grade 4 hemorrhoids. Their pre-operative presentation were prolapse haemorrhoids in all the patients (100%), bleeding per rectum in 22 (68.7%), constipation in 12 (37.5 %) and pain with or without discharge in 5(15.6%) patients. (Table 2) All of them underwent stapled haemorrhoidectomy under spinal anaesthesia with mean operating time of 40 – 60 minutes.

Post operatively, 12 (37.5%) patients over the age of 50 years developed acute urinary retention that was managed with indwelling Foley's catheterization for 24 hours. One patient presented in the emergency 24 hours after discharge with severe per rectal bleeding from the stapled line which was controlled successfully by haemostatic resuturing under spinal anesthesia (Table 2).

2. Pre-operative and post operative Presentation

SN	Pre operative Clinical Presentation	Number (%)
1	Prolapse Mucosa	32 (100)
2	Bleeding per rectum	22 (68.7)
3	Constipation	12 (37.5)
4	Anal Pain	5 (15.6)
	Post Operative Complication	
1	Urinary Retention	12 (37.5)
2	Mild peri-anal pain	2 (6.2%)
3	Grade II Haemorroids	2 (6.2%)
4	Bleeding per rectum	(3.1%)

The assessment of post operative pain at 24 - 48 hours was interpreted the requirement of analgesia. In the first 24hours 6 (18.7%) patients required single dose intramuscular diclofenac sodium 75mg, 9 (28.1%) patients required double dose and 17 (53.2%) patients were given three doses. In the subsequent 24 hours, this analgesic requirement was significantly decreased. In this period, 10 (31.3%) patients requiring one dose and 5 (15.6%) requiring two doses and none requiring three doses. (Table 3)

3. Assessment of Post operative pain as requirement of analgesia

Assessment of pain 24 hrs after operation		
SN	**Dose of Inj Diclofenac Sodium (75 mg)**	**No. of patients (%)**
1	Single dose	6 (18.7)
2	Double dose	9 (28.1)
3	Three doses	17 (53.2)
Assessment of pain 48 hrs after operation		
SN	**Dose of Inj Diclofenac Sodium (75 mg)**	**No. of patients (%)**
1	Single dose	10 (31.3)
2	Double dose	5 (15.6)
3	Three doses	None

Six months after surgery 2 patients had recurrent 2nd degree hemorrhoids which were managed by Barron's gun banding at the outpatient department. Other than this, there were no complaints with other patients during follow up.

DISCUSSION

Many theories have been coined about the etiopathogenesis of hemorrhoids including venous varicosities of the anus, vascular hyperplasia in the hemorrhoidal vascular tissue, and a mucosal prolapse of the anal canal mucosa resulting in elongation and kinking of the upper and middle hemorrhoidal vessels.[5,6] The operative time for stapled haemorrhoidectomy has been demonstrated to be shorter than open procedure in several trials and is generally reported at 15-25 minutes. [7] Our average operating time of 40-60 minutes is almost double while comparing with other studies.[8]

Stapled haemorrhoidectomy is a simple, safe and effective alternative method for the treatment of symptomatic haemorrhoids with a relatively painless post operative period as it excises a circumferential portion of the lower rectal and upper anal canal mucosa and submucosa and performs a re-anastomosis with a circular stapling device. The strongest argument in favour of this operation is that it leaves the richly innervated anal canal tissue and perianal skin intact, thus reducing the pain usually associated with open method. Its indication includes

grade III and uncomplicated grade IV haemorrhoides. In the (Procedure for Prolapse and Hemorrhoids) PPH Multicentre Study Group trial it was demonstrated that stapled haemorrhoidopexy offers the benefits of less post operative pain, less analgesics requirements, and less pain at bowel movement.[9] We have noted similar results in our series and no recurrence of symptoms on follow up. Similarly other studies presented that SH results in significantly lesser immediate postoperative pain than conventional excision techniques and offers more comfort to the patient. [10, 11, 12]

Urinary retention is a common complication of anorectal surgery with an incidence between 1.5 to 32%.[13] in our experience, urinary retention occurred in 37.5% of cases. The causes of urinary retention are uncertain, but perioperative fluid intake and perioperative pain are the possible precipitating factors.[14] Other complications such as perforation of the rectum,[15] pneumoretroperitoneum,[16] pelvic sepsis[17] and rectal obstruction[18] have also been reported but were not encountered by us.

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Spectrum of bacterial pathogens and their antibiogram from cases of urinary tract infection among renal disorder patients

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ABSTRACT

Urinary tract infection (UTI) is the commonest bacterial infection occurring in renal disorder patients and is associated with significant morbidity. Resistance to antibiotics is highly prevalent in bacterial isolates and is an emerging problem in UTI. A hospital based cross sectional study was conducted from April 2011 to September 2011 to determine the frequency and bacterial profile of urinary tract infections in the patients with renal disorders visiting KIST Hospital along with their antimicrobial susceptibility pattern. Urine samples were collected from 300 clinically-suspected cases of UTI among renal disorder patients and investigated by conventional semi-quantitative culture technique, microscopy and antibiotic susceptibility test. Significant bacteriuria were detected in 34% of the total subjects, mostly from patients with Chronic Kidney Disease. Incidence of bacteriuria was found higher in females (40.40%) than in males (27.52%) and mostly occurred in elderly patients. *Escherichia coli* (62.75%) was the predominant isolate followed by *Klebsiella pneumoniae* (10.78%), *Staphylococcus aureus* (9.80%), Coagulase negative *Staphylococcus aureus* (CoNS) (5.88%), *Enterococcus spp* (3.92%), *Klebsiella oxytoca* (2.00%), *Pseudomonas aeruginosa* (2.00%), *Proteus mirabilis* (2.00%) and *Proteus vulgaris* (1.00%). Multidrug resistance was observed in 68.82% of the total bacterial isolates.

Key words: Urinary tract infection, Renal disorder, Bacteriuria, Multidrug resistance

INTRODUCTION

Urinary tract infection (UTI) is an important complication of certain conditions like diabetes, renal disease, renal transplantation and structural and neurologic abnormalities that interfere with urine flow[1]. Many kidney disorders exist and major renal disorders encountered are acute renal failure (ARF), nephrotic syndrome (NS), nephrolithiasis, hydronephrosis, glomerulonephritis. Chronic Renal Failure (CRF) which is the permanent loss of kidney function, is the most severe of these. UTI complicating kidney diseases are often caused by *E. coli*. *Enterococcus spp, Pseudomonas aeruginosa, Klebsiella spp, Proteus spp* are also pathogens frequently associated with UTI. *Staphylococcus epidermidis* and *Enterococcus* spp are more often associated with UTI in hospitalized patients[2]. Since patients with kidney disorders are usually on prophylactic therapy, pathogens causing UTI are often resistant to the commonly prescribed drugs. This study was conducted to find the profile of bacteria and their antibiogram from UTI cases among the renal disorder patients attending our hospital since very few such studies have been done.

MATERIALS AND METHODS

During April 2011 to September 2011, a total of 300 mid- stream urine samples from patients suspected of UTI among renal disorder patients were collected and processed according to standard laboratory methods. These samples were cultured on MacConkey agar and Blood agar plates and incubated at 37°C for 24 hrs. The test by Kirby- Bauer's disc diffusion method. Bacteria resistant to >2 classes of antibiotics were isolated bacteria were identified by colony characters, Gram's reaction and biochemical tests. Bacterial growth $\geq 10^5$ cfu/ml were considered as significant. The isolates were then subjected to antibiotic susceptibility considered as multi drug resistant[3]. Data were then analysed by SPSS version 16.

RESULTS

Overall 102 (34.00%) showed significant growth whereas 198 (66.00%) showed no growth. As shown in figure 1, the most common renal condition yielding significant bacteriuria was CKD, 58 (56.86%) followed by ARF, 14 (13.73%). More number of female patients (40.40%) had significant growth as compared to males (27.52%). This difference was statistically significant.

Among the 300 patients, 37% were asymptomatic but 26.13% of them showed significant growth. Similarly, 38.62% of the symptomatic patients had significant growth. Inpatient comprised 43.31% of the growth positive cases while 27.17% were from outpatients which were statistically significant.

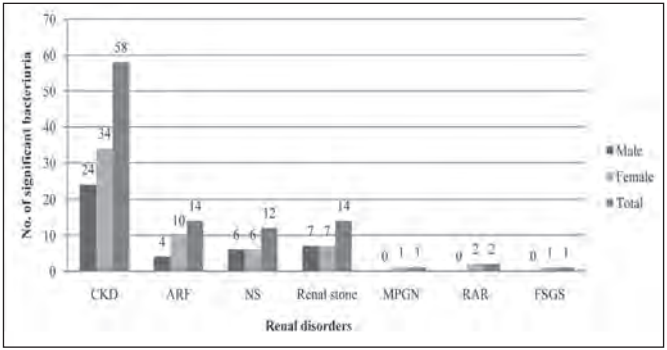

Fig 1: Gender wise distribution of significant bacteriuria in renal disorders patients

Note: CKD: Chronic Kidney Disease, ARF: Acute Renal Failure, NS: Nephrotic Syndrome, MPGN: Membrano Proliferative Glomerulonephritis, RAR: Renal Allograft Recipients, FSGS: Focal Segmental Glomerulosclerosis

A total of 102 isolates belonging to 9 different genera were isolated among which *Escherichia coli* 64 (62.75%) was the commonest followed by *Klebsiella pneumoniae* 11 (10.78%) and *Staphylococcus aureus* 10 (9.80%). The highest number 34 (58.62%) of *E. coli* were isolated from Chronic kidney disease (CKD). Table 1 shows the frequency of different bacterial isolates from different renal disorder.

Among the first line antibiotics used against Gram negative bacteria, most of the isolates were sensitive to nitrofurantoin (75.61%) followed by gentamicin (60.98%). Among the second line drugs, the isolates were found to be sensitive to amikacin 39 (76.47%) followed by ofloxacin 13 (25.49%) and ceftazidime 9 (17.65%).

