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My right is to my work

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Dengue (Break bone fever): An Emerging Disease in Nepal

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Dengue fever is a mosquito-borne tropical infectious disease with potential fatal complications, and highly complex pathophysiological, economic and ecologic problems, caused by **dengue virus** (DENV). This disease occurs primarily in the equatorial regions of Africa, the Americas, South-East Asia, and the Western Pacific. The incidence of dengue fever has increased dramatically since the 1960s, with current estimates of incidence ranging from 50 million to 528 million people infected yearly, leading to half a million hospital admissions and about 25,000 deaths. This increase is believed to be due to several factors, including global warming, urbanization, and increased international travel. Dengue has become a global problem involving newer areas, newer populations and is increasing in magnitude epidemic after epidemic, and is endemic in more than 110 countries.

Dengue fever was first referred as 'water poison' associated with flying insects in a Chinese Medical Encyclopedia from the Jin Dynasty (265 - 420 AD). The first clinically recognized dengue epidemics occurred almost simultaneously in Asia, Africa, and North America in the 1780s. The first clinical case dates from 1789 report of 1780 epidemic in Philadelphia is by physician Benjamin Rush, who coined the term '**break bone fever**' because of the symptoms of myalgia and arthralgia. In the report's title he also used the term "**billous remitting fever**". The term **dengue fever** (DF) came into general use only after 1828. The word "dengue" is derived from the Swahili phrase Ka-dinga pepo, meaning "**cramp like seizure**".

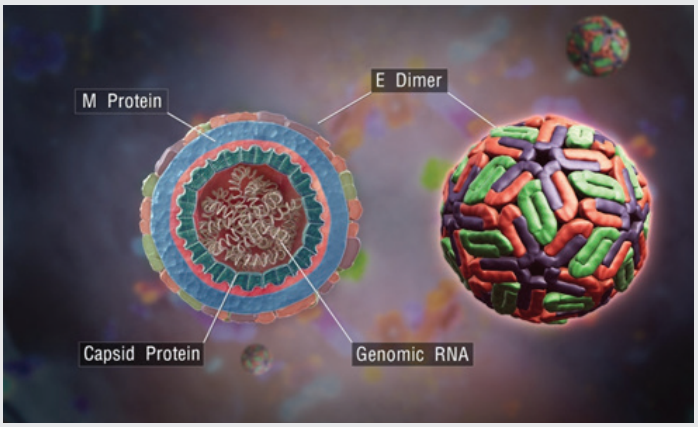
The first epidemic of DF like illness was recorded in Madras (now Chennai) in 1780, and the first virologically proved epidemic of DF occurred in Calcutta (now Kolkata) and Eastern Coast of India in 1963-1964. The first major epidemic of the Dengue hemorrhagic fever (DHF) occurred in Philippines followed by a quick global spread of epidemics of DF/DHF. The DHF started simmering in various parts of India since 1988. The first major wide spread epidemics of DHF and dengue shock syndrome (DSS) occurred in India in 1996 involving areas around Delhi and Lucknow and then it spread all over the country.

Although dengue virus infections have been found in India over a long period of time, there was no documented case of DF in Nepal. For the first time in 2004, dengue virus was identified as a causative agent in a patient with acute febrile illness in Nepal. Thereafter, minor dengue outbreaks were confirmed in six districts of Terai region in 2006. In addition to these, acute dengue virus infection

has been reported from Western Nepal during 2007–2008, indicating that dengue virus infection is becoming one of the major emerging infectious disease in Nepal. It is plausible to assume that dengue virus could have been introduced into Nepal from India, due to the open border between the two countries. This hypothesis is further supported with the finding of nucleotide sequences of the Nepalese dengue strain that have been described to be very similar to the dengue virus strains circulating in India. During the 2006 outbreaks, all four dengue serotypes were found to be circulating in Nepal. In 2010, Nepal had experienced major outbreaks of dengue fever in several districts (24 districts), particularly in Chitwan and Rupendehi districts. A total of 265 cases from across the country were admitted to Sukraraj Tropical and Infectious Disease Hospital (STIDH), Kathmandu, between July and December 2010. There was one death reported due to dengue shock syndrome (DSS). Taken together, a sudden resurge of severe dengue disease can be, therefore, assumed to occur in the near future in Nepal.

Dengue virus (DENV) is a single stranded, positive-sense RNA virus of the family *Flaviviridae* and the genus *Flavivirus.* Other members of the genus include Japanese encephalitis virus, yellow fever virus, Kyasanur forest disease virus etc. Most are transmitted by arthropods (mosquitoes or ticks) and are therefore also referred to as ***arbo*viruses** (***ar*** = arthropod; ***bo*** = borne). The genome is approximately 11 kb in length, that encodes for three structural proteins, the capsid (C), membrane (M), and envelope (E) glycoproteins that form the virus particle (Fig 1), and seven nonstructural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5), of which NS1 has diagnostic and pathological importance. E glycoproteins are responsible for the important biological properties such as binding to receptors, hemagglutination of RBC, and the induction of neutralizing antibodies and the protective immune response. There are four serotypes of the virus (DENV-1, DENV-2, DENV-3, and DENV-4) and recently a fifth serotype (DENV-5) has been reported in 2013.

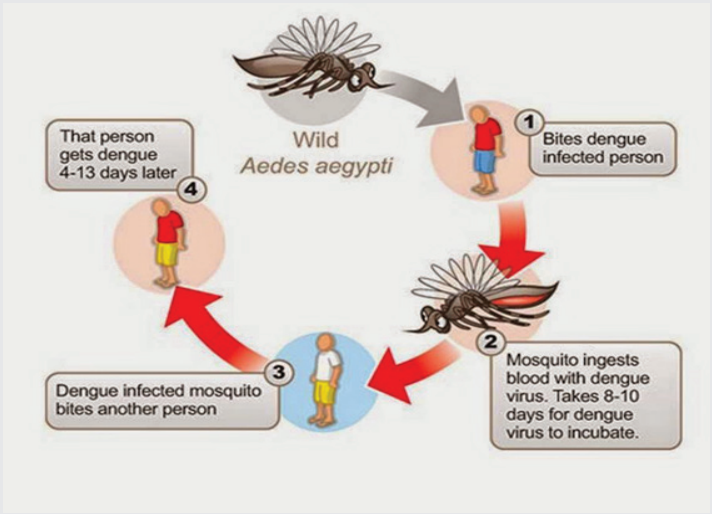
Fig 1: Dengue virus structure (Source: en.wikipedia.org)



Dengue virus is transmitted primarily by domesticated day biting mosquitoes ***Aedes aegypti*** (Fig 2) and also *A. albopictus*. Although the mosquitoes are of Asian origin, they now occur in Africa, Europe, and USA. International travel and the transportation of goods favored the spread of both vector and virus. A female mosquito that takes a blood meal from an infected person (during the potential 2 to 12 day range of the febrile, viremic period) becomes infected (Fig 3). The virus passes from the mosquito gut to the salivary glands in 8 – 10 days (extrinsic incubation period), and is subsequently released into its saliva. The virus seems to have no detrimental effect on the mosquito, which remains infected for life.

Fig 2: *Aedes aegypti* mosquito (Source: www.livemint.com)

Fig 3: Transmission of dengue virus infection (Source: www.eliminatedengue.com)



When an infected mosquito bites a person, the virus enters the skin along with the mosquito's saliva. In the skin, dengue viruses infect immature dendritic cells through the non-specific receptor (Dendritic cell-specific ICAM3-grabbing non-integrin; DC-SIGN). Infected dendritic cells mature and migrate to local or regional lymph nodes where they present viral antigens to T cells, initiating the cellular and humoral immune responses. There is also evidence of abundant replication of DENVs in liver parenchyma cells and in macrophages in lymph nodes, liver, and spleen, as well as in peripheral blood monocytes. Both *in vitro* and *in vivo*, macrophages and monocytes participate in **antibody-dependent enhancement** (ADE). This ADE occurs when mononuclear phagocytes are infected/ coated through the Fc receptors of **immune complexes** (Antibody-virus complexes) that form between DENVs and nonneutralizing antibodies. These nonneutralizing antibodies result from previous heterotypic DENV infection or from low concentrations of dengue antibodies of maternal origin in infant sera. The co-circulation of four DENV serotypes in a given population might be augmented by the ADE phenomenon.

Immune complexes (Antibody-virus complexes) when coated on mononuclear phagocytes by their Fc fragments of Immunoglobulins suppresses innate immune responses, increasing intracellular infection and generating inflammatory **cytokines** (Gamma interferon, tumor necrosis factor-α, and interleukin-10) that lead to vascular endothelial cell dysfunction, which result in plasma leakage

DENVs produce several syndromes that are conditioned by age and immunological status.

During initial/ primary dengue infections, most children experience subclinical infection or mild undifferentiated febrile syndromes. During secondary dengue infections, the pathophysiology of the disease changes dramatically, particularly sequential (or multiple infections with different serotypes) in which infection with DENV-1 is followed by infection with DENV-2 or DENV-3, or infection with DENV-3 is followed by infection with DENV-2. Such severe infections can result in **dengue hemorrhagic fever (DHF)** or **dengue shock syndrome (DSS)**. Dengue-associated deaths are usually linked to DHF/DSS.

Infection with a given serotype confers life-long immunity to that serotype, but only short-term immunity to others. Dengue can be life-threatening for people with chronic diseases such as diabetes mellitus and asthma.