Table 1: Pattern of bacterial isolates from urine samples in renal disorder patients

Organism isolated	CKD No. (%)	ARF No. (%)	NS No. (%)	Renal stone No. (%)	MPGN No.(%)	RAR No.(%)	FSGS No.(%)	Total No.(%)
Gram negative bacteria								
E. coli	34(58.62)	11(78.57)	8(66.67)	9(64.29)	1(100)	1(50.00)	-	64(62.75)
K.pneumoniae	8(13.79)	1(7.14)	-	2(14.29)	-	-	-	11(10.78)
K. oxytoca	2(3.45)	-	-	-	-	-	-	2(2.00)
P.aeruginosa	2(3.45)	-	-	-	-	-	-	2(2.00)
P. mirabilis	-	-	2(16.67)	-	-	-	-	2(2.00)
P. vulgaris	1(1.72)	-	-	-	-	-	-	1(1.00)
Gram positive bacteria								
S. aureus	5(8.62)	1(7.14)	2(16.67)	1(7.14)	-	1(50.00)	-	10(9.80)
CoNS	4(6.89)	1(7.14)	-	1(7.14)	-	-	-	6(5.88)
Enterococcus spp	2(3.45)	-	-	1(7.14)	-	-	1(100)	4(3.92)
Total	58(56.86)	14(13.73)	12(11.76)	14(13.73)	1(1.00)	2(2.00)	1(1.00)	102

Note: CKD: Chronic Kidney Disease, ARF: Acute Renal Failure, NS: Nephrotic Syndrome, MPGN: Membrano Proliferative Glomerulonephritis, RAR: Renal Allograft Recipients, FSGS: Focal Segmental Glomerulosclerosis

Table 2: Antibiotic Susceptibility Pattern of Gram negative bacteria

Antibiotic used		E coli N=64	Klebsiella pneumoniae N=11	Klebsiella oxytoca N=2	Proteus mirabilis N=2	Proteus vulgaris N=1	Pseudomonas aeruginosa. N=2
CIP (%)	R	49(76.56)	4(36.36)	1(50.00)	2(100)	1(100)	0
	I	0	0	0	0	0	0
	S	15(23.44)	7(63.64)	1(50.00)	0	0	2(100)
G (%)	R	24(37.50)	3(27.27)	1(50.00)	2(100)	1(100)	0
	I	1(1.56)	0	0	0	0	0
	S	39(60.94)	8(72.73)	1(50.00)	0	0	2(100)
NA (%)	R	55(85.94)	6(54.55)	1(50.00)	2(100)	1(100)	2(100)
	I	0	1(9.09)	0	0	0	0
	S	9(14.06)	4(36.36)	1(50.00)	0	0	0
COT (%)	R	44(68.75)	6(54.55)	0	2(100)	1(100)	2(100)
	I	0	0	0	0	0	0
	S	20(31.25)	5(45.45)	2(100)	0	0	0
AMP (%)	R	56(87.50)	10(90.91)	2(100)	2(100)	1(100)	-
	I	2(3.13)	0	0	0	0	-
	S	6(9.38)	1(9.09)	0	0	0	-
CZ (%)	R	54(84.38)	10(90.91)	2(100)	2(100)	1(100)	-
	I	2(3.13)	0	0	0	0	-
	S	8(12.50)	1(9.09)	0	0	0	-
NIT (%)	R	9(14.06)	3(27.27)	1(50.00)	2(100)	1(100)	2(100)
	I	2(3.13)	0	0	0	0	0
	S	53(82.81)	8(72.73)	1(50.00)	0	0	0
TOB (%)	R	-	-	-	-	-	0
	I	-	-	-	-	-	0
	S	-	-	-	-	-	2(100)
PI (%)	R	-	-	-	-	-	2(100)
	I	-	-	-	-	-	0
	S	-	-	-	-	-	0
OF (%)	R	32(50.00)	2(18.18)	1(50.00)	2(100)	1(100)	0
	I	0	0	0	0	0	0
	S	7(10.94)	4(36.36)	0	0	0	2(100)
AK (%)	R	6(9.38)	1(9.09)	0	2(100)	1(100)	0
	I	2(3.13)	0	0	0	0	0
	S	31(48.44)	5(45.45)	1(50.00)	0	0	2(100)
CAZ (%)	R	33(51.56)	2(18.18)	1(50.00)	2(100)	1(100)	2(100)
	I	1(1.56)	0	0	0	0	0
	S	5(7.81)	4(36.36)	0	0	0	0

Note: R= resistant, S= sensitive, I= intermediate, CIP=Ciprofloxacin, G= Gentamicin, NA= Nalidixic Acid, COT= Cotrimoxazole, AMP= Ampicillin, CZ= Cefazolin, NIT= Nitrofurantoin, TOB= Tobramycin, PI= Piperacillin, OF= Ofloxacin, AK= Amikacin, CAZ= Ceftazidime

Among the first line drugs tested for Gram positive bacteria, most isolates were found to be sensitive to nitrofurantoin 18 (90.00%) followed by gentamicin 15 (75.00%). Among the second line drugs, most were sensitive to amikacin 9 (81.82%). All the gram positive isolates showed 100% sensitivity towards vancomycin (Table 3). Out of 102 bacterial isolates, 70 (68.82%) were multidrug resistant (MDR); 76.56% of *E. coli* were MDR (Table 4).

Table 3: Antibiotic Susceptibility Pattern of Gram positive bacteria

Isolates tested		CIP (%)	G (%)	COT (%)	P (%)	OX (%)	NIT (%)	OF (%)	AK (%)	VA (%)
S. aureus N=10	R	3(30.00)	0	4(40.00)	9(90.00)	9(90.00)	0	3(30.00)	0	0
	I	0	0	0	0	0	0	1(10.00)	0	0
	S	7(70.00)	10(100)	6(60.00)	1(10.00)	1(10.00)	10(100)	1(10.00)	5(50.00)	10(100)
CoNS N=6	R	2(33.33)	2(33.33)	1(16.67)	5(83.33)	4(66.67)	0	1(16.67)	0	0
	I	0	0	0	0	0	0	0	0	0
	S	4(66.67)	4(66.67)	5(83.33)	1(16.67)	2(33.33)	6(100)	1(16.67)	2(33.33)	6(100)
Enterococcus spp N=4	R	4(100)	3(75.00)	3(75.00)	2(50.00)	3(75.00)	2(50.00)	3(75.00)	2(50.00)	0
	I	0	0	0	0	0	0	0	0	0
	S	0	1(25.00)	1(25.00)	2(50.00)	1(25.00)	2(50.00)	1(25.00)	2(50.00)	4(100)

Note: R= resistant, S= sensitive, I= intermediate, CIP=Ciprofloxacin, G= Gentamicin, COT= Cotrimoxazole, P= Penicillin, OX= Oxacillin, NIT= Nitrofurantoin, OF= Ofloxacin, AK= Amikacin, VA= Vancomycin

Table 4: Percentage of single drug, two drugs and multidrug resistant bacterial isolates

Organisms	Total no. of isolates	Resistant to				
		0 Drug (%)	1 Drug (%)	2 Drug (%)	MDR Strains	
					> 2 drugs	%
E. coli	64	3	5	7	49	76.56
K. pneumoniae	11	1	0	4	6	54.55
K. oxytoca	2	0	1	0	1	50
P. mirabilis	2	0	0	0	2	100
P. vulgaris	1	0	0	0	1	100
P. aeruginosa	2	0	0	0	2	100
S. aureus	10	0	6	1	3	30
CoNS	6	1	2	1	2	33.33
Enterococcus spp	4	0	0	0	4	100
Total	102	5 (4.90)	14 (13.73)	13 (12.75)	70	68.82

DISCUSSION

In the present study, the culture positivity was found to be only 34%. Similar low growth rate was observed in other studies[4].The low yield might be due to the presence of either slow growing organisms or organisms that cannot be grown on ordinary media or the sample collected after initiation of antibiotics. Significant bacteriuria was found in 31.82% among patients with acute renal failure which is similar to the study carried out by Jadav *et al.*,[5]. Similarly, the significant growth was found in 30.00% of nephrotic syndrome cases which is in contrast with the study done by Adeleke *et al.*,[6]. UTI prevalence was higher in females (40.40%) than in males (27.52%) which was found statistically significant ($p<0.05$). The higher prevalence of UTI in females is also supported by the findings in Nepal[7] and elsewhere[8]. There are no specific reasons behind the higher isolation rate in female patients with renal disorder besides the anatomical proximity of the female urethra to the anus. This may be also due to inclusion of more female patients suffering from renal disease in this study.

Since 26.13% of the asymptomatic patients had significant bacteriuria, diagnosis based on symptoms alone is highly inaccurate. Leigh *et.al.*[7], have derived a >50% false negative rate with symptomatic diagnosis alone. Similar findings were shown in studies by Karkkainen[8] and Dhakal[9].

Higher rate of isolates were seen among inpatients as compared to outpatients. Interventions, manipulations, exposure to hospital flora, antibiotics pressure, patient's immunocompromised state, all contribute to acquisition of infection in a hospital setup. Some of these patients were admitted for complicated UTI.

Gram negative bacilli 82 (80.39%) were the predominant organism and the Gram positive cocci were only 20 (19.61%). This finding agrees with the studies done in Nepal[10,11] and elsewhere[12,13]. *E. coli* is by far the most common bacteria isolated from urine samples in both outpatient and inpatient in renal disease patients. This finding is in agreement with other studies[14]. *E. coli* possess a number of virulence factors specific for colonization and invasion of the urinary epithelium such as P-fimbriae and S-fimbriae adhesions which make it a successful uropathogen.

In this study, 75.61% gram negative isolates were sensitive to nitrofurantoin. Although most *E. coli* were susceptible to nitrofurantoin, this antimicrobial agent has demonstrated poor invitro activity against Enterobacteriaceae other

than *E. coli*. However, nitrofurantoin should not be used in patients with significant renal dysfunction. Prolonged use of nitrofurantoin in chronic renal failure is associated with increased risk of neuropathies due to systemic drug accumulation[15]. Aminoglycosides like gentamicin and amikacin may be ototoxic and nephrotoxic, especially in patients with diminished renal function. Therefore, if they have to be used, the dosage should be reduced according to GFR (Glomerular Filtration Rate) and serum creatinine and antibiotic levels should be monitored[15,16] .

Quinolone (nalidixic acid) and Fluroquinolone (ciprofloxacin, ofloxacin) are the drugs of choice for the treatment of pyelonephritis in patients with renal dysfunction unless bacterial sensitivities indicate otherwise. However in the current study, large number of isolates (>50%) were resistant to them. These were isolated from urine from elderly people with complicated UTI. Among Gram positive bacteria, most isolates were sensitive to vancomycin (100%) and similar result was obtained in the study carried out by Hasan *et al.*,[17]. Several factors are responsible for the development of drug resistant uropathogens which include misuse of antimicrobials, frequent oral use of broad spectrum antimicrobials that may change intestinal flora and inappropriate dosages and duration of treatments[18]. In this study, 68.82% (70/102) were found to be multi drug resistant (MDR) which is similar to the results of Pokhrel *et al.,*[19].

The highest percentage of resistance was seen with the beta-lactam drugs (88.75% for ampicillin and 86.25% for cefazolin). These beta-lactam agents are less effective in bacteriuria eradication, leading to increased rate of recurrence and therefore are not preferred for treatment of UTI [20]. However, the high numbers of resistant isolates reflect the fact that these are the agents often prescribed and procured over the counter without prescription for different ailments.

To conclude, UTI is a common complication occurring in patients with various renal diseases. Symptomatic diagnosis alone can miss out a number of UTI cases among these patients. So, a urine culture and antibiotics sensitivity test will help establish the diagnosis as well as help choose the antibiotic which is effective, without nephrotoxicity and with minimal risk of building up resistance.

ACKNOWLEDGEMENT

We are grateful to Dr. Amita Pradhan, Statistician, KIST Hospital for her help in Statistical Analysis.

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Computed tomogram guided fine-needle aspiration cytology of lung and mediastinal masses with cytological correlation: A study of 257 cases in Western region of Nepal

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ABSTRACT

Computed tomogram guided fine needle aspiration cytology (FNAC) is an important and useful investigation to differentiate between benign and malignant lesions of lungs. To evaluate the lung and mediastinal masses and to analyze and compare the results with cytological findings, 257 patients were retrospectively studied who underwent CT guided FNAC over a period of 2007 to 2013. The study was done in patients who presented with respiratory symptoms with a localized lung lesion which was confirmed radiologically. 252 cases of lung masses and 5 cases of mediastinal cases were included. Patients' age ranged from 24 to 84 year and the male to female ration was 1.2:1. Radiologically, out of 257 cases, 225 cases were given as malignant, 8 cases as benign and 24 cases as inflammatory lesions. Cytologically, 212 cases were malignant, 12 cases were benign and 21 cases were inflammatory. Most common lung malignancy was adenocarcinoma (87 cases) followed by squamous cell carcinoma (56 cases). 8 cases of lung metastasis were seen. Compared to biopsy, CT guided FNAC shortens the diagnostic interval and helps in differentiating lung malignancy into different cytopathological types which aids in proper management of the malignant lesion.