Typically people infected with dengue virus are asymptomatic (80%) or have only mild symptoms, such as uncomplicated fever. Others have more severe illness (5%), and in a small proportion of cases (<1%), it is life-threatening and causes death, despite treatment.

The incubation period ranges from 3 – 14 days, but most often it is 4 to 7 days. The onset of symptoms is usually abrupt. Fever is characteristic symptom (**Febrile phase**) and is often abrupt in onset with high spikes of 39.4 – 40.5°C. **The fever pattern is classically biphasic or saddleback**, and generally lasts for five to seven days. In young children fever may cause febrile seizures or delirium. Patients with rapid defervescence may enter the **critical phase** of infection.

Aches and pains, particularly backache, **arthralgia**, **myalgia**, and **bone pain** are common. **Headache** is also typical of infection and is generally constant and towards the front of the head. Severe retro-orbital pain on eye movement or with a little pressure applied to the eyeball is also usual.

Gastrointestinal symptoms (e.g. anorexia, nausea or vomiting, epigastric discomfort or pain), lethargy or restlessness, collapse or dizziness may also be present. Patients often report a lack of appetite or changes to taste sensation. Gastrointestinal symptoms, weakness, and dizziness may be more noticeable in dengue hemorrhagic fever. Upper respiratory tract symptoms, such as sore throat and cough, are usually absent.

Diffuse skin flushing of the face, neck, and chest develop early with infection. This evolves into a **maculopapular or rubelliform rash** of the whole body, usually on third or fourth day of the fever. Blanching may occur when the skin is pressed. The rash fades with time, and during the **convalescent phase** appears as pallid areas.

Hemorrhagic signs include **petechiae**, purpura, or a **positive tourniquet test** (blood pressure cuff inflated to a point midway between systolic and diastolic pressures for five minutes, and then counting any petechial hemorrhages that occur. The test is positive if ≥10 petechiae per square inch appear on the forearm). More major hemorrhages can manifest as **epistaxis**, **gingival bleeding**, hematemesis, melaena, vaginal bleeding (in women of child bearing age), or bleeding from a venepuncture site. These signs can occur with either DF or DHF.

Hepatomegaly may be present. **Plasma leakage** is a sign of dengue hemorrhagic fever, and clinical evidence of this includes the presence of ascites, postural dizziness, or pleural effusion.

Circulatory collapse (that is, cold clammy skin, rapid and weak pulse with narrowing of pulse pressure <20 mmHg with decreased diastolic pressure, postural drop of blood

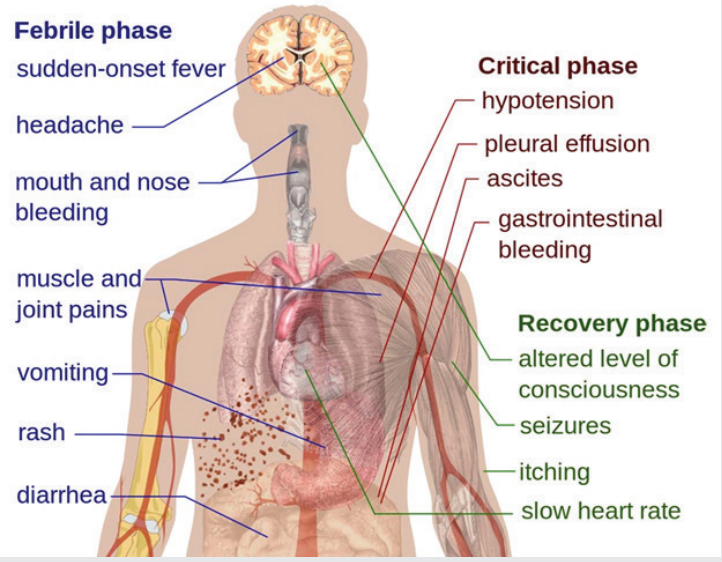
pressure >20 mmHg, capillary refill time greater than three seconds, reduced urine output) indicates the presence of shock and supports a diagnosis of DSS.

Dengue infection has three distinct phases (Fig 4): Febrile, critical, and convalescent. The **febrile phase** is characterized by a sudden high grade fever and dehydration that can last two to seven days. The **critical phase** is characterized by plasma leakage, bleeding, shock, and organ impairment and lasts for about 24 to 48 hours. It usually starts around the time of defervescence (this does not always occur), typically third to seventh day of the infection. Patients with DHF or DSS go through all three stages. The critical phase is bypassed in patients with DF.

Table 1: Main characteristic manifestations of dengue illness

1. Continuous high fever lasting 2 – 7 days
2. Hemorrhagic tendency as shown by a positive tourniquet test, petechiae or epistaxis
3. Thrombocytopenia (platelet count <100,000 mm^{-3}), and
4. Evidence of plasma leakage manifested by hemoconcentration (an increase in hematocrit 20% above average for age, sex and population), pleural effusion and ascites etc.

Fig 4: Symptoms of dengue fever (Source: www.medicalnewstoday.com)



The **WHO 2009 classification** divides dengue fever into two groups: Uncomplicated and severe. According to this system, dengue that is associated with severe bleeding, severe organ dysfunction, or severe plasma leakage is considered severe, whereas all other cases are uncomplicated. This simplified system replaces the 1997 WHO classification, which was found to be too restrictive, although it is still widely used. The **1997 WHO classification** divided dengue into undifferentiated fever, dengue fever, and dengue hemorrhagic fever (DHF). DHF was subdivided further into grades I to IV, where grade I is the presence of only easy bruising, or a positive tourniquet test result in someone with fever, grade II is the presence of spontaneous bleeding into the skin and elsewhere, grade III is clinical evidence of shock, and grade IV is shock so severe that blood pressure and pulse cannot be detected. In this system grades III and IV are referred to as '**dengue shock syndrome (DSS)'**.

The diagnosis of dengue is typically made clinically, on the basis of reported symptoms and physical examination, especially in endemic areas. A probable diagnosis is based on findings of fever and any few of the following: nausea and vomiting, rash, generalized pains, leucopenia, positive result on tourniquet test, or any warning sign (Table 2) in someone who lives in an endemic area.

Table 2: Warning signs of impending critical phase of dengue infection

• Abdominal pain or tenderness
• Persistent vomiting
• Enlargement of the liver >2 cm
• Mucosal bleeding
• Increase in hematocrit with rapid decrease in platelet count
• Lethargy or restlessness
• Accumulation of clinical fluid (e.g. ascites, pleural effusion)

The earliest change detectable on laboratory investigations is **leucopenia**, which may be followed by **thrombocytopenia**. Leucopenia in combination with a **positive torniquet test**, in a dengue endemic area has a positive predictive value of 70 – 80%.

Table 3: Laboratory criteria for diagnosis of dengue hemorrhagic fever or dengue shock syndrome

• Rapidly developing, severe thrombocytopenia
• Decreased total WBC count and neutrophils and changing neutrophil to lymphocyte ratio
• Increased hematocrit (20% increase from baseline is objective evidence of plasma leakage)
• Hypoalbuminemia (serum albumin <35 g/L suggests plasma leakage)
• Increased liver function test results (aspartate aminotransferase : alanine aminotransferase >2)

The hematocrit may also rise about 10% in patients with dengue fever owing to dehydration. The results of liver function tests are usually increased, particularly for aspartate and alanine aminotransferases. Clotting studies are not required for diagnosis but may play a useful role in the management of the infection in patients with hemorrhagic signs.

Confirmatory tests should be carried out, because dengue fever can be confused with many non-dengue illnesses.

1) Detection of viral nucleic acid by PCR, and nucleic acid–sequence based amplification assay (NASBA).
2) Detection of viral antigen (NS1) in tissues such as liver, spleen, and lymph nodes as well as tissues from fatal cases (slides from paraffin-embedded, fresh or frozen tissues) by antigen-capture ELISA, and lateral flow antigen detection.
3) Serological demonstration of virus-specific antibodies (IgM, IgG) by ELISA, and

neutralization tests.Detection of viral nucleic acid or viral antigen is primarily done in the first five days of illness, and serological tests after the fifth day.

4) Virus isolation is possible during the initial viremic phase. *Aedes albopictus* mosquito C6/36 cell line is the method of choice for isolation, although other mosquito (*Aedes pseudoscutellaris* AP61) and mammalian cell lines (Vero, LLC-MK2, BHK21 cell lines) can be used.

Imaging studies are required only if DHF or DSS is suspected. A lateral decubitus chest radiograph of the right side of the chest can be ordered to detect clinically undetectable pleural effusion in the early phase of plasma leakage.

Ultrasonography of the abdomen is useful to detect the presence of ascites and plasma leak or other disease related changes in abdominal organs, including the liver, gall bladder (edema may precede plasma leakage), and kidneys.

Treatment is supportive, as no specific antiviral therapy is available for dengue infection, and is based on guidance produced by WHO and other region specific authorities. The only recognized treatment in dengue fever is maintaining adequate hydration, and in DHF and DSS treatment is fluid replacement therapy, by judicious use of intravenous fluids to maintain sufficient urinary output and perfusion, and to achieve stabilization of vital signs, and normalization of vital signs. For patients presenting with unstable vital signs in the face of decreasing hematocrit, blood transfusion should be initiated early.

There is no vaccine available. A tetravalent vaccine is under development and may be available in the near future. Prevention thus depends on control of, and protection from the bites of, the mosquito that transmits it. The primary method of controlling *Aedes aegypti* is by eliminating its habitats, which include standing water in urban areas (e.g. discarded tyres, ponds, drainage ditches, and open barrels), and by applying insecticides. The mosquito bite can be avoided by appropriate clothing to cover exposed skin, especially during the day, and the use of insecticides, mosquito repellants, mosquito coils, and mosquito nets etc.

WHO is working together with several partnerships (e.g. Asia-Pacific dengue prevention partnership, European Union's DENFRAME and DENCO projects) and national Governments to develop new tools and strategies to improve diagnostics and clinical treatments and a successful vaccine.

A Comparative Study between Staplers and Suture (Silk 2-0) for Skin Closure in Cesarean Sections at Gandaki Medical College Teaching Hospital

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Keywords
Cesarian section, Skin closure, Stapler, Suture.

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ABSTRACT

Background: Skin closure in the abdominal surgeries is an important factor that affects the prognosis of wound in terms of hospital stay as well as overall outcome of the surgery.

Objectives: Cesarean section being the commonly performed operation, choice of suture material has the unexceptional role on it. This study has been performed with an objective to look for the merits and demerits of the skin closure by suture (Silk 2-0) and stapler.

Methods: Prospective comparative study conducted among the patients admitted in a Maternity Ward of Gandaki Medical College Teaching Hospital for elective and emergency cesarean section. The comparison has been made in terms of time taken during the skin closure, presence or absence of soakage and wound dehiscence, day of suture removal and pain during the suture removal.

Results: The average time taken for skin closure for suture group was found to be 5.46 min (±0.97) and the same for stapler group was found to be 1.22 min (±0.15) respectively. Similarly, the mean day of stitch removal in suture and staples were found to be 6.94 (±1.75) and 7.95 (±1.89) respectively. Surgical site infection (SSI) i.e. soakage was present in eight percent of those in suture group and 20% in stapler group. Wound dehiscence was present in two percent among the suture group and five percent among the stapler group. The severity of pain is more in stapler group than that of suture group during its removal.

Conclusions: Our study concluded suture being superior to staplers for skin closure during cesarean section. Though time taken for the closure is less in the stapler group, other factors like wound complications, duration of hospital stay, pain during its removal favored for the suture to be used.

INTRODUCTION

Skin closure is one of the important determining factors for success of a surgery. It's been over 50000 years physicians have been searching for ideal suture material[1]. The skin is protective and self repairing barrier between the body and external environment. Every surgeon would wish to see wound healthy and scar cosmetically better. The method of closure should be simple, quick to use and cost effective. Many factors are involved in the choice of the skin closure material, including the type and place of the wound, available materials, physician expertise and preferences, patient age and health[2].

Cesarean section is one of the most commonly performed abdominal surgeries worldwide with recent increase to unprecedented levels[3]. Mostly obstetricians use staples, interrupted suture or subcuticular suture immediately below the skin layer to close the skin during cesarean section[4]. The choice of the suture materials may affect the overall prognosis of wound in terms of time taken for skin closure, duration of hospital stay, wound infection, pain during the suture removal cosmetic outcome of the scar[5]. The existing literature still does not provide enough evidence to say whether one of the suture material either staplers or suture is better than one another for closure of abdominal wall in cesarean section[6].

This comparative study has been done with objective to see the merits and demerits of skin closure by stapler and suture. The comparison has been made in terms of time taken during the skin closure, presence or absence of soakage and wound dehiscence, day of suture removal and pain during the suture removal.

METHODS

Study design: Prospective comparative study conducted among the patients at Medical College Teaching Hospital of Western Region of Nepal.

Study site: Gandaki Medical College Teaching Hospital

Study population: The admitted patients in the Maternity Ward who went for elective or emergency cesarean section and agreed to participate were included in the study.

Sample size: In this study 200 pregnant women of aged 16 to 36 were enrolled.

Study Period: 15th April, 2016 to 14th May, 2017

Sampling procedure: Convenience sampling was applied as the patients admitted in Maternity Ward for cesarean section during the period of April 2016 to May 2017 were enrolled in the study.

Instruments and techniques: The patients were divided into two groups on the basis of material used for skin closure in cesarean section i.e. stapler and suture.

Suture group: Skin was approximated with vertical mattress suture using non absorbable silk 2-0 at a distance of one cm from each other.

Stapler group: The staplers were used to close the wound placed at a distance of 5 mm from one another.

Each patient was given same antibiotics for the same duration. Wound of every patient was assessed at fourth, sixth, seventh and eighth post operative day. Wound dehiscence was noted.

Pain occurring during the removal was evaluated as mild, moderate or severe as described by the patient on 3 point severity scale.

Data analysis: Data was entered in SPSS 18 software and analyzed. Proportions, percentage were calculated for descriptive statistics and Chi square test was performed for the statistical analysis of the observations. P-value was set at 0.05 level of significance.

Ethical consideration: Informed consent for the enrollment in study was taken for all the patients before the surgery. Ethical clearance was taken and the ethical approval was obtained from the Institutional Ethical Committee of Gandaki Medical College.

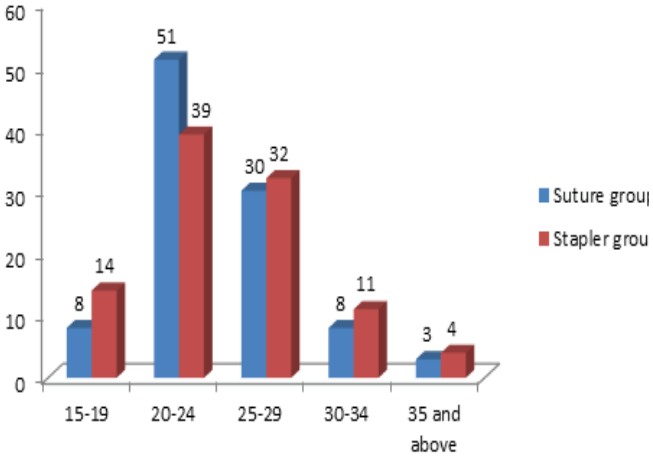
RESULTS

Total 200 cases studied were divided into two groups each of 100 cases. Those cases whose skin was closed with suture are categorized as "Suture group" and those with stapler are categorized in "Stapler group" to compare the time taken during the skin closure, presence or absence of soakage and wound dehiscence, day of suture removal and pain during the suture removal. The results were analyzed from the observations made as follows:

3. 1. Age distribution

The mean age of the participants in the suture group and stapler group were 24.12 (±4.073) and 24.74 (±4.769) respectively. The age wise distribution of the two different groups is shown in fig 1.

Fig 1: Age wise distribution of suture and stapler groups



3.2. Time taken for skin closure

The average time taken for skin closure for suture group was found to be 5.46 min (±0.97) and the same for stapler group was found to be 1.22 min (±0.15) respectively (p<0.001).

3.3. Day of stitch removal

The mean day of stitch removal in suture and stapler were found to be 6.94 (±1.75) and 7.95 (±1.89) respectively (p<0.001).

3.4. Surgical site infection (Soakage)

Surgical site infection (SSI) i.e. soakage was present in eight percent of those in suture group and 20% in stapler group (p = 0.014) as shown in table 1.

Table 1: Surgical Site Infection in suture and stapler group

Surgical Site Infection	Group A		Group B		P value
	N	%	N	%	
Present	8	2	20	20	0.014
Absent	92	98	80	80	

3.5. Wound dehiscence

Wound dehiscence was present in two percent among the suture group and five percent among the stapler group (p=0.248) as shown in table 2.

Table 2: Wound dehiscence in suture and stapler group

Wound dehiscence	Group A		Group B		P value
	N	%	N	%	
Present	2	2	5	5	0.248
Absent	98	98	95	95	

3.6. Pain during stitch removal

The severity of pain is more in stapler group than that of suture group during its removal as shown in table 3.

Table 3: Pain during stitch removal

Severity	Group A		Group B		P value
	N	%	N	%	
Mild	94	94	74	74	0.000
Moderate and Severe	6	6	26	26	

DISCUSSION

Every surgeon wishes for the early healing of post operative wound with minimal complications. Indeed, it's the responsibility of the surgeon to choose the method of skin closure that would be suitable for the patient in terms of early wound healing without post operative surgical site infection, cost effectiveness, minimal pain during the suture removal and duration of hospital stay. In our study, the two groups with similar demographic characteristics are evaluated with skin closure by suture on one and stapler on other.

In our study, time taken for the skin closure by staplers was significantly less than by suture which is in consistent with the other studies[7-10]. Use of staplers will thus shorten the duration of surgery and anesthesia on the patients considerably reducing the perioperative risk. It will also be of great value in the busy obstetric care settings where high number of patients needs to undergo cesarean section.

The mean day of stitch removal in suture group was found to be earlier than that of stapler which was statistically significant (p<0.001). This may be due to the fact that less evidence of surgical site infections was seen in the patient with suture group. This favors for the early discharge of patient from the hospital and indeed results in decrease in post operative complications. Similar study conducted by Basha *et al*[9] showed no significant difference between two groups in the length of hospital stay.

As far as surgical site infections i.e. soakage of the wound is concerned, it was seen more in the patient with stapler group than that of suture which was statistically significant (p=0.014). Similarly the wound dehiscence was also found to be more on the patient with stapler group that of suture (p=0.248). The findings of our study were consistent with the studies by Mackeen *et al*[5] and Basha *et al*[9] where wound complication rates were higher in stapler group. A meta-analysis of randomized control trails by Clay *et al*[11] comparing staples and subcuticular suture had also shown high wound complications rate in staples group than that of suture. The exact reason behind the high infection rates among the staples group is not clear. We hypothesize that it may be due to increased bacterial migration to the incision site because of the gap between staples. Furthermore studies are needed to make this understanding clear.