Keywords: Computed tomogram, cytology, guided FNAC, lung mass

INTRODUCTION:

Computed tomography (CT) guided fine needle aspiration cytology (FNAC) is a well known modality for characterization of lung masses. It has been used to differentiate lung masses into benign, malignant and inflammatory types. Furthermore its use has been extended in differentiating lung malignancy into different cytopathological types which aids in proper management of the malignant lesion. CT guided FNAC is widely recognized technique in indeterminate mass. It is a simple diagnostic method of relatively low cost, with negligible mortality and limited morbidity.[1] The accuracy of CT guided FNAC for discriminating benign from malignant lesion has been recorded to vary from 64% to 97%.[2]

Several post procedural complications have been reported for CT guided FNAC such as pulmonary hemorrhage, hemoptysis and pneumothorax. The risk for developing pneumothorax has been observed to be 22% - 45% due to high sensitivity of CT in detecting pneumothorax.[3] Relative contraindications to image guided FNAC are severe chronic obstructive airway disease, bleeding diathesis, contralateral pneumonectomy and pulmonary arterial hypertension.[4]

The purpose of our study is to evaluate the accuracy of CT and CT guided FNAC in differentiating and recording the pathological spectrum of the lung masses.

MATERIALS AND METHOD:

This is a retrospective study conducted in Manipal Teaching Hospital (from Jan 2007 to Dec 2013) and Gandaki Medical College (Jan 2012 to Dec 2013) for a period of 7 years. The study was carried out in 257 patients who presented with intrathoracic and mediastinal mass that attended the outpatient/inpatient department of Medicine in the respective hospitals and were sent for chest CT in the department of Radiology. Relevant clinical history and investigations were obtained from the patient to narrow down the differential diagnosis and to see if patient was eligible for FNAC, such as history of bleeding disorder, thrombocytopenia, dyspnea, uncontrolled cough, chronic obstructive airway diseases, pulmonary arterial hypertension etc. CT guided FNAC was performed in patients with peripheral lung mass or mass which were only approachable by spinal needle. Patient inclusion criteria included: cooperative patient who was able to hold breath for a short while, no bleeding tendency, indeterminate lung mass, patient who was to undergo chemo- or radio-therapy and lesions not approachable by USG. Informed and written consent was taken from the patient explaining the risk and benefits of the procedure.

Axial section of the area of interest was taken after a scanogram. A feasible approach was judged and the patient positioned accordingly with raiopaque marker placed at the site of puncture. Then under all aseptic

precaution a 20-gauge spinal needle was introduced in suspended respiration into the lesion (Fig 1).

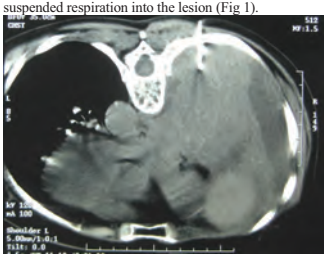

Fig. 1. Ct guided FNAC of lung mass with a needle.

Repeat scan of the area of interest was taken to check the position of the needle tip. With satisfactory position of the needle, the stylet was removed, 10 cc syringe attached, negative suction maintained and aspiration was carried out with to and fro movement. Slides were prepared from the aspirate. Both air dried and alcohol fixed (95% alcohol) smears were made for cytological evaluation. A final CT scan of the area of interest was taken to rule out any complications. The smeared slides were stained with Giemsa and Papanicolaou stains for cytological examination.

All the relevant data were collated and analysis was performed using SPSS version 17 program.

RESULTS:

The data were collected from January 2007 to December 2013. Our study included 257 patients, out of which 252 with intrathoracic and 5 with mediastinal lesions were subjected to CT guided FNAC. Their ages ranged from 24 to 84 years with mean age of 67.32 years. The male to female ratio was 1.2:1. The distribution of cases according to radiological and cytological diagnosis is given in table 1.

Table 1. Distribution of cases according to radiological and cytological diagnosis.

	Malignant	Benign	Inflammatory
Radiological diagnosis (n=257)	225	8	24
Cytological diagnosis (n=245)	212	12	21
No of inadequate cases (n=12)	9	1	2

Comparison between radiological and cytological diagnosis is given in table 2.

Table 2. Comparison between radiological and cytological diagnosis.

		Cytological diagnosis		
		Malignant	Benign/ Inflammatory	Total
Radiological diagnosis	Malignant	202	14	216
	Benign/ Inflammatory	10	19	29
	Total	212	33	245

Sensitivity – 95.28%, Specificity – 57.57%

Out of 212 malignant cases, adenocarcinoma (87 cases) was the commonest followed by squamous cell carcinoma (56cases). 10 cases of small cell carcinoma were seen. Out of 8 cases of metastatic tumors, 3 cases were from gastrointestinal tract and 2 cases each were from thyroid follicular carcinoma and renal cell carcinoma (Table 3).

Table 3: Details of cytological diagnosis

Organ (n=257)	Malignant	Inflammatory	Benign lesion	Inadequate
INTRATHORACIC				
Lung (n = 252)	TOTAL 210 Adeno carcinoma 87 Squamous carcinoma 56 Nonsmall cell carcinoma 49 Small cell carcinoma 10 Anaplastic large cell carcinoma 1 Non Hodgkin Lymphoma 1 Metastasis from Breast adenocarcinoma 1 GIT adenocarcinoma 3 Thyroid follicular carcinoma 2 Renal cell carcinoma 2	TOTAL 19 Abscess 12 Granulomatous 6 Hydatid cyst 1	TOTAL 11 Reported as "Benign aspirate" 11	12
Mediastinum (n = 5)	TOTAL 2 Seminoma 1 NHL 1	TOTAL 2 Abscess 2	TOTAL 1 Benign Dermoid 1	-
	212	21	12	12
Adequacy of sample	90.20%			

DISCUSSION:

Fine needle aspiration cytology is a diagnostic procedure for cytological evaluation of lung mass lesions. Mentrier in 1886 used the FNAC technique for the first time to diagnose lung cancer. In spite of high diagnostic yield, the post-procedure complications are widely variable as reported in the literature.[5] CT-guided FNAC plays a crucial role in diagnosing lung mass lesions in which accurate needle placement is possible by avoiding injury to the surrounding structures, thus, limiting the complications of the procedure.[5]

CT-guided FNAC procedure has been widely used in clinical practice to diagnose lung mass lesions with variable yield (50%-98%).The advances in imaging techniques and improved cytological techniques and expertise have improved the diagnostic yield.[6,7] The diagnostic accuracy of CT guided FNAC in our study was 90.20%. This is consistent with most of the studies where Emara MM et al, Jayashanker et al, Duenasa et al, and Saha A et al have reported the accuracy to be 96.9%, 90% ,93.5% and 94.7 % respectively.[8-11] However Sing JP et al have reported their diagnostic accuracy to be 85.3% while Ahmed S et al found malignancy in 82.10% of cases. [12,13]

Among the malignant cases there is variation in prevalence in the most common malignant lesions. Saha A et al and Basnet et al have reported the incidence of squamous cell carcinoma to be 58.33% and 50% respectively.[11, 14] The frequency of squamous cell carcinoma have been proclaimed to vary from 22- 29% by others.[8, 11, 12, 15]

The prevalence of adenocarcinoma of lungs have been disclosed to be variable with lower measure being 9.3%, higher of 49.4% as disclosed by Tan KB et al and 52.63% as reported by Mondal SK et al.[11, 16, 17] In our series we have observed that adenocarcinoma

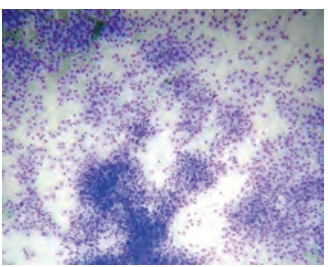

Fig 2. was more common as compared to squamous cell carcinoma

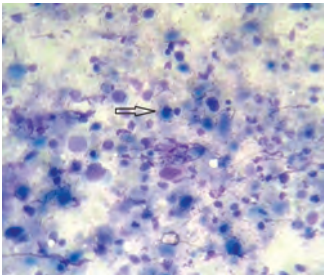

Fig 3. amounting to 41.03% and 26.41% respectively .

The metastatic tumors encountered in our study were from gastrointestinal adenocarcinoma (3 cases), breast (1 case), thyroid (2 cases) and renal cell carcinoma (2 cases) comprising of 3.77% of malignant lesion in the chest. Malignant tumors of the mediastinum (0.94% of cases) included a case each of seminoma and non-Hodgkins lymphoma (NHL). Saha A et al in their series have reported a case of metastasis from kidney and three (5.6%) cases of mediastinal mass comprising of NHL (2 cases) and Hodgkin's lymphoma (1 case).[11]

Non neoplastic cases comprised of 17.9% of the lesion as presented by Ahmed S et al and 2.5% of cases in the series by Madan M et al.[13, 15] Non neoplastic cases comprised of 13.46% of cases in our data. Non neoplastic cases were segregated as benign aspirate (5.18%) and inflammatory (9.90%) lesion. Abscess was the most common inflammatory lesion amounting to 57.14% (12cases).

The adequacy of samples in our data was 90.20% which almost correlates with those obtained by Tan et al (93%).[16] Emara MM et al and Prashant C et al have demonstrated that as the average number of attempts needed for final diagnosis is increased the obtained material would be sufficient for cytological diagnosis.[8, 18] Only one pass was attempted in our study and the material was inadequate in 12 (4.66%) cases. However it has been suggested that an unsatisfactory aspiration must be repeated, particularly when there is strong suspicion of possible malignancy.[8]

Post procedure pneumothorax, perilesional hemorrhage and hemoptysis are occasionally encountered and rarely require major intervention.[19] Post procedural complication was very negligible in our study with few patients developing small pneumothorax (3cases) or minimal perilesional hemorrhage (2cases) accounting

to 2 % of the cases which is in compliance with findings of Mondal SK who demonstrated the complication rate to be 2.4%.[17] In most instances the patient can be discharged after a check x-ray and few hours of observation, the hospital stay can thus be shortened for the patient which in turn also reduces the economic burden to the patient.[2,3]

We have observed the sensitivity and specificity to be 95.28% and 57.57% respectively for differentiating malignant and non- malignant lesions by CT. Similarly, Seemann MD et al have reported the sensitivity of 88.9% and specificity of 60.9 in differentiating malignant from non neoplastic lesion.[20] Basnet S B et al in their study of 100 cases reported a sensitivity and specificity of 88% and 84% respectively.[14] While Jayashankar E et al who conducted the study in 60 patients presented with a sensitivity of 84.5% and specificity of 76% in diagnosing malignancy with CT guided FNAC in chest and mediastinal mass and stated that a large scale of study would be more authentic to establish a statistical significance.[9]

CT guided FNAC is a well accepted, simple, accurate, safe and cost effective method for diagnosing a lung lesion with low morbidity rates. Combined with CT the aspiration needle can be guided safely into the lesion to improve the diagnostic yield of the cytological material. Thus improving the predictability of positive cases in malignant lesion. CT guided FNAC provides early diagnosis and subclassification of the lung masses hence directing the clinicians in proper management.

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Dental caries status and oral health practice among 12-15 year old children in Jorpati, Kathmandu

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ABSTRACT

Oral health is an essential component of health throughout life. There has been a decline in dental caries and periodontal disease in developed countries which can be attributed to the implementation of preventive programmes but in developing countries dental diseases are still on the rise. Therefore this cross sectional study was carried out to assess the prevalence of dental caries and oral hygiene practices among 12 to 15 years old children. Self administered close ended questionnaires were used to assess the oral hygiene practice. The overall dental caries prevalence was 58.3% and the mean DMFT score was 1.2 (± 1.79) and the deft score was 0.6 (± 1.24). Majority of the children (84.1%) presented with the practice of brushing their teeth once everyday using tooth brush and toothpaste. Regular dental check up was very poor (5.6%) but 77.4% reported that they visited a dentist in case of pain or presence of stains in the teeth. Females (63.4%) and children studying in higher secondary class (74.2%) showed a "good" level of oral hygiene practice than males and children in secondary class respectively. Children having "good" practice presented with "low" dental caries severity. The utilization of dental services was poor in the children, therefore highlighting the necessity to implement preventive programmes is important which would help in reducing the incidence of the dental caries as well as aiding in prompt treatment of dental caries at its initial stages.

Key words: dental caries, oral hygiene practice, toothbrushing

INTRODUCTION

Oral diseases such as dental caries, periodontal disease, tooth loss etc., are major public health problems worldwide and poor oral health has a profound effect on general health and quality of life.[1]Increased level of dental caries in children and adolescents have been observed in developing countries, in contrast to developed countries. [2] Regarding the etiology of dental caries the multifactorial relationship of plaque, sugar consumption, tooth susceptibility and time has been demonstrated in the previous studies.[3] Nutritional transition with easy access to refined carbohydrates, low use of fluoridated toothpaste and irregular tooth brushing habits lead to increasing trend in dental caries in developing countries.[4,5]

Although children have a basic knowledge of dental health, such as importance of proper brushing and diet in preventing dental caries, many fail to brush their teeth effectively and tend to consume cariogenic foods and may underestimate health risks.[6] Hence the aim of this study was to assess the oral hygiene practice in the children of 12 to 15 years old and also to determine the prevalence of dental caries in this population which would help us in planning and implementing necessary preventive measures.

MATERIALS AND METHODS

This was a cross sectional study carried out in 252 school children of age group of 12 -15 years old from January 2014 to February 2014. Four Schools were selected randomly in the locality and all healthy children of age group 12 to 15 were included in the study. The children of age group 12 is the index age recommended by WHO,[7] and the mean age group in this study was 13.04(±1.1). The date of birth of these children was obtained from school register. Consent was obtained from the school headmaster and the parents prior to the screening of the school children. Prevalence of dental caries was determined using decayed, missing, and filled permanent teeth (DMFT) index.[7] All children were examined by a single trained examiner. The children were seated on the chair and examined in day light using mouth mirror and an explorer. Data on oral health practice was collected by means of 10 self- administered close ended questionnaires, which were distributed in the class room and filled by the children under the supervision of the class teacher to ensure that all questions were answered by the children. The questionnaire contained questions regarding the brushing frequency, use of tooth paste and toothbrush, consumption of the sugar containing food and practice of visiting a dentist.

Statistical analysis:

Data were entered using SPSS package 11.5 and descriptive data were obtained. The association of different variables with dental caries was analyzed using Pearson's chi square test.

RESULTS

This cross sectional study was done among 252 school children of age group of 12 to 15 years old, the mean age being 13.04(±1.1). There were 118 males and 134 females who participated in the study.

Table 1: General characteristics of the children

Gender	N	%
Males	118	46.8
Females	134	53.2
Age	115	45.6
12	51	20.2
13	46	18.3
14	40	15.9
15		
Mean age= 13.04		
Secondary school	163	64.7
Higher Secondary school	89	35.3
Total	252	100

The prevalence of the dental caries was 58.3%. The mean DMFT(decayed, missing and filled teeth due to caries for permanent dentition) score was 1.2 (± 1.79) and for deft(decayed, indicated for extraction and filled teeth in primary dentition) was 0.6 (± 1.24), the D/d component was dominating in the DMFT and deft score . For analyzing the dental caries status, the severity of dental caries was taken into reference and categorized as <1.26= "Low", 2.7- 4.3="Moderate" and > 4.4="High".[8] In the studied population most of the children had "low" severity of dental caries (67.5%).

Table 2: Caries status of the children

	N	%	
Caries free	105	41.7	
At least one caries	147	58.3	
Gender	63	53.4	(p value= 0.1)*
Caries present (N=147)	84	62.7	
Males			
Females			
Age (N=147)	78	67.8	(p value= 0.008)*
12	27	52.9	
13	18	39.1	
14	24	60.0	
15			
Mean DMFT 1.2 (± 1.79)			
Mean deft 0.6 (± 1.24)			

*P value from pearson's chi square test

Among the studied sample the females presented with more caries (62.7%) than the males (53.4%), but this difference was not statistically significant (p= 0.135). Regarding the different age groups, children belonging to 12 years of age presented with maximum dental caries (67.8%). However, this difference was not statistically significant.

Regarding the oral hygiene practice of the children, it was observed that 84.1% of the children brushed their teeth everyday with toothbrush (89.7%) and toothpaste (97.6%) but only 36.9% had the habit of brushing twice a day. The proportion of population which consumed sweet snacks more than 3 times a day was 51.6% but only 43.3% had the habit of rinsing the mouth after taking sweet snacks. 74.6% of children had the habit of rinsing their mouth after every meal. 29.8 % of children uses toothpick as an inter dental aid. Regular visit to dental hospital was seen in only 5.6% among the studied population, but 77.4% reported that they visit dental hospital if only they have pain or stains in the teeth.

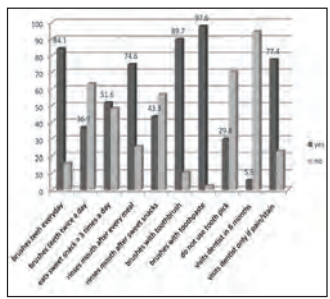

Fig: 1. Graph showing oral hygiene practice of the children

The scoring of practice was regrouped as "Poor" (0-5.9) and "Good" (6-10) taking the mean practice score (5.9) as the cutoff point. 72.5% of the children aged 15 years fell in the "Good" category whereas children of 12 years of age fell most into the "Poor" category. Regarding gender, it was seen that females (63.4%) showed better oral hygiene practice than males (59.3%).This difference was not statistically significant. Children in higher secondary level (74.2%) had better practice compared to that of children in secondary level (54.6%) and this difference was statistically significant (p= 0.002).

Table 3: Oral health practice

	good	poor	
Gender			
Males	59.30%	40.70%	(p=0.50)*
Females	63.40%	36.60%	
Education			
Secondary	54.60%	45.4%	(p=0.002)*
higher sec	74.20%	25.80%	

*P value from pearson's chi square test

The children who had a "good" practice (62.9%) presented with "low" severity of dental caries, but the difference was not statistically significant.

Table 4: Relation of oral hygiene practice and dental caries

Caries(N)	Practice		
	Poor	Good	
Low (170)	37.1%	62.9%	(p= 0.04)*
Moderate (68)	41.2%	58.8%	
High (14)	38.5%	61.5%	

*P value from pearson's chi square test

DISCUSSION

This study was conducted to evaluate the oral hygiene practice and dental caries status in 12 – 15 years old children with the mean age of 13.04(±1.1). The 2004 national oral pathfinder survey in Nepal has shown the dental caries prevalence of children aged 12-16 years to be 25.6%.[9] But in our study it was seen that caries prevalence was 58.3% in the studied sample with the children having at least one dental caries. This is somewhat similar with the dental caries prevalence studies by Lonim et al and B Subedi et al which showed that 12-13 years old children of different population had 41% and 53.23% caries prevalence respectively.[4,10]This increase in the caries status be could be attributed to the changing pattern of lifestyle, the diet habit and the ignorance shown by the people regarding periodic visit to the dentist.[10] It was seen that the practice of visiting dentist for a regular check up was very poor (5.6%) in the studied population and most of them had a habit of going to the dentist only when they have pain or stains in the teeth (77.4%). The D/d component was dominating in the DMFT and deft score with cavitated, unrestored teeth but when the caries severity was categorized most of the children fell into the "low" category.

Among the studied population the children of age group 12 (67.8%) had the maximum number of dental caries. The decrease in the dental caries status in the older age might be because of the reduction in the number of primary teeth due to exfoliation.[5] Regarding the gender, females (62.7%) showed a good oral hygiene practice compared to that of males (53.4) which was similar to the study done by Yee et al where female presented with better oral hygiene than males which can be attributed to behavioural differences between two genders.[3] Even though the oral hygiene practice was seen to be good in females they had more dental caries (63.4%) than the males. This was concurrent with another study done among the Nepalese population where dental caries was found to be more prevalent in females than males.[10] This could be due to chances of interviewer bias [2] and the caries' multifactorial etiology.[5,11]

In the studied population majority of the children used tooth brush(89.7%) and toothpaste (97.6%) to brush their teeth and had the habit of brushing the teeth everyday (84.1%) but only (36.9%) brushed their teeth twice a day. Prasai et al and Humagain have reported that the habit of brushing the teeth twice in a day was poor 24% and 40% respectively in different Nepali population.[4,12] Rinsing mouth with water after meal is common practice in Nepal,[4] similar practice was observed in the studied population with maximum children rinsing their mouth after every meal (74.6%).

Humagain have reported in his study that 100% of children had the knowledge that the sweets have an negative impact on the dental health,[12] eventhough it was seen in our study that there were 51.6% of children who were consuming sweet snacks more than 3 times in a day and there were 43.3% of children who reported that they do not have the habit of rinsing their mouth after taking sweet snacks.

When the practice score was re-categorized into "good" and "bad" it was seen that children studying in secondary higher class had a "good" level of practice which was statistically significant (p=0.002) and the children having good practice had "low" severity of caries. However it has been seen in previous literature that oral health practice cannot be understood simply based on the Knowledge attitude practice chain and other factors should be considered as well.[13]

In this study dental caries was found to be highly prevalent, eventhough the oral hygiene practice was found to be good in the studied population. It was seen that most of the children had untreated dental caries which was due to poor utilization of the available dental services in the community. Regular dental check up facilitates early diagnosis and treatment of the dental caries. Therefore, rehabilitative care targeted towards treatment of the existing problems of dental caries should be made available and more accessible to the children. Along with this, oral health education programs at individual, and community levels should be implemented aimed primarily at improving the knowledge and awareness of the communities towards the risk factors associated with oral problems in order to decrease overall incidence and prevalence of dental caries.

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D test: A simple test with big implication for *Staphylococcus aureus* Macrolide-Lincosamide-Streptogramin$_B$ Resistance Pattern

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ABSTRACT

D test is a simple disc diffusion test giving high throughput results. It is used to study the macrolide lincosamide streptogramin resistance (MLS_B), both constitutive and inducible as well as macrolide streptogramin resistance (MS_B) in *Staphylococcus aureus*. In this test, erythromycin (macrolide) and clindamycin (lincosamide derivative) discs are placed adjacent to each other over the Mueller Hinton agar medium inoculated with the test organism. The growth of the organism up to the edges of the disc, flattening of the clindamycin zone (D test positive) near the erythromycin disc (resistant) and susceptible to both antibiotics implicate that the organism is having constitutive MLS_B ($CMLS_B$), inducible MLS_B ($IMLS_B$) and no resistance respectively. Further, the organism susceptible to clindamycin without any flattening of the zone (D test negative) near clindamycin disc (resistant) implicates that the organism is having macrolide streptogramin resistance (MS_B). The test is performed in the same MHA plate in which the antibiotic sensitivity test is being done, taking into consideration that the discs are placed adjacent to each other maintaining the distance. Since clindamycin and streptogramin are among the few drugs of choice in the treatment of methicillin resistant *S. aureus* (MRSA) infections, knowing the resistance to these antibiotics is imperative.