Similarly, our study suggests the severity of pain is more in staples group than that of suture group during its removal. This finding is consistent with other studies conducted in orthopedic surgeries[12,13], where the patient reported more pain during the stapler removal than that of suture. The reason for this may be more traumatic and

rigid fixation of the tissues by staplers.

Another important factor to be discussed here is the cost effectiveness of the suture and staplers for the abdominal closure. The market price of the staplers is relatively more than that of suture used for the abdominal wound closure here in our settings. Similar finding has been demonstrated in the study by Abdus-Salam *et al*[7].

The limitation of our study is that we didn't evaluate other parameters for the comparison between the stapler and suture like patient satisfaction and scar cosmetic outlook after the healing. Other studies[10,14] that evaluated these parameters also didn't find any significant differences on it among the stapler and suture group.

The results of our study advocated for the use of suture rather than staplers during cesarean section for the skin closure. Though time taken for the closure is less in the stapler group, other factors like wound complications, duration of hospital stay, pain during its removal favored for the suture to be used. The cost effectiveness among the two is also an important factor determining for the selection of stapler and suture in our resource limited settings which furthermore emphasized for the use of suture rather than staplers.

CONCLUSIONS

We compared the merits and demerits of the stapler and suture based on the different parameters. Staplers are less time consuming but have high rate of wound complications, results in lengthy duration of hospital stay and more painful during its removal. Furthermore staplers are expensive than suture. Our study provided the baseline comparison of outcomes between the staplers and suture for the skin closure in the cesarean section. Furthermore it's the choice of the operating surgeon and availability of the suture or staplers that ultimately decides the selection of suture materials for the skin closure in cesarean section.

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Profile of Ocular Trauma in a Tertiary Centre in Western Nepal

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Keywords
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ABSTRACT

Introduction: Ocular trauma is an important cause of blindness and ocular morbidity throughout the world. The present study was done to establish the common causes of ocular trauma in a Tertiary Care Hospital of Western Nepal.

Methods: In this prospective study, all the patients with ocular trauma visiting eye Out Patient Department (OPD) and Emergency Department of Gandaki Medical College Teaching Hospital (GMCTH) from June 2015 to June 2016 were included. A complete history and detailed ophthalmological evaluation was done.

Results: Over a period of one year, 226 patients attended to the OPD and Emergency Department of GMCTH. Mean age of patients was 30.41 ±15.7 years. Males were 70.8% and females 29.2%. About 82.5% patients had visual acuity better than 6/18 while 17.7% had visual acuity <3/60. Road traffic accident (RTA) was the most common cause followed by foreign bodies.

Conclusions: Males are more prone to ocular trauma than females and majority of ocular trauma occurred at workplace. RTA is the commonest cause of ocular trauma. Very few patients used protective device. Public awareness and strict legislation for the use of personal protective devices can also help reduce the occurrence of ocular injury.

INTRODUCTION

Although eyes represent only 0.1% of the total body surface and only 0.27% of the anterior body surface, their significance to individuals and society is disproportionately higher.

Ocular trauma is an important cause of blindness and ocular morbidity throughout the world. The available literature on ocular trauma mainly comes from the developed countries with modern facilities of management. There are almost 2.5 million incident cases of eye injuries each year in the United States alone[1]. The annual incidence of hospitalisation for eye injuries is 8.1, 12.6, 13.2 and 15.2 in Scotland[2], Singapore[3], United States[4] and Sweden[5] respectively.

In Nepal, with developing economy, poor health facilities and poor access to health care system, trauma is a significant cause of ocular morbidity. According to Nepal blindness survey[6] done in 1981 blindness due to ocular trauma was 2.4%. Bhaktapur eye survey showed the prevalence of ocular trauma 0.7%[7]. These types of studies are important to define the target groups for prevention and education on ocular trauma.

It is believed that over 90% of all eye injuries can be prevented, making ocular trauma one of the most important preventable causes of blindness[21,22]. To prevent eye injuries and to develop effective treatments an adequate data is essential. Therefore we performed this study in Gandaki Medical College Teaching Hospital, a Tertiary Centre in Western Nepal with an objective to determine the magnitude of ocular trauma and to identify the factors leading to ocular trauma.

METHODS

In this descriptive and prospective study, all the patients with ocular trauma visiting eye Out Patient Department (OPD) and Emergency Department of Gandaki Medical College Teaching Hospital from June 2015 to June 2016 were included. Detailed histories of all 226 patients were taken including demographic data, mode of injury, type as well as object of injury. The time of injury and the time of presentation as well as the distance travelled to reach hospital was noted.

Mechanism of injury was categorized as mechanical, chemical or thermal. Mechanical injury was further classified according to 'Ocular Trauma Classification Group' by Kuhn and associates into closed globe and open-globe injury[8]. Closed-globe injury was divided into contusion injury and superficial injury. Complete ophthalmologic evaluation was done including presenting visual acuity, measured with internally illuminated Snellen's chart. Detailed anterior segment evaluation was done with slit lamp bimicroscopy, which included an examination of the lids and adnexae (lacerations, abrasions and ecchymosis), conjunctiva (tear, hemorrhages, abrasions), cornea (foreign body, abrasion, laceration) anterior chamber (depth, reaction, hyphaemia), iris, pupil (size, shape, light reaction), lens (clarity, position), vitreous (hemorrhage, detachment, reaction) and fundus (hemorrhages, detachment). The posterior segment evaluation was done with direct or indirect ophthalmoscopes (Heine and Volk 78D/90 D Aspheric Lens). Intraocular pressure of both eyes was measured by applanation or by schiotz tonometer (whenever possible and whenever required). Relevant investigations like USG, X-ray orbit/skull, CT scan and MRI were done whenever indicated. All the patients were examined by the ophthalmologist and appropriate intervention was taken (Medical, surgical). Data was analyzed using a SPSS program.

RESULTS

Over a period of one year, 226 patients attended to the OPD and Emergency Department of Gandaki Medical College Teaching Hospital. Mean age of patients was 30.41 ±15.7 years. Males were more prone to trauma accounting for 70.8% than 29.2% females.

Regarding laterality of the eye involved, 48.2% patients had right eye involvement, 34.4% had left eye involvement and 17.4% had both eye involvements.

82.5% patients had visual acuity better than 6/18 while 17.7% had visual acuity <3/60.

Majority of the patients were in age group 21 - 30 years. Table 1 shows age distribution of patients.

Table 1: Age distribution of patients

Age of patients in years	Frequency	Percent (%)
0 - 10	21	9.2%
11 - 20	38	16.7%
21 - 30	68	30.1%
31 - 40	40	17.9%
41 - 50	36	15.9%
51 - 60	11	4.7%
≥60	12	5.5%
Total	226	100%

Road traffic accident (RTA) was the most common cause followed by foreign bodies. Table 2 shows different causes of injuries.

Table 2: Distribution of different causes of ocular trauma

Cause	Frequency	Percentage (%)
RTA	63	27.9%
Physical assault	26	11.5%
Foreign body	46	20.4%
CP hair	5	2.2%
Chemical injury	5	2.2%
Vegetative material	14	6.2%
Welding arc	18	8%
Fall injury	8	3.5%
Thunder injury	4	1.8%
Animal bite	3	1.3%
Insect bite	11	4.9%
Miscellaneous	23	10.2%

This study showed 61% of ocular injury occurred in workplace. In this study it was observed that out of 226 patients, 15 patients gave history of wearing protective devices while working specially while welding. About 82% of our patients didn't use any medication, 17% used herbal preparation, 0.5% used antibiotics eye drop and 0.5% used unknown medication.

Common ocular findings were corneal foreign bodies, eyelid edema and ecchymosis, subconjunctival hemorrhage. Summary of ocular findings are given in Table 3.

Table 3: Summary of ocular findings

	Frequency	Percentage
Lids		
Lids laceration	22	9.7%
Oedema and ecchymosis	32	14.2%
Conjunctiva		
Subconjunctival hemorrhage	28	12.4%
Laceration	4	1.8%
Foreign body	22	9.7%
Hyperemia	7	3.1%
Cornea		
Foreign body	48	21.2%
Corneal abrasion	14	6.2%
Corneal ulcer	2	0.9%
Laceration	6	2.7%
Uvea		
Uveitis	5	2.2%
Hyphema	2	0.9%
Iridodialysis and sphincter tear	5	2.2%
Iris prolapse	2	0.9%
Lens		
Cataract	3	1.3%
Vitreous		
Hemorrhage	3	1.3%
Retina and optic nerve	5	2.2%
Orbit injuries	7	3.1%
Globe rupture	4	1.8%
Chemical injuries	3	1.3%
Thermal injuries	2	0.9%
Total	226	100%

DISCUSSION

Ocular trauma is a major cause of monocular blindness and visual impairment throughout the world[8]. The age group most vulnerable to trauma was 21 - 30 years which was consistent with other studies[9,10]. Similar result showing 21 - 30 years of age as most common age group was seen in the study done in Dhulikhel[11].