Keywords: Resistance, erythromycin, clindamycin, streptogramin, *Staphylococcus aureus.*

INTRODUCTION

Macrolide, lincosamide and type B streptogramin (MLS) are chemically distinct antibiotic having similar target site and mode of action.[1,2] They all have a narrow spectrum of activity against Gram positive cocci especially staphylococci, streptococci and enterococci. Three mechanisms account for acquired resistance to these MLS antibiotics and they are modification of the target of the antibiotics, active efflux of the antibiotics and inactivation of the antibiotics. Target site modification is the most common mechanism of acquired resistance to MLS antibiotics in staphylococci. A single alteration in 23S rRNA confers broad cross-resistance to macrolides, lincosamides, and streptogramin B-type antibiotics and hence known as macrolide lincosamide streptogramin B resistance (MLS_B resistance).[3] MLS_B resistance can be either constitutive MLS_B ($CMLS_B$) or inducible MLS_B ($IMLS_B$).[4] MLS_B resistance phenotype accounts for nearly all of the resistant clinical isolates. In staphylococci, the prevalence of this resistance phenotype in hospital settings is between 15 and 45%, but generalization cannot be made because of important local variations.[5] Active efflux of antibiotic, less frequently encountered mode of acquired resistance is mediated by an ATP-dependent pump mediated by msrA.[6] Inactivation of antibiotic yet another mode does not confer cross resistance[7] and has limited value.

Macrolides consist of 14-, 15-, and 16- membered lactone ring macrolides. Erythromycin, oleandamycin, clarithromycin, dirithromycin and roxythromycin are macrolides having 14- membered lactone ring, Spiramycin, jasomycin, midecamycin, kitasamycin and rokitamycin are having 16-membered lactone ring and Azithromycin is having 15-membered lactone ring (also called azalide structure).

Clindamycin is a derivative of lincomycin, the lincosamide antibiotic that inhibits protein synthesis by the target modification. Clindamycin is a useful antibiotic for the treatment of skin and soft tissue infection, and infections caused by *Staphylococcus* spp. especially methicillin resistant *S. aureus* (MRSA). Clindamycin has excellent tissue and bone penetration, and accumulates in abscesses. Good oral absorption and no requisition of renal dosing adjustment make it an important therapeutic agent.[8]

Streptogramin antibiotic consists of at least 2 structurally unrelated molecules: group A (M) streptogramins (macrolactones) and group B (S) streptogramins. Pristinamycin and virginiamycin are naturally occurring streptogramins, whose use in clinical practice has been limited due to their complex and irregular composition, and insolubility.[9] Streptogramins A and B act synergistically and the mixture of the two

compounds is more powerful than the individual components in inhibiting protein synthesis. Group A or group B compound alone has a moderate bacteriostatic activity, whereas the combination of the two exhibit strong bacteriostatic activity and often bactericidal activity.[10] Streptogramins are effective in the treatment of vancomycin resistant *S. aureus* (VRSA) and vancomycin resistant enterococci (VRE).[11]

These three antibiotics though are structurally different their mode of action is similar working in the same site during protein synthesis. Cross resistance among these antibiotics is due to modification of drug target. Erythromycin and other macrolides bind reversibly to 50S ribosomal subunit and methylate ribosomal protein in the 23S ribosomal RNA. Such rRNA methylation leads to conformational change in ribosome resulting into co-resistance between macrolides, lincosamide and streptogramin due to their common target of action. Therefore, erythromycin mediated methylase confers resistance to lincosamide and streptogramin in the presence of erythromycin. Clindamycin and streptogramin do not induce methylase.[12] In the absence of erythromycin to induce the enzyme, organisms appear susceptible to these antibiotics.

RESISTANCE TO MACROLIDE, LINCOSAMIDE AND STREPTOGRAMIN

Resistance of bacteria against these antibiotics may be intrinsic or acquired. Gram negative bacteria like members of Enterobacteriaceae family, *Pseudomonas* spp. and *Acinetobacter* spp. are intrinsically resistant to MLS antibiotics due to the impermeability of the bacterial cell membrane. However in the gastrointestinal tract (GIT) infection the MIC is achieved in the range of 2-256 µg/ml, hence can be used in the infection occurred in the GIT.

Three mechanisms that account for the acquired resistance among bacteria against these antibiotics are target modification, active efflux of the antibiotic and inactivation of antibiotics.

Target modification: Single alteration in 23S rRNA confers broad cross resistance to macrolide, lincosamide and streptogramin B antibiotics. *erm* genes [*erm*(A), *erm*(B) and *erm*(C)] encoded methylase enzyme, methylate the ribosome at 23S thus target of the antibiotic is altered. As a result antibiotic cannot act upon the target and resistance is observed.

Active efflux of antibiotics: There are antibiotic resistance genes encoding for transport of proteins (efflux). They do not modify the antibiotic or the antibiotic target, rather pump (efflux) the antibiotics out of the cell or the cellular membrane such that intracellular concentration becomes low and ribosomes are free from the antibiotics.[2]

Macrolide and streptogramin resistant *msr*(A), macrolide efflux *mef*(A) in *Streptococcus Pyogenes* and *mef*(E) in *S. pneumonia*; and virginiamycin factor A *Vga*(A) and *vga*(B) in staphylococci are three different efflux systems that have been described in gram positive cocci.[2]

msr(A), *msr*(B) [also *msr*(A') and *msr*(B')] are different from *mef* genes in the aspect that they confer resistance to both macrolide and streptogramin B whereas the later confer efflux of macrolide only. A lincomycin specific efflux pump encoded in *lmr*(A) has been described in *Streptomyces lincolnensis*.[2]

Inactivation of antibiotics: There are arrays of genes encoding for the enzymes that inactivate the antibiotics. There is no cross resistance when the mode of action is by inactivation of antibiotics.[7] In the members of Enterobacteriaceae and in *S. aureus*, macrolide inactivation occurs by ErmA and ErmB enzymes that hydrolyze the lactone ring of the macrocyclic nucleus and also phosphotransferase [type I (*mph*(A) and type II] inactivate the macrolide.[13] *lin*(A) gene conferring resistance only to lincosamide[13] has been detected in *S. aureus, S. haemolyticus, S. epidermidis, S. cohnii* and *S. hominis*. Similarly *lin*(A') has been reported in *S. aureus, S. epidermidis* and *S. cohnii*.[7] *vgb* gene in staphylococci encoding lactonase is capable of cleaving macrolactone of streptogramin B. Similarly *vat*(A) and *vat*(B) genes encoding acetyltransferases inactivate streptogramin A.[13]

The multiplicity and complexity of MLS resistance phenotypes of bacteria observed today are largely due to the recent detection of new mechanisms of resistance mainly the inactivation of antibiotics. However, these new mechanisms have a limited importance in practical point of view due to their low incidences. Inactivation of lincosamide has been reported in 2 % of *S. aureus* and 4-8 % in coaglulase negative *Staphylococcus* (CoNS). Less than 5 % of *S. aureus* inactivate streptogramin antibiotics. This is in contrast to that MLS resistance conferring nearly all the resistance observed among the clinical isolates which accounts for 15-45 % of resistance among *S. aureus* isolated from hospital settings. Erythromycin resistance in MRSA has been reported to be higher than 90 % in numerous countries.[7] However, generalization is difficult due to the importance of local variation.

Macrolide-lincosamide-streptogramin B (MLS_B) resistance: Cross resistance occurring between macrolide, lincosamide and streptogramin B also known as Macrolide-lincosamide-streptogramin B resistance

is an acquired resistance encoded in erythromycin methylase (*erm*) genes. Three distinct methylase genes *erm*(A), *erm*(B) and *erm*(C) have been detected in staphylococci.[3] Expression of these methylase genes is controlled by translational attenuation.[3]

MLS_B resistance in *S. aureus* may be constitutive or inducible. When the expression is constitutive, the organisms are resistant to all macrolides, lincosamides and type B streptogramin antibiotics. In contrary, when the resistance expression is inducible, the organisms are resistant to 14- and 15-membered macrolides; and are sensitive to 16 membered macrolide, lincosamide and streptogramin B in the absence of inducer erythromycin.[14] Since, 14- and 15-membered macrolides are effective inducers of methylase synthetase, methylase is produced only in the presence of an inducer (erythromycin). Azithromycin, the 15-membered macrolide also induce resistance in clindamycin.[15] Strains with inducible resistance are resistant to erythromycin and appear susceptible to clindamycin and streptogramin B in the absence of inducer the erythromycin. They are resistant to these antibiotics in the presence of inducer.

Erythromycin ribosome methylase gene: Till 1999, 22 classes of rRNA methylase (*erm*) genes had been reported. Twenty one classes contained the identified and characterized *erm* genes and in 22nd class contained all unclassified and uncharacterized genes.[2] In 2009, 33 classes of *erm* genes have been reported. Of those, only 9 classes [*erm*(A), (B), (C), (F), (G), (Q), (T), (Y), *erm*(33)] have been identified in *S. aureus*.[16] The most prevalent genes encoding the methylase in *S. aureus* have been designated *erm*(A), *erm*(B), and *erm*(C). Of these three too, *erm*(A) and *erm*(C) are the most common ones and *erm*(B) is found in the *Staphylococcus* isolates from animal origin. *erm*(A) and *erm*(C) genes are located in chromosome and plasmid respectively. The distribution of *erm*(A) and *erm*(C) is often species specific. Rarely occurring e*rm*(B) gene is located in transposon of *S. aureus*.

Genetic basis of MLS_B resistance: *erm* genes code for MLS_B resistance irrespective of their constitutive of inducible nature of resistance. The methylase enzyme produced by *erm* gene methylates the 23S ribosomal RNA, specifically adenine 2058 in 23S rRNA.[17] The methylation alters the conformation of ribosome leading to resistance to macrolide. The *erm* mediated methylase produced by erythromycin resistant *S. aureus* is also responsible for cross resistance to clindamycin and streptogramin due to their common site and mode of action.

The inducible or constitutive expression of resistance is not related to class of *erm* gene. It solely depends on the regulatory region sequence present upstream of the methylase structural gene. The regulation of expression of MLS_B resistance occurs by translation attenuation, where translation of methylase encoding genes occurs depending on the presence of inducer. Two point mutations in the control region convert the inducibly resistant strain to constitutively resistant strain irrespective of the presence or absence of the inducer.[18]

Macrolide-streptogramin B (MS_B) resistance: Staphylococci that exhibit resistance to 14- and 15-membered ring macrolide and streptogramin B but are sensitive to 16 membered ring macrolide and lincosamide are said to have MS_B resistance.[1, 2, 19] MS_B resistant staphylococci harbor macrolide streptogramin resistance [*msr*(A)] gene or a similar gene that encodes an ATP dependent efflux pump mechanism.[20] MS_B resistant strains remain Clindamycin susceptible in disc diffusion test.