This comprises the economically productive age group and ocular injury in this age group results in great economic loss. Increased incidence of ocular injuries among young can be explained by their frequent social activity. Decreased visual acuity in elderly population could be due to poor vision as a result of various ocular conditions like cataract, glaucoma, age related macular degeneration and previous ocular surgeries[12]. Also 16.7% of the patients were in age group 11 - 20. This can be explained by the fact that young patients are more involved in occupations, sports and a risky and adventure seeking behavior[13].

In this study, 70.8% of the patients were males and 29.2% were females. This may be due to the difference in exposure risk between males and females due to different social behavior. This again can be attributed to the increased outdoor, occupational and sports, risky adventure seeking behavior and recreational activities with higher risk of injuries in men. Also this could be because of more attention being paid for the health of males. Other studies also showed higher incidence of eye injuries in males than in females[9-11,14-16].

This study shows that closed globe injuries occurred more frequently than the open globe injuries. Other studies also have reported closed globe injuries more frequently than open ones[9,11,14,17].

In our study, the most common ocular injury documented was corneal foreign body followed by eye lid edema and ecchymosis, subconjunctival hemorrhage, conjunctival foreign body, eye lids laceration, corneal abrasion. Corneal abrasion was seen in 6.2% of patients in our studies. While studies conducted by Upadhyaya M *et al* in Bhaktapur[7] and Oum BS *et al* in South Korea[17], corneal abrasion has been observed to be the most common finding.

Corneal foreign body was the most common findings in our study. Iron particles accidently falling on eye during welding was one of the most common causes of corneal foreign body. GMCTH is located at the centre of Pokhara city so these patients had easy access to GMCTH. Other causes of cornea foreign body were dust particles, insect wings.

We observed that major cause of ocular trauma was RTA followed by foreign body, physical assault. Similar result was seen in the study done in Manipal[9].

This study showed 61% of ocular injury occurred in workplace. Work place injuries were the commonest cause of injury, similar to studies from India and other countries[18-20].

In this study it was observed that out of 226 patients, 15 patients gave history of wearing protective devices while working specially while welding. It is believed that with education about use of proper eye protection, 90% of eye injuries could be prevented[12].

About 82% of our patients didn't use any medication, 17% used herbal preparation, 0.5% used antibiotics eye drop and 0.5% used unknown medication. This could be due to ignorance, lack of transportation facilities and lack of eye service facilities lots of patients are first seen by medical shop keepers, family members and traditional healers before coming to the hospital.

CONCLUSIONS

Males are more prone to ocular trauma than females and majority of ocular trauma occurred at workplace. RTA is the commonest cause of ocular trauma. Very few patients used protective device. The planning of treatment and development of strategies should be done according to these findings to prevent ocular blindness due to trauma. Public awareness and strict legislation for the use of personal protective devices can also help reduce the occurrence of ocular injury.

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| Original Article

A Hospital Based Study of 2015 Earthquake Injured Patients Attending the Medical College Hospital in Western Region of Nepal

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ABSTRACT

Introduction: On April, 2015, at 11:56 Nepal Standard Time (06:11:26 UTC), a catastrophic earthquake with a magnitude of 7.8 - 8.1 on Richter scale and lasted approximately fifty seconds with Mercalli intensity of IX (Violent) hit the North West of Kathmandu, Nepal. Its epicenter was East of Gorkha District at Barpak, Gorkha, and its hypocenter was at a depth of approximately 8.2 km. It was the worst natural disaster to strike Nepal since the 1934 earthquake. The earthquake caused nearly 9,000 individuals death, injured 22,000 people and 3.5 million people were homeless.

Objectives: The main objective of this investigation was to study the demography profile, morbidity pattern, duration of hospital stay and mortality incidence among the 2015 earthquake injured patients visiting Emergency Department of Gandaki Medical College Teaching Hospital.

Methods: A retrospective analysis of the earthquake injured patients attending through the Emergency Department. All the earthquake injured patients attending the Emergency Department from 25th April to 24th May, 2015 were enrolled in our study. The collected data has been complied and analyzed using Statistical Package for the Social Science software package 16 version.

Results: Hundred and seventy patients were triage and 63% were females. Majority (44.2%) of patients were of age group of 15 - 34 years. Eighty one percent of patients were from Gorkha district, the epicenter site of the earthquake. The three most common diagnoses were trauma and orthopedic injuries (52.4%), mental health issues and psychological problems (21.2%) and reproductive health issues (16%). Among the 83% of patients who had been hospitalized, almost 34% were discharged within one week. Mortality rate was 1.2%.

Conclusions: Since 1993, earthquakes of more than or equal to 5.0 on the Richter scale have occurred in Nepal every year and this makes Nepal 11th most vulnerable country in world. Therefore, every hospital should have well functioning Earthquake Disaster Management Plan to handle this high intensity emergency situation in our country.

Keywords
Disaster, Earthquake, Hospital, Morbidity, Nepal.

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INTRODUCTION

Earthquake is unpredictable and devastating natural disaster. Since 1993, earthquakes of more than or equal to 5.0 on the Richter scale have occurred every year in Nepal. In 2012, almost 95 earthquakes were reported in Nepal. According to the United Nations, Nepal is the 11th most vulnerable country to earthquakes, and Kathmandu the most at-risk city[1]. Natural Calamity Relief Act, 1982 has formed a Central Natural Disaster Committee chaired by Prime Minister along with Relief and Treatment Subcommittee chaired by Health Minister has formulated number of policies and guidelines for the management of health emergencies and to control the epidemics and outbreaks[2].

On 25 April, 2015 at 11:56 Nepal Standard Time (06:11:26 UTC), a catastrophic earthquake with a magnitude of 7.8 - 8.1 on Richter scale and lasted 50 seconds with Mercalli Intensity of IX (Violent) hit the North West of Kathmandu, Nepal. Its epicenter was East of Gorkha District at Barpak, Gorkha. It is also known as Gorkha earthquake affected 30 out of 75 districts in Western and Central Regions, including Kathmandu Valley districts. This was the worst natural disaster that killed nearly 9,000 individuals, injured 22,000 people and 3.5 million people were homeless[3,4]. Centuries-old buildings were destroyed at UNESCO world heritage sites in the Kathmandu valley, including some at the Kathmandu Durbar Square, the Patan Durbar Square, the Bhaktapur Durbar Square, the Changu Narayan Temple, the Boudhanath Stupa and the Swayambhunath Stupa. The earthquake triggered an avalanche on Mount Everest and in the Langtang valley, where over 300 people were reported missing[5].

Natural disaster may occur with or without a warning. A devastating and unpredictable event such as an earthquake becomes alarmingly insurmountable in a resource constrained geographically challenging scenario such as ours in Nepal. Earthquake is a sudden rapid shaking of the earth. Based on a study published in 2014, of the main frontal thrust, on average a great earthquake occurs every 750 ±140 and 870 ±350 years in the East Nepal region[6]. A study from 2015 found a 700 year delay between earthquakes in the region. The study also suggests that because of tectonic stress buildup, the earthquake from 1934 in Nepal and the 2015 quake are connected, following a historic earthquake pattern. A 2016 study on historical great (M ≥8) earthquake pairs and cycles found that associated great earthquakes are likely to occur in West China region through the 2020[7,8].

The rationale and objective of the paper is to study the demography profile, injury epidemiology after earthquakes by examining injury patterns, treatment, and patient's outcomes based on retrospective analysis of patient's record at the Gandaki Medical College Teaching Hospital, as studies relating to earthquakes related epidemiology are very limited in our country and this is the first disaster related study of our hospital.

METHODS

This was a retrospective study of earthquake related injured patients which occurred on 25th April 2015 in Nepal. The injured patients attending the Emergency Department of Gandaki Medical College Teaching Hospital from 25th April to 24th May, 2015 were enrolled in our study. Approval of this study was obtained from Institutional Ethics Committee and informed consent was taken from the patients or their family members.

The inclusion criteria included the earthquake injured patients visiting the Emergency Department of Gandaki Medical College Teaching Hospital. The collected data was reviewed verified and statistically analyzed using the Statistics Packed for Social Science (SPSS) version 16 and Microsoft Excel 2007. Descriptive statistics was used for all studied variables.

RESULTS

One hundred and seventy patients were included in the study. Injured patients aged ranged from four days neonates to 89 years. There were 107 females and 63 males. Maximum number of patients (44.2%) belonged in the age group category of 15 - 34 years (Fig 1). We had patients from 13 different districts of Western Development Region. Eighty one percent of patients were from Gorkha district, the epicenter site of the earthquake followed by Kaski (7.1%). One International female from France Nationality had undergone treatment at our Hospital. Most common diagnosis in our study (Fig 2) were trauma and orthopedic injuries in 89 (52.4%), mental health issues and psychological problems were present in 36 (21.2%), reproductive health problems were present in 27 (16%) of them 14 patients were pregnant and one of them had normal delivery at our Hospital, Infectious diseases 11 (6.5%) and non communicable diseases seven (4.1%).

Fig 1: Age and gender distribution of earthquake injured patients

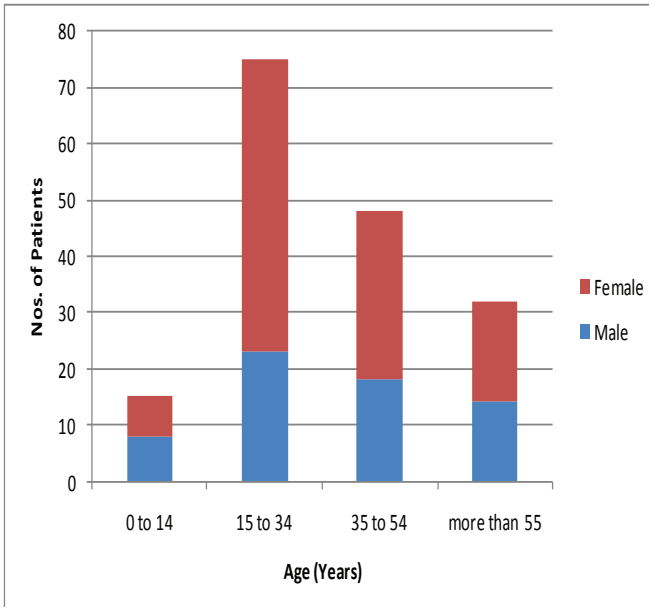


Fig 2: List of diagnosis of the earthquake injured patients

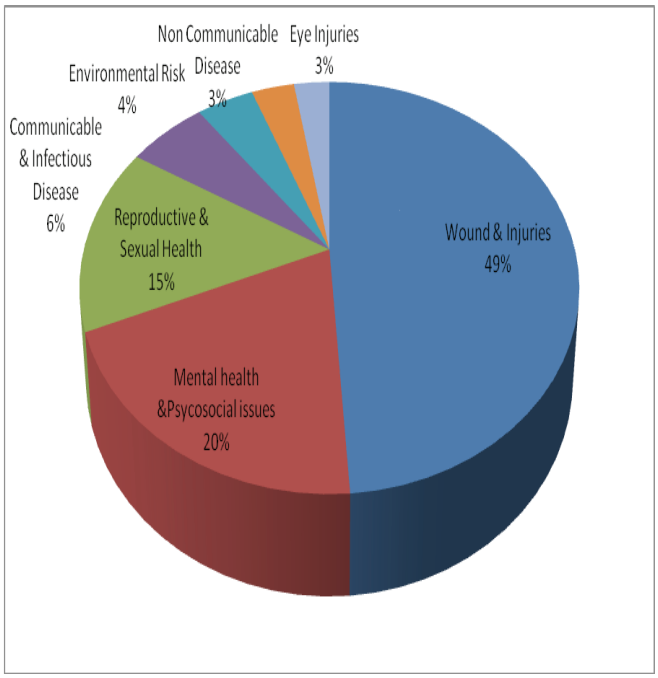
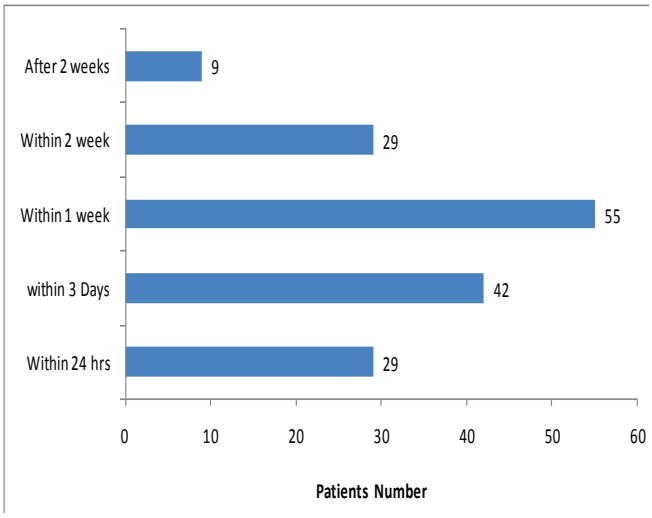


Figure 3 shows the duration of hospital stays of the earthquake injured patients at Gandaki Medical College Teaching Hospital. Almost 34% of patients were discharged within one week followed by 25.6% within three days. Six percent of patients stayed for more than two weeks duration at our hospital. Four of our patients were referred to Kathmandu on family request and two mortalities had occurred while undergoing treatment at our Hospital.

Fig : 3 : Duration of hospital stay



DISCUSSION

Every major disaster warrants retrospective studies so that we can learn how to improve all levels of emergency medical care. Lack of precise data from immediate aftermath is seen as a remarkable weak point in disaster epidemiology[9,10]. Management of earthquake aftermath is a complex and challenging. The causality rates of earthquake is one to eight percent, however the number of causality dependents on magnitude, proximity to populated areas, geographic topology, time and duration of the earthquake, degree of disaster preparedness implemented at the earthquake affected area. In 2005, a hypothetical scenario earthquake near Kathmandu for 8.1 Richter scale had expected numbers of fatalities between 21,000 and 42,000[11].

The 2015 April/May earthquake killed more than 8,800 people and injured nearly three times as many and almost 3.5 million people were left homeless[3]. It killed only about 10,000 people because it was Saturday and the children were not in the collapsing school buildings. This saved the lives of about 10,000 children. The original estimate was correct within a factor of 2.5 and would have been exactly correct, had it not been for the lucky break children got due to Saturday being a holiday[12].

The study showed female to male ratio of 1.7:1, involving almost 63% of females. The age group of our patients varied from four days neonates to 89 years old individuals. There were total of four infants below one year of age. Highest number ((44.2%) of individuals belonged within the category of 15 - 35 years age group. This age group is the most commonly involved population during

earthquake would be more likely been active population in the community. Our findings were similar with two studies done from Gujarat reported more female patients than males[13]. In our study we had 15 pregnant mothers and one had delivered at our Hospital after admission in the maternity ward.

Our findings showed trauma and orthopedic injuries of 52.4% were in similar with other earthquake studies reporting higher incidence of orthopedics injuries particularly extremities fractures[14,15,16]. Mental health issues and psychological problems (21.2%) were second in the list with reproductive health problems (16%). The findings were similar with Tanaka *et al*[17] done in Hanshin-Awaji earthquake study of 1995. The noticed crush injuries and trauma, respiratory diseases, cardiovascular diseases, obstetrics complications most commonly.

The average duration of hospital stay in our study patients was less than one week, although patient's hospital stay duration varied from within 24 hours to 2 months. About 34% of patients had been discharged within less than one week period. Within 24 hours period 29 (17.7%) patients was discharged. Duration of hospital stay after the disaster is an important variable particularly to measure the humanitarian response strategies within the countries.

CONCLUSIONS

Our country Nepal lies in the earthquake prone region within the Southern limit of the diffuse collisional boundary where the Indian Plate under thrusts the Eurasian Plate, occupying the central sector of the Himalayan region. Earthquake and other disasters can have a serious impact in the developing countries like ours. Hospitals need to develop, practice and continuously update an effective disaster/ emergency medical response plan and also perform a mock drill at least once every year. Therefore it is recommended for every Institute to be prepared for immediate response and mobilize the hospital personnel in effective way to meet the needs of the affected populations at the time of an earthquake and other disasters. Lastly we would like to recommend for earthquake response training and capacity building activities for our health workers throughout the country to specialize their skills in management of large numbers of victims with a spectrum life threatening injuries emergently.

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Conflict of Interest

The authors declare that they have no competing interests and have not received any funding or benefits to conduct this study.

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Analysis of Risk Factors for Incisional Hernias and its Management

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Keywords
Incisional hernia, Lower abdominal midline incision, Mesh repair.

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ABSTRACT

Introduction: Incisional hernia is a common problem after abdominal surgery. Patients present with pain, swelling and intestinal obstruction. It may be repaired by either anatomical suturing or mesh repair.

Methods: It is a prospective observational study conducted in Western Regional Hospital and Fewa City Hospital, Pokhara from 2013 to 2016. A total of consecutive 100 patients admitted in these hospitals during the study period were included.

Results: Incisional hernia is more common in females (M : F = 1 : 3.8), and in 30 - 50 years age group (60%). Major risk factors were wound infection (30%), overweight (25%), and postoperative cough (10%). It is found to be more associated with gynecological (65%), than gastro-intestinal operations, and more so with lower abdominal midline incision (65%). It is found to occur mostly within one year (60%) of primary surgery than later. Even 24% of the patients had first symptom within six months. Mesh repair (92%) was the preferred standard surgical treatment for incisional hernia.

Conclusion: Overweight females of age range between 30 - 50 years with history of gynecological operations by lower abdominal midline incision are more prone to develop incisional hernia. This incidence increases when there is wound infection. Mesh repair is the choice of operation for incisional hernia.

INTRODUCTION

Incisional hernia is a common presentation in general surgery. It account for 15 to 20% of all abdominal hernias. They develop in two to 11 percent of patients undergoing laparotomy. The figure rises to 26% in those who develop wound infection[1]. In 80 to 95% of the patients they develop within six months to three years after initial surgery[2]. Hernias that develop within three years of operation are more likely to be troublesome and larger in size than those that develop later[3].

Approximately 200,000 incisional hernia repairs are performed each year in the United States and it is estimated that another 200,000 incisional hernias may be going unrecognized or untreated. Incisional hernias occur mostly with midline and transverse incision. It also occurs with paramedian, subcostal, McBurney, Pfannenstiel, flank incisions, and laparoscopic port sites. An incisional hernia occurs early in the healing process due to biochemical failure of the acute fascial wound when wound tensile strength is very low or absent[4].

During this time, the wound strength depends entirely on suture integrity. So when patients start returning to the increased levels of activity and place loads across their acute wounds, the wound gives away. However, the hernia may not be obvious for days or even years.