Macrolide streptogramin resistance gene: In *S. aureus*, the MS_B resistance is conferred by the macrolide streptogramin resistance *msr*(A) gene.[21] This is the most prevalent gene conferring MS_B resistance. Another gene conferring MS_B resistance is *msr*(B) which has not been reported much. The *msr*(B) gene homologous to *msr*(A) is significantly shorter than the *msr*(A) gene sequence which is roughly half the size of *msr*(A).[2] Recently in 2009, *msr*(B) along with *msr*(SA), *msr*(SA') have been included in *msr*(A) gene.[22,23]

Genetics of MS_B resistance: The *msr*(A) gene encodes for a hydrophilic ATP binding protein, MsrA that functions as a drug efflux pump, an ATP dependent process.[20] MsrA protein belonging to ATP binding cassette (ABC) transporters super family exports antibiotics across the cell membrane. *msr*(A) gene expression is regulated by translational attenuation and removal of the control region of the gene leads to constitutive expression of *msr*(A).[24]

EPIDEMIOLOGY

In 2 hospitals in the USA (Chicago) occurrence of $CMLS_B$ resistance has been stated to be much higher among MRSA (84 % and 82 %) compared to that among methicillin sensitive *S. aureus,* MSSA (3 % and 18 %).[4] In the same hospitals, the incidence of $IMLS_B$ resistance has been reported to be low (7 and 12 %) among MRSA and among MSSA (20 % and 19 %). However, in another US hospital MSSA isolates (34%) has been reported to be almost three times more likely to have $IMLS_B$ resistance compared to MRSA isolates (11%).[25] In yet another report from Atlanta USA 32 % of *S. aureus* isolates had $IMLS_B$ and 13.7 % had $CMLS_B$ resistance in

a collection of *S. aureus* strains from Center of Disease Control and prevention and project, and Rockefeller University, USA.[14] Association of MRSA with $IMLS_B$ resistance has been put forward by Maple et al.[26] They have stated that clindamycin resistance emerge readily a common event in MRSA.

In Spain Significantly higher prevalence of $IMLS_B$ than $CMLS_B$ resistance among *S. aureus* has been reported.[27] In a European study from 24 university hospitals, majority of the macrolide resistant MRSA strains were $CMLS_B$ phenotype, whereas $IMLS_B$ resistance was predominant among MSSA.[28] Similar higher occurrence of $IMLS_B$ resistance among MSSA has been reported in Birmingham.[29] On the contrary, in Greece Higher prevalence of $CMLS_B$ (60 %) followed by $IMLS_B$ (35 %) and clindamycin susceptible phenotype (5 %) has been reported in *S. aureus*.[30] Similarly, in Turkey a higher occurrence of $CMLS_B$ resistance in MRSA (44.2 % versus 24.4 %) and $IMLS_B$ in MSSA, (14.8 % versus 4.5 %) has been reported.[31] Comparatively higher occurrence of $CMLS_B$ resistance in MRSA has been put forward in a Turkish study.[15]

In Nepali context, Mohapatra *et al* have reported association of $CMLS_B$ and $IMLS_B$ with MRSA.[32] In similar Nepali study, MLS_B resistance was found associated with MRSA (97.7 %).[33] MRSA having $CMLS_B$ resistance has been stated to be 94.7% and 100% of the $IMLS_B$ resistant isolates were MRSA.[33]

In USA (Atlanta), 8.5% of the *S. aureus* exhibited MS_B resistance.[14] In another report from two US hospitals (Chicago), quite a low occurrence of MS_B among MRSA and MSSA has been reported.[4] O'Sullivan et al. have stated that MS_B resistance occurs less commonly than $IMLS_B$ but they have also stated that the resistance pattern show great geographical variation.[34] On the contrary, Merino-Diaz et al. have reported that in Spain MS_B resistance was the most common resistance type comprising of 7.2 % in *S. aureus* of the erythromycin-resistant strains. In the same study, the occurrence of $IMLS_B$ resistance has been reported to be higher (5.2 %) than rate of $CMLS_B$ resistance (1.7 %) in *S. aureus*.[27] In a Turkish study Azap et al. have reported that MS_B resistance was found among MSSA. Again in another Turkish study, almost equal occurrence of MS_B resistance among MRSA and MSSA has been reported.[31] In Nepal, no association of MS_B resistance with MSSA or MRSA has been reported.[35] MS_B resistance was found in small frequency that occurred mostly among MSSA and heterogeneous MRSA.[33]

Factors affecting the prevalence of different resistance phenotype strains

The differences in the occurrence of $CMLS_B$, $IMLS_B$, MS_B resistance among MRSA, and MSSA could be due to geographic variation.[34] It has been stated that the incidence of resistance is highly variable with regard to the country, type of infections among the patients,[21] geographical region and specific clones of MRSA may differ in different hospitals and regions.[29] Further, Patel *et al* has stated that the prevalence of resistance phenotypes, and specific clones of MRSA may vary in different regions. [29] The incidence of $IMLS_B$ resistance is important in a setting where clindamycin is prescribed empirically, and this incidence is known to differ between hospitals.[4,15] Further, Maple *et al* have stated that clindamycin resistance emerge readily which is common in MRSA.[26] Hence, local statistics are of crucial value for empiric therapy. Surveillance of incidence of macrolide resistance and the respective prevalence of the various resistance types should be done in each hospital and D test is the simple and highly indicative test for the purpose.

METHODOLOGY OF D TEST

D test is a simple disc diffusion test where erythromycin and clindamycin discs are placed adjacent to each other on a lawn of the test organism. D test has a high throughput indicating different types of resistance phenotypes in a single test. This easy to read test can be done along with the antibiotic susceptibility test or even in the same plate hence does not require any extra energy, cost and effort.

For D test, guidelines of recent Clinical Laboratory Standard Institute (CLSI) 2007[36] should be followed. 5/6 colonies of the test isolate grown on blood agar is directly suspended in physiological saline (0.85% sodium chloride in distilled water) and is matched with 0.5 McFarland's turbidity standard ($1.5x10^8$ bacterial load of per ml). Within 15 minutes of the preparation of the bacterial suspension, it is inoculated onto a dried (37^0 C for 30 minutes) Mueller Hinton agar (MHA) plate having a depth of 4 mm ± 0.5 mm and pH 7.3 ± 1. A sterile swab is dipped in the matched inoculum suspension and pressed against the inside of tube to express excess of the inoculum, and is inoculated onto MHA plate. The plate is allowed to stand on bench for 5 -10 minutes. Erythromycin (15 µg) and clindamycin (2 µg) antibiotics discs that have been stored at 2-8^0 C and have been brought to room temperature are used. The antibiotic discs are placed over the inoculated MHA plate at a distance of 15 mm edge to edge, allowed to stand on bench for 30 minutes and then incubated at 35^0 C for 18 hrs.[36]

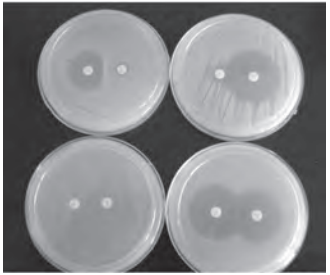

Fig. 1. Top left $IMLS_B$ resistance, Top right MS_B resistance, Bottom left $CMLS_B$ resistance and B ottom right No resistance

D TEST INTERPRETATION

The susceptible phenotypes are susceptible to both erythromycin and clindamycin. Presence of flattening of clindamycin zone adjacent to erythromycin disc is a characteristic known as D zone and the isolate is referred to as D test positive.

Any test strain that is resistant to erythromycin and is D test positive is exhibiting $IMLS_B$ resistance and any strains that are resistant to both erythromycin and clindamycin are having $CMLS_B$ resistance. The genes encoding such resistance may carry either one of *erm*(A), *erm*(B) or *erm*(C) conferring methylation of adenine 2058 in 23S rRNA of ribosomal RNA.

D test also detects strains with macrolide-streptogramin B (MS_B) resistance. The strains which are resistant to erythromycin, susceptible to clindamycin and are D test negative (no flattening of clindamycin zone adjacent to erythromycin disc) are having MS_B resistance. These strains are resistant to macrolide and streptogramin and are susceptible to clindamycin. Such resistance is encoded in macrolide streptogramin resistance (*msr*) genes, which are either *msr*(A) or *msr*(B)[21] conferring active efflux of antibiotics[20] such that intracellular concentration becomes low and ribosomes are free from the antibiotics.[2] (Figure 1)

Steward, Raney, Morrell et al. have described two distinct phenotypes induction phenotypes and non-induction phenotypes.[14] Induction phenotypes consists of two $IMLS_B$ resistance phenotypes namely D and D^+. Non-induction phenotypes consist of four phenotypes and are Neg (MS_B), HD ($CMLS_B$), R ($CMLS_B$) and S (susceptible) among the isolates of *S. aureus* (Table-1).

Debate over the use of clindamycin in $IMLS_B$ resistance phenotype infection

Clindamycin, one of the drugs of choice in the treatment of infections by homogeneous MRSA cannot be used for those exhibiting $CMLS_B$. MS_B resistance phenotypes do not develop resistance to clindamycin during therapy.[14] There is doubt in usefulness of clindamycin for the treatment of infections by homogeneous MRSA exhibiting $IMLS_B$. Although $IMLS_B$ resistance phenotype isolates appear susceptible to clindamycin in the absence of an inducing agent macrolide, there is widespread reluctance to prescribe clindamycin for treatment of patients with infections caused by such organisms due to the concerns that resistance to clindamycin will develop during therapy.[4]

Lewis et al. have recommended avoidance of clindamycin

Table-1: Additional characteristics of D test for clindamycin susceptibility/resistance pattern.

Induction test phenotype	Resistance phenotype	Erythromycin result	Clindamycin result	Test description
D	Inducible MLS_B	R	S	Blunted D shaped clindamycin inhibition zone adjacent to erythromycin disc
D^+	Inducible MLS_B	R	S	Blunted D shaped clindamycin inhibition zone near erythromycin disc and small colonies in the zone
Neg	MS_B	R	S	Clear inhibition zone around clindamycin disc
R	Constitutive MLS_B	R	R	Growth up to clindamycin and erythromycin discs
HD	Constitutive MLS_B	R	R	Double Clindamycin zones, one zone is light, hazy growth extending from clindamycin disc to second zone where the growth is heavy. The inner light zone exhibit flattened zone like in D phenotype
S	No resistance	S	S	Clear susceptible zone around clindamycin and erythromycin discs

for the treatment of complicated infections having a high bacterial burden, such as abscesses or osteomyelitis.[37] Clindamycin if used for treatment of a less severe $IMLS_B$ *S. aureus* infection, the patient must be closely monitored for signs of treatment failure or relapse of infection. Non-$IMLS_B$ infections can be treated with clindamycin.[29] Nevertheless, clindamycin is a frequent choice for treating some staphylococcal infections because it can be given orally and is well tolerated.[4]

CONCLUSION

The sharp rise in staphylococcal infection all over the world and changing pattern of antimicrobial resistance including the emergence of MRSA have led to the use of clindamycin therapy in the treatment of staphylococcal infections.[8] Increasing frequency of $CMLS_B$ resistance phenotype may be the reflection of the increased use of clindamycin in the treatment of staphylococcal infection.[38] Occurrence of $CMLS_B$ and $IMLS_B$ resistance in MRSA[15,30,31,32] and also in MSSA[4,25] has made it necessary to perform D test in all *S. aureus* isolates. Further, association of both $CMLS_B$ and $IMLS_B$ resistance with MRSA has also been reported.[32,35] It has been suggested that $IMLS_B$ phenotypes determined by disk diffusion methods correlate well with genotypic test and the degree of correlation is so strong that disk diffusion results may be used to predict genotype.[33,38]

Use of clindamycin in MRSA expressing $IMLS_B$, is a matter of debate due to its ability to develop clindamycin resistance in vitro[39] and in vivo during clindamycin therapy.[40] However, there are reports of successful clindamycin treatment of infection by MRSA expressing $IMLS_B$ resistance.[40] Hence, D test should be included in routine susceptibility test of all *S. aureus* isolates. Any *S. aureus* isolate positive in D test ($IMLS_B$ resistance phenotype) should be reported as clindamycin resistant with a comment that the organism is presumed to be resistant based on the detection of inducible clindamycin resistance and clindamycin may still be effective in some patients.[36]

ACKNOWLEDGEMENTS

The authors are grateful to Prof. T. M. Mohapatra and Prof. B. M. Pokhrel for their immense support.

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Bedside sonographic evaluation of the diaphragm in ventilator dependent patients with Amyotrophic Lateral Sclerosis. A report of two cases

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease with progressive and inexorable loss of bulbar and limb functions. Respiratory muscle weakness and failure is a common complication late in the course of disease. Bedside ultrasonography of the diaphragm was done in two ventilator dependent patients with ALS. Thickness of the diaphragm was markedly reduced during both end expiration and end of deep inspiration. The degree of diaphragmatic thickening was also significantly reduced. The diaphragmatic excursion during deep inspiration was sub-optimal. The findings were consistent with diaphragmatic atrophy and paralysis. Sonography of the diaphragm can be a useful non-invasive bedside tool for the diagnosis and follow up of diaphragmatic involvement in patients with amyotrophic lateral sclerosis.

Keywords: Amyotrophic lateral sclerosis, diaphragmatic paralysis, ultrasonography, ventilator dependent.

Amyotrophic lateral sclerosis (ALS) is the most common form of progressive motor neuron disease.[1] The clinical course is inexorably progressive and over 60% of patients die within three years of presentation. Respiratory muscle weakness and failure is an important cause of morbidity and mortality in these patients.[2] Ultrasonography of the diaphragm can help in prompt identification and follow up of patients with diaphragmatic dysfunction and paralysis.[3] Here, I report bedside sonography of the diaphragm of two ventilator dependent patient with ALS, which revealed diaphragmatic atrophy and paralysis.

FIRST CASE

A 51 years old male patient presented with the history of gradually progressive weakness of the extremities for five months duration. It started in bilateral lower limbs, which was followed by the weakness of upper extremities for two months duration. Later in the course of disease, he had involvement of respiratory muscles manifested as progressive shortness of breath, to the point that he required mechanical ventilatory support. All four extremities were flaccid with preserved deep tendon reflexes. There was marked atrophy of thenar and hypothenar muscles. There was no bowel and bladder involvement. Gag reflex was absent. A panel of investigations was done including antinuclear antibody, serum ceruloplasmin level, serum protein electrophoresis, MRI of brain and cervical spine, nerve conduction study, needle electromyography, muscle biopsy and nerve biopsy. The reports were suggestive of ALS. He was tracheostomized following prolonged mechanical ventilation. He was started on tablet Riluzole 50 mg twice daily. Attempts to wean from mechanical ventilation were unsuccessful and the patient remained ventilator dependent.

Bedside ultrasonography of the diaphragm was done as described by Sarwal A et al,[4] using curvilinear transducer C60X (frequency range of 5 to 2 MHz) and linear array transducer HFL38X (frequency range of 6 to 13 MHz) (MicroMaxx®; SonoSite, USA). Patient was placed in supine position with the tracheostomy tube attached to T-piece with oxygen supplementation at 8 litres per minute. Thickness of the diaphragm was assessed at the zone of apposition by placing the linear array transducer at the anterior axillary line to obtain an intercostal view. The transducer was positioned to obtain a sagittal image of the diaphragm between eighth and ninth ribs in both sides. Two dimensional B-mode ultrasonography was used to measure the diaphragm thickness at end expiration (at functional residual capacity) and at end of deep inspiration (at total lung capacity).

In the right side, the thickness measured at end expiration was 0.11 cm (Fig. 1) and was 0.12cm (Fig. 2) at the end of deep inspiration. In the left side, it was 0.16 cm (Fig. 3) at end expiration and 0.18 cm (Fig. 4) at the end of deep inspiration. The degree of diaphragmatic thickening was measured using the formula: (thickness at end inspiration – thickness at end expiration) / thickness at

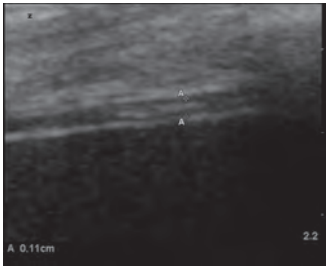

Fig. 1. End expiratory diaphragmatic thickness in right side (0.11 cm) at functional residual capacity.

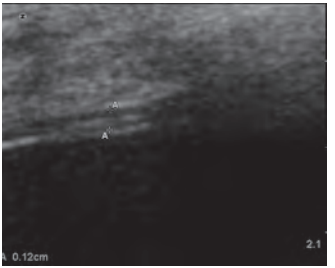

Fig. 2. End inspiratory diaphragmatic thickness in right side (0.12 cm) at total lung capacity.

end expiration. It was 9.09% in the right side and 12.50% in the left side. Diaphragmatic excursion was measured by placing the micro-convex transducer between the midclavicular and anterior axillary lines in the anterior subcostal region. The transducer was directed medially, cranially and dorsally. Right hemidiaphgram was visualized throught hepatic window and left hemidiagphragm through splenic window. B-mode was used to visualize the diaphragm and then the imaging was changed to M-mode to measure the excursion during deep breathing (from end expiration to end of deep inspiration). It was 0.71 cm in right (Fig. 5) and 0.60 cm in left (Fig. 6).

SECOND CASE

A 60 years old gentleman, a military veteran, with no significant past medical history, presented with shortness of breath of two to three months duration. It was insiduous in onset and gradually progressive. He also had generalized weakness of one to two months duration, involving all four limbs. Shortness of breath progressed to the point that he required endotracheal intubation to support oxygenation and ventilation. He had spastic extremities with muscle wasting and visible fasciculations. Sensory examination was normal. He had no involvement of extraocular muscles and never had diplopia or ptosis. Sphincter function and mentation was intact. He had a pannel of investigations to reach the diagnosis. Complete blood count and electrolytes were normal. Calcium and phosphate levels were normal. Cerebrospinal fuild analysis was within normal limits. Erythrocyte sedimentation rate was normal. Thyroid function test and serum electrophoresis was normal. Acetylcholine receptor antibody in serum was negative. Creatine kinase level was within normal limit and deltoid muscle biopsy was negative for myopathy. Electromyography was suggestive of ALS. MRI of head and neck was normal. He continued to have gradual,

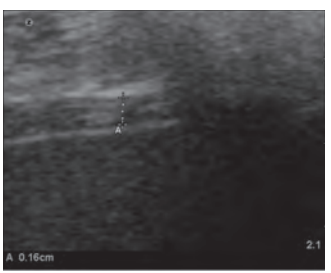

Fig. 3. End expiratory diaphragmatic thickness in left side (0.16 cm) at functional residual capacity

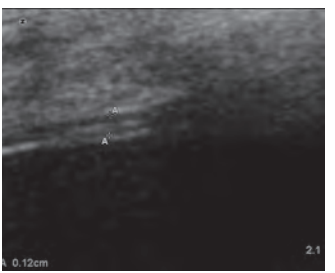

Fig. 4. End inspiratory diaphragmatic thickness in left side (0.18 cm) at total lung capacity.

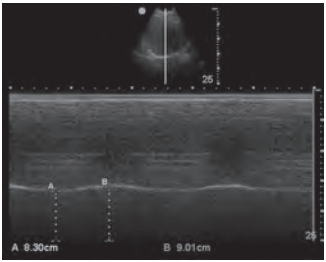

Fig. 5. Diaphragmatic excursion in right side (9.01cm – 8.30cm = 0.71 cm) during deep respiration.

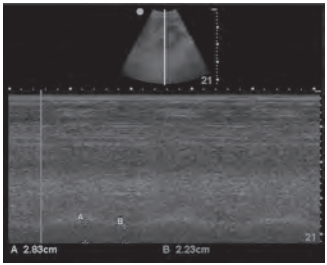

Fig. 6. Diaphragmatic excursion in left side (2.83cm – 2.23cm = 0.60cm) during deep respiration.

but progressive worsening of muscle strength and attempts at weaning from mechanical ventilation were unsuccessful. He underwent tracheostomy after two weeks of intubation. He was started on tablet Riluzole 100 mg daily. He continued to be ventilator dependent. Noninvasive positive pressure ventilation was attempted, but was unsuccessful.

Bedside ultrasonography of the diaphragm was performed as described in the first case. In the right side, the diaphragmatic thickness at end expiration was 0.12 cm and at end of deep inspiration was 0.14 cm. In the left side, the thickness at end expiration was 0.11 cm and was 0.13 cm at the end of deep inspiration. The degree of diaphragmatic thickening was 16.67% in right and 18.18% in left side. Diaphragmatic excursion was 0.34 cm in right side. The hemidiaphragm could not be visualized in left side due to poor sonographic window.

DISCUSSION

Patients with ALS present with progressive neurological deterioration involving the corticospinal tract, brainstem and anterior horn cells of the spinal cord.[5] It is more common in men than in women, with the peak incidence between 50 to 75 years of age. The incidence being 2-3 people per 100,000 of the general population.[2] Majority of the patients present with limb symptoms. Respiratory onset disease, presenting with failure of respiratory muscle function, as in the second case, is seen in only 5% of patients with amyotrophic lateral sclerosis.[6,7] Majority of the patients with ALS die from respiratory failure and the presence of respiratory muscle weakness is an independent predictor of quality of life.[2] Diaphragmatic dysfunction is common in patients with ALS.[8]

The diaphragm is the major respiratory muscle. Ultrasonography is a non-invasive and portable method for assessing the diaphragm.[4] Ultrasound has been shown to be similar in accuracy to most other imaging modalities for diaphragm assessment.[9,10] Ultrasound measurement of thickness of the diaphragm and diaphragmatic thickening during inspiration was found to be helpful in diagnosing diaphragmatic paralysis[11] and to assess for potential functional recovery from diaphragmatic weakness or paralysis.[12] Average thickness of the diaphragm is 0.22 – 0.28 cm in healthy volunteers[13] and 0.13 – 0.19 cm in paralyzed diaphragm. The thickness of less than 0.2 cm, measured at the end of expiration has been proposed as the cutoff to define diaphragm atrophy.[4] In both patients, the thickness was below the cutoff value. In the first case it was 0.11 cm in right and 0.16 cm in left. In the second case it was 0.12 cm in right and 0.11 cm in left. Diaphragmatic thickening of less than 20% has been proposed to be consistent with diaphragmatic paralysis.[12] In the first case it was 9.09% in right and 12.50% in left. In the second case, it was 16.67% in right and 18.18% in left. The findings are similar to those reported in a case series of patients with ALS reported by Yoshioka *et al*.[14]

The normal range of diaphragmatic motion during deep breathing in adults is 1.9 – 9.0 cm.[4] Diaphragmatic paralysis is indicated by the absence of excursion or paradoxical motion on sniffing and diaphragmatic weakness is indicated by less than normal ampitude of excursion. Excursion of more than 2.5cm has been proposed as a cutoff for excluding severe diaphragmatic dysfunction.[15] In the first case, it was 0.71 cm in right and 0.60 cm in left. In the second case, the excursion was only 0.34 cm in right side.

The patients discussed in this case report had atrophied and severly dysfunctional or paralyzed diaphragm.

Ultrasonographic findings correlate well with the clinical scenario of failure to wean from mechanical ventilation and prolonged ventilator dependence. Only a few studies have evaluated the role of ultrasonography in assessing diaphragmatic function in patients with ALS. Early identification of diaphragmatic involvement in ALS is curcial, since early application of non-invasive positive pressure ventilation improves the quality of life[16] and prolongs survival.[17] Bedside ultrasonography can be a valuable tool for the diagnosis and followup of diaphragmatic dysfunction, atrophy and paralysis in patients with ALS and other motor neuron diseases.