According to Jack Abrahmson, many factors singly or in combination may cause failure of the wound to heal. The factors responsible for the failure include obesity, wound infection, closure of fascia with catgut, drainage tube through the index incision, senility, early wound dehiscence, immunosuppressant therapy, anemia, diabetes mellitus, malnutrition, jaundice and azotemia. Suture length and technique also play a role. Repair of abdominal incisional hernia is difficult with recurrence. Recurrence rates of up to 33% after first repair and 58% after second repair have been reported[5] with anatomical repair. Meshes have reduced the recurrence rate to 1 –10%.

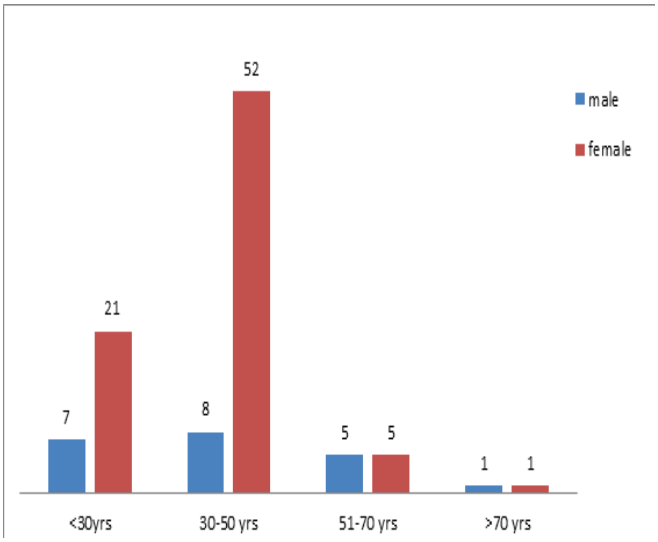
The aim of this study is to determine the common risk factors for incisional hernias such as age and sex of the patient, predisposing factors like overweight, previous wound infection, chronic urinary retention, diabetes mellitus, postoperative cough, COPD, type of previous surgery, type of previous incision, duration from previous surgery. This study will also determine the common surgery for incisional hernia.

METHODS

It was a prospective, observational study conducted in Western Regional Hospital and Fewa City Hospital, Pokhara from 2013 to 2016. A total of one hundred consecutive patients of incisional hernia admitted in those hospitals during the study period were included in the study. After detailed history and a thorough clinical examination, information of the patients was collected in various aspects like age, sex, mode of presentation, previous operation (indication of surgery, incision site, and post-operative wound infection). Patients were also evaluated for other risk factors like obesity, diabetes mellitus and malignant diseases. Routine laboratory investigations of blood, and urine, chest X-ray, and ECG were done. After complete evaluation they were operated either by anatomical closure or with mesh repair. Data were collected on a structured proforma. Statistical Analysis was done using Microsoft Excel software. Result obtained from the study was discussed with reference to current world literature.

RESULTS

Fig 1: Age and sex distribution of patients with incisional hernia



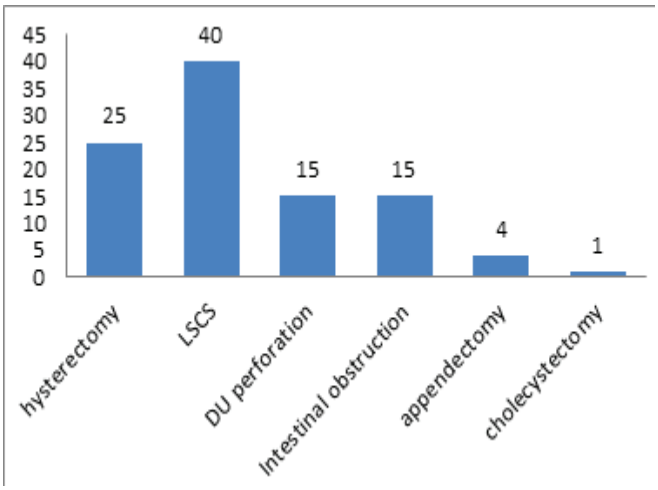
Among one hundred patients enrolled in the study, 21 were males and 79 were females in the ratio of 1 : 3.8. Most of the patients (60%) were in the age group of 30 - 50 years. The youngest patient was 21 years old and the oldest was 81 years of age.

Table 1: Predisposing factors in patients with incisional hernias

Predisposing factors	Number
Overweight BMI >25	25
Previous wound infection	30
Chronic urinary retention	3
Diabetes mellitus	5
Postoperative cough	10
Chronic obstructive pulmonary disease	2
No associated factors	25

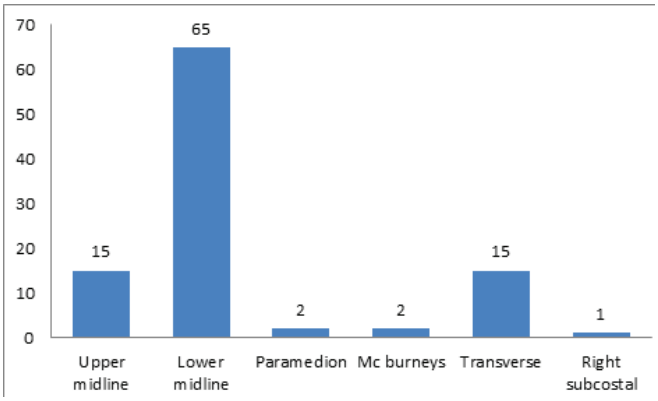
In our study, 30% of the patients had previous surgical wound infection, 25% were overweight, 10% had postoperative cough, 5% were diabetic, 3% had chronic urinary retention, and 2% had chronic obstructive pulmonary disease. But 25% of the patients had developed incisional hernia in spite of having not any associated predisposing factors.

Fig 2: Type of previous surgery



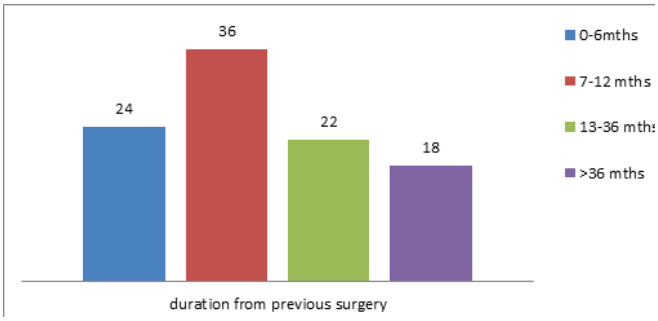
This study showed that 65% of patients with incisional hernia had undergone gynecological procedures. Among which LSCS was the most common (40%), followed by abdominal hysterectomy (25%). Among other surgical procedures followed were laparatomy for duodenal ulcer perforation (15%), for intestinal obstruction (15%), appendectomy (4%) and open cholecystectomy (1%).

Fig 3: Type of previous incision



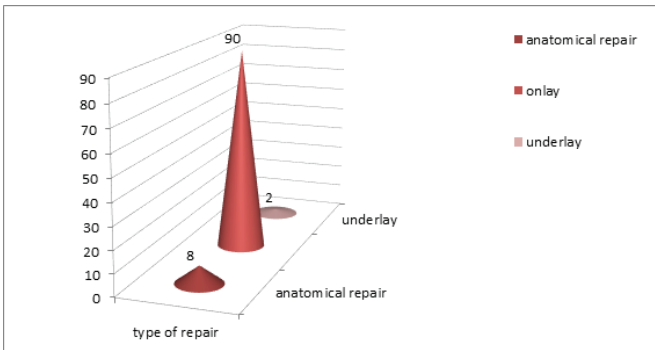
In this study incisional hernias were more common in patients with lower midline incision (65%), followed by upper midline (15%), transverse (15%), McBurney's (2%), paramedian (2%), and right subcostal (1%).

Fig 4: Duration from previous surgery



It was found that 24% of the patients had developed incisional hernia within six months, 36% of the patients had developed hernia from seven to 12 months, 22% developed in 13 to 36 months and the rest 18% after 36 months of primary surgery.

Fig 5: Therapeutic modality



In our study most of the operated incisional hernias (92%) were repaired by placing a mesh. Among them 90% had onlay placement of mesh and two had underlay mesh. Only 80% of the incisional hernias were repaired anatomically. No recurrence of incisional hernia was found after repair.

DISCUSSION

Incisional hernia is defined as any abdominal wall gap with or without bulge in the area of a postoperative scar perceptible or palpable by clinical examination or by imaging. Any condition which inhibits natural wound healing leads to the development of an incisional hernia. Those conditions are previous wound infection, wound dehiscence, poor surgical technique, obesity, smoking, diabetes mellitus, immunosuppressive conditions, excessive wound tension, malnutrition, connective tissue disorders, chronic obstructive pulmonary disease, and other co-morbidities. Emergency surgery increases the risk of incisional hernia.

In our study the incidence of incisional hernia was more in 30 to 50 years age group. It was common in females (M : F = 1 : 3.8). This is because of laxity of abdominal muscles due to multiple pregnancies and increased incidence of obesity in females. A retrospective study by Hoer J *et al* found that age around 45 years, female gender, BMI >25, and previous laparotomies played an important role in the development of incisional hernia[6]. Similarly another retrospective study by Bhat M *et al* showed that incisional hernia was common in females in the fifth decade and gynecological operation with lower midline incision was

the most common predisposing factors for development of incisional hernia[7]. These studies were comparable with our study.