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Axillary arch and other neurovascular anomalies in a cadaver –

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ABSTRACT

The co-existence of multiple variants in the axilla has been rarely documented. Hence, we report the multiple variations of axillary structures and axillary arch. During a dissection of axilla of adult male cadaver, following variations were encountered. a) One superficial and complete type of axillary arch on left axilla only. b) Venous chiasma between the basilic vein and brachial vein of both sides. c) Abnormal course of intercosto-brachial nerve on left side d) Presence of two medial cutaneous nerve of forearm and the absence of medial cutaneous nerve of arm on left side only.The presence of such variations should be kept in mind while performing various invasive and surgical techniques.

Keywords: Axillary arch, basilic vein, brachial vein, intercosto-brachial nerve, medial cutaneous nerve of forearm.

Anatomical variations are common in axilla but the co-existence of multiple variant in the axilla is rarely documented. Here we present a case where we observed multiple variations in a single cadaver during routine dissection.

Axillary arch: Occasional presence of an anomalous muscle "Axillary arch" and its relative closeness of vital structures are important to the surgeons and also for academic purposes. This variation was described by Carl Langer[1] in 1846 as "Achselbogen" a fibrous thickening of the medial edge of axillary fascia between the pectoralis major and latissimus dorsi. Later, in 1884 Testut[2] called it as Langer's axillary arch to the muscular variation.

The axillary arch (AA) is a variant muscular slip of latissimus dorsi crossing from the edge of latissimus dorsi to join the tendons of pectoralis major, coracobrachialis or the fascia over the biceps brachii.[3] The AA can receive nerve fibers from the lateral pectoral, medial pectoral, intercostobrachial or thoracodorsal nerve.[4] The AA brought about hyper-abduction syndrome, thoracic outlet syndrome, costoclavicular syndrome, shoulder instability syndrome, median nerve and radial nerve entrapment, and venous thrombosis.[5,6]

Basilic vein: Usually, the distal basilic vein lying superficially pierces the deep fascia at the elbow and joins the venae comitantes of the branchial artery to form the axillary vein.[3]

Intercostobrachial nerve (ICBN): Usually lateral cutaneous branch of the second intercostal nerve supplies the axillary floor and upper medial surface of the arm via communication with the medial cutaneous nerve of arm.[3] The ICBN preservation provides a potential anatomical landmark in axillary lymph node dissection and prevent the postoperational arm lymphedema.[7]

Medial cutaneous nerve of forearm (MCNF) pierces the deep fascia along with the basilic vein midway in the arm and divides into anterior and posterior branches.[3]

MATERIALS AND METHODS

Over a span of two years, during dissection different congenital anomalies were observed and recorded on 24 embalmed cadavers in the Department of Anatomy of Nepal Medical College. Axillary regions and the arm of both sides were dissected as a part of routine dissection. Multiple variants in a single cadaver with presence of axillary arch and other variations regarding the axillary vessels, brachial plexus and its branches along with superficial vein of arm were observed. It is being reported because of its unique nature.

RESULTS

During the dissection of axilla of 45 years old male cadaver, several anomalies were recorded on both arms. Most importantly an unusual muscle band was encountered on left axilla and identified as axillary arch. A venous chiasma between the basilic vein and branchial vein were also observed in both sides. On the left side there were abnormal course of intercosto-branchial nerve; presence of two medial cutaneous nerve of forearm and the absence of medial cutaneous nerve of arm.

Axillary Arch

AA extended from axillary border of the fleshy portion of latissimus dorsi to the inferior surface of pectoralis major and had a small fascial extension to the fascia of coracobrachialis and short head of biceps brachii (Fig. 1). It was rectangular-shaped muscle of 7.5cm in length and 1.2 cm in width. On the basis of clinical classification of axillary arch, our finding belonged to superficial group as it passed across the axilla, crossing the axillary vein, artery and nerves of brachial plexus.[4] The AA was in close association with intercosto-brachial nerves near its distal attachment and to median nerve near its proximal attachment. The thoracodorsal nerve innervating LD had supplied this extension.

Variant branchial-basilic vein anatomy:

In the middle of the left arm, we observed the basilic vein receiving the tributaries from lateral vena-comitant of brachial artery in front of median nerve and again got bifurcated into two basilic vein. The lateral vena comitant after sending a tributary to basilic vein runs upward, medial to biceps brachii and coracobrachialis and then rejoined with lateral basilic vein in the middle of coracobrachialis. The bifurcated basilic veins run in the axilla between AA and axillary artery. It received medial vena commitant and united to form a single axillary vein (Fig. 1).

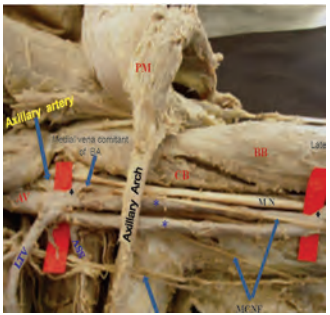

Fig 1: Left axilla showing superficial and complete type of Axillary Arch and upper extremity showing various brachial-basilic vein junction and double basilic veins.

AV-Axillary Vein, BB-Biceps Brachaii, BV- Basilic Vein, CB- Coracobrachialis, ICBN- Intercostobrachial Nerve, LD- Lattisimus Dorsi, LTV- Lateral Thoracic Vein, MCNF- Medial Cutaneous Nerve of Forearm, MN- Median Nerve, PM- Pectoralis Minor, SSV- Subscapular Vein, TDN- Thoracodorsal Nerve..

Note the Double Basilic Veins (**) and brachial- basilic vein junction (♦)

In the right arm, the vena commitants of brachial artery joined with basilic vein, medial to median nerve and coracobrachialis. Subsequently it got divided and after a short distance rejoined with basilic vein forming the axillary vein (Fig. 2). The medial cutaneous nerve of forearm on the right axilla passed through the two divisions of basilic veins and ran medial to basilic vein (Fig. 2).

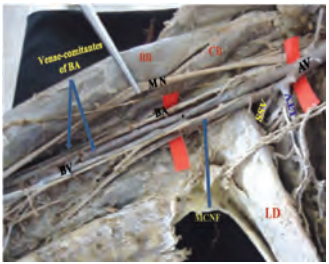

Fig 2: Right upper extremity showing brachial-basilic vein junction and abnormal course of MCNF passing through the two divisions of basilic veins.

AV-Axillary Vein, BA- Brachial Artery, BB-Biceps Brachaii, BV- Basilic Vein, CB-Coracobrachialis, LTV-Lateral Thoracic Vein, MCNF- Medial Cutaneous Nerve of Forearm, MN- Median Nerve, SSV- Subscapular Vein.

Note the brachial- basilic vein junction (♦) and absence of axillary arch.

Intercostobrachial nerve

ICBN of the left side was seen piercing 2nd intercostal space and divided into anterior and posterior branches, hooking around the lateral thoracic artery (Fig. 3).

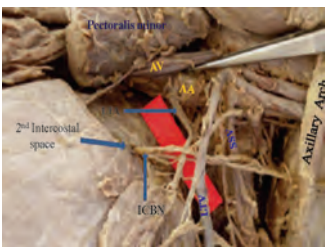

Fig 3: Left axilla showing intercostobrachial nerve hooking around the lateral thoracic artery.

AA- Axillary, AV-Axillary Vein, ICBN- Intercostobrachial Nerve, LTA- Lateral

Thoracic ArArterytery, LTV- Lateral Thoracic Vein, SSV-Subscapular Vein.

Anterior division communicated with the posterior division and divided into two branches. The lower branch innervated the medial aspect of arm where as the upper branch joined further with medial cutaneous nerve of forearm and divided in a M shaped pattern and supplied the medial aspect of forearm, while posterior division of ICBN supplied medial aspect of the arm (Fig. 4).

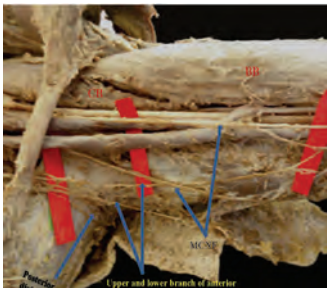

Fig 4: Left upper extremity showing the abnormal course of Intercostobrachial nerve.
BB-Biceps Brachaii, CB- Coracobrachialis , ICBN-Intercostobrachial Nerve, MCNF- Medial Cutaneous Nerve of Forearm.

Medial cutaneous nerve of Forearm

In the left arm, two MCNF arose from medial cord of brachial plexus and absence of medial cutaneous nerve of arm (Fig. 4). Upper one innervating the ventral aspect of forearm where as lower one gave cutaneous supply to posterior aspect of the elbow. In spite medial side of arm was innervated by ICBN.

DISCUSSION

Multiple variations in a single body are a rare occurrence. Particularly these variations reported in the same arm are rare.

Axillary Arch

In the present study, we found one axillary arch in 24 cadavers i.e. 4.16% within the limited no. of cadavers we studied. The different authors had mentioned about different percentage of AA ranging from 1-12% (Table-1). Miguel *et al*,[8] reported a frequency of 3% out of 100 cases. Our observation of superficial and complete AA was also described by others.[5,9,10]

Various authors highlighted the significance of AA. During physical examination, a loss of the normal concavity of the axilla [12] and also can be confused with lymphadenomegaly.[13] The phylogenetic history suggested it as remnant of panniculos carnosus which is present in low ranking rodents to protect themselves from insects. In human it mostly disappeared since its functional significant being lost.[13]

Venous Chiasma Between Basilic Vein And Brachial Vein:

There were few studies addressing the anatomy of basilic vein but none of them were similar to our findings. Anaya-Ayala et al., [14] have reported 34% variant brachial-basilic vein anatomy in 290 patients. But our finding did not match any of the type mentioned by them. Another observer mentioned the basilic vein and the brachial vein joined with one another in the middle of the arm to form a unique venous chiasma.[15] The unusual position of brachial-basilic junction near the antecubital fossa led to an inadvertent distal brachial vein ligation and failure of subsequent graft placement during basilic vein transposition.[16] As the limb enlarges, the marginal vein gets subdivided and forms the superficial veins. It was suggested that as a result of different hemodynamic influences, some anastomosis develop between superficial and deep vessels, where as others don't develop or regress. In this way, the chiasmatic patterns between superficial and deep blood vessels arise.[17]

Table-1: Comparison of the axillary arch cases in the literature

Authors	Total axillae (n)	AA (%)	Complete AA	Incomplete AA	Innervation/ cases
Miguel et al, 8 2001	100	3(3%)	2	1	Thoracodorsal nerve / 3
Merida- Velasco et al,5 2003	64	4(6.25%)	2	2	Thoracodorsal nerve/ 3 Medial pectoral nerve/ 1
Pai MM et al,9 2006	68	1(1.47%)		1	Branch from lateral cord
Bertone VH et al,11 2009	78	9 (12%)	6	3	Intercostobranchial nerve/ 8
Orhan M et al,10 2012	20	2 (10%)	2		Medial pectoral nerve /1
Present study	24	1(4.16%)	1		Thoracodorsal nerve

Intercostobrachial nerve

Our finding belongs to one of the variety of type I reported by Loukas et al.[18] in which ICBN provide a branch to MCNF. In an extensive study, he had observed 8 different pattern of ICBN in 200 axilla.

Medial cutaneous nerve of Forearm

The medial cutaneous nerve of the forearm, which passing through the divisions of basilic veins, could be the result of entrapment of the persistent axonal growth cone within the venous plexus during embryological development.[19] A similar variation was reported by Roy *et al*.[20]

CONCLUSION

We are reporting the variations of AA because it is of special interest for the anesthesiologist during nerve block and surgeons during axillary lymph adenectomy. This type of a venous chiasma between basilic and brachial vein may mislead the doctor during cardiac catheterization. The knowledge regarding the variation of ICBN is important in prevention of postoperational arm lymphedema.

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