In our study major predisposing factors were previous wound infection (30%), overweight (25%), postoperative cough (10%), diabetes mellitus (5%), COPD (2%) and chronic urinary retention (3%). These results were comparable with those of Bose *et al*[8] in which the predisposing factors were wound infection (53.63%), obesity (30%), COPD (20.90%) and urethral stricture (9%). Similarly a prospective study by Agbakwuru EA *et al* found that midline incisions, wound sepsis, and overweight are the major risk factors[4]. Colombo *et al* also reported that the incisional hernia occurring within three years after midline abdominal incision for gynecologic diseases was associated with obesity and anesthesiological risk factors[9].

Incisional hernia in diabetic patients occurs due to altered regulation of collagen metabolism at the level of the fascial scar. When diabetes was optimally controlled, wound strength and extensibility were similar in diabetic and non-diabetic[10]. So the association between incisional hernia and diabetes might be explained by the suboptimal glycemic control often found in elderly diabetic subjects and not by the disease per se.

Wound dehiscence is defined as failure of the incision to heal and maintain a normal abdominal wall anatomy. It presents with serosanguinous discharge from the wound in the first week of surgery. The severity ranges from superficial breakdown of the skin to a complete failure and an exposure of the viscera. Incisional hernia is chronic wound failure[11]. A study carried out in Egypt concluded that incisional hernia is a complication of wound healing after surgery which can be prevented by good care and precaution[12].

In our study incisional hernia was common in patients with previous lower abdominal midline incision like in LSCS (40%), hysterectomy (25%), intestinal obstruction (15%). Lower abdominal incisions have a higher incidence of development of incisional hernia due to absence of posterior rectus sheath below arcuate line, increased intra-abdominal hydrostatic pressure in erect position and laxity of abdominal muscles in females. This study was comparable with the study by Parekh JN, Shah DB, Thakore *et al*[13]. A randomized trial comparing vertical and transverse incisions for abdominal aortic aneurysm repair found that incisional hernia was more likely to occur with vertical laparotomy (20 out of 22 patients with vertical incisions versus six out of 15 patients with transverse incision laparotomy)[14]. Incisional hernias have been described following paramedian, subcostal, McBurney, Pfannenstiel, and flank incisions. Incisional hernias are also reported in laparoscopic port sites due to weakness in the fascia.

Our study result of 60% of incisional hernias occurring within one year of primary surgery is comparable with Akmans series with 65% of hernia occurring within a year. In our study, 24% of cases had developed incisional hernia within six months, 36% cases had it in seven to 12 months, 22% from 13 to 36 months and 18% after 36 months of previous surgery.

Most of the incisional hernias should be repaired in fear of more sinister complications. Upper abdominal hernias, hernias less than one cm in diameter, and hernias larger than seven to eight cm are less likely to incarcerate. Surgery should be considered when it is symptomatic, has potential for incarceration, and when the hernia size is sufficient enough to hamper activities of daily living.

There are two basic types of repair: anatomical repair and mesh repair. Mesh repairs are preferable for incisional hernias. In a retrospective study from Europe, the incidence of recurrence of incisional hernias after simple sutured repair was over 60%, and the use of mesh decreased the recurrence rate to approximately 17%[15]. Continued smoking and occupational straining may be the risk factors for the recurrence of mesh repair in ventral abdominal hernia[16]. Mesh repair is important for incisional hernia with a diameter greater than four cm as the risk of recurrence is related to the tension placed on the repair in large hernias. Suture repairs have certain advantages like nidus for infection is less; less chance of adhesion to bowel and ability to close some very small hernias without tension.

Mesh repairs minimize the amount of tension that must be put on the abdominal wall in order to cover the hernia. The mesh can be placed above the fascia (onlay), below the fascia (sublay), or simply bridge the gap of the defect by suturing the mesh to the fascial edges and underlay. Laparoscopic repair of incisional hernia is a relatively new procedure. Laparoscopic repair most closely mirrors the sublay technique of open repair. A large piece of mesh with a minimum of four to five cm overlap over the hernia is secured with transfascial sutures and intra-abdominally placed tacks.

SUMMARY

Incisional hernia is common in females of age range 30 to 50 years with history of gynecological operation by lower abdominal midline incision like in LSCS and abdominal hysterectomy. Previous wound infection, overweight and post operative cough are important predisposing factors. It usually occurs within the first year of previous surgery. Mesh repair is the preferred choice of repair.

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Cutaneous Adverse Drug reactions: A Four-Year Study from Western Nepal

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Keywords
Antibacterial agents, Cefixime, Exanthema, Stevens-Johnson syndrome

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ABSTRACT

Background: Cutaneous adverse drug reactions (CADRs) range from minor reactions to several life threatening complications.

Objectives: To study the clinical spectrum of cutaneous adverse drug reactions, determine the causative drugs responsible for the reactions and to assess the preventability.

Methods: The study was carried out in the Department of Dematology of Gandaki Medical College Teaching Hospital from June 2011 to June 2015. All the patients attending the Dermatology Outpatient Department and the patients admitted in the wards with suspected cutaneous adverse drug reactions to systemic drugs were included in the study. A detailed clinical history, including the history of drug intake was noted. Each case was assessed for its causality by using the WHO definitions. Data analysis was done using SPSS software.

Results: There were 102 patients in total. The mean age of the patient was 32 ±15.7 years. Maximum patients belonged to the 21 to 30 years age group. There were 59 female patients and 43 male patients. Severe type of cutaneous adverse drug reactions was noted in 7.8% of patients. Antibiotics were responsible for most of the cutaneous adverse drug reactions. Cefixime was the most commonly incriminated drug. Exanthematous drug reaction was the most common type seen in 45%. Stevens-Johnson syndrome was the commonest type noted among the serious adverse drug reactions. Drug preventability was noted in 6% of patients.

Conclusions: The commonest type of CADR noted was exanthematous type. Antibiotics were the commonest drug group involved in CADR. Six percent of CADR were preventable.

INTRODUCTION

Drug reactions are the unwanted effects on the body exerted by the drugs, which are not the characteristic of the desired pharmacodynamic effects[1]. Cutaneous adverse drug reactions (CADRs) range from minor reactions to several life threatening complications. They are often under reported because of their resemblance with viral exanthemas, collagen vascular diseases and neoplastic processes. A high index of suspicion is required for the prompt diagnosis so that the culprit drug is withdrawn as early as possible and the treatment initiated early to prevent a grave outcome. The list of drugs causing a particular type of reaction is ever expanding due to the introduction of newer drugs for the treatment of diseases.

This prospective study was conducted to study the clinical spectrum of cutaneous adverse drug reactions, determine the causative drugs responsible for the reactions and to assess the preventability.

METHODS

It was a prospective descriptive study carried out over a period of four years from June 2011 to June 2015 in the Department of Dermatology of Gandaki Medical College Teaching Hospital, Pokhara, Nepal. All the patients attending the Dermatology Outpatient Department and the patients admitted in the wards with suspected cutaneous adverse drug reactions to systemic drugs were included in the study. Prior approval was obtained from the Institutional review committee. Informed consent was obtained from each patient in our study. Cutaneous adverse drug reactions caused by the use of topical medications were excluded from the study. Data collection was done in a preset proforma that included the demographic details of the patients and a detailed clinical history. A detailed history of drug intake, reaction time, previous history of drug reaction, duration of reaction, type of cutaneous reaction, and improvement after the dechallenge were noted. Relevant investigations were done to rule out any infectious etiology. The reaction was considered as preventable if a previous exposure to the suspected causative drug(s) or another drug of the same family had already caused an adverse skin eruption.

Each case was assessed for its causality by using the WHO definitions and was categorized as 'certain', 'probable', 'possible', 'unlikely', 'conditional/ unclassified' and 'unassessable /unclassifiable'[2]. Only the 'certain' and 'probable' cases were included in the study. Data analysis was done using SPSS software.

RESULTS

According to the census, there were altogether 31,396 patients in the Department of Dermatology in four years period, including the outpatients and inpatients. Among them, 102 patients were diagnosed with cutaneous adverse drug reactions. So, the incidence of cutaneous drug reaction was 0.32%. The mean age of the patient was 32 ±15.7 years. Age of the patients ranged from one to 77 years. Maximum patients belonged to the 21 to 30 years age group (Table 1). There were 59 (58%) female patients and 43 (42%) male patients. Eight patients (7.8%) had severe type of cutaneous adverse drug reactions and the remaining had milder CADR.

Table 1: Age distribution of patients

Age group (Years)	Frequency	Percentage
Up to 10	6	5.9%
11 - 20	17	16.7%
21 - 30	32	31.4%
31 - 40	25	24.5%
41 - 50	9	8.8%
51 - 60	6	5.9%
61 - 70	5	4.9%
71 - 80	2	2.0%
Total	102	100.0%

Table 2: Clinical pattern of cutaneous adverse drug reactions

Type of drug reaction	Frequency	Percentage
Exanthematous	46	45.1%
Fixed drug eruption	17	16.7%
Photosensitivity	8	7.8%
Urticaria	7	6.9%
Steven-Johnson syndrome	5	4.9%
Hair loss	4	3.9%
Pityriasis rosea	2	2.0%
Pigmentation	2	2.0%
Pruritus	2	2.0%
AGEP	1	1.0%
Purpura	1	1.0%
Acneiform	1	1.0%
Urticarial vasculitis	1	1.0%
Psoriasiform	1	1.0%
Lichenoid	1	1.0%
Erythroderma	1	1.0%
Toxic epidermal necrolysis	1	1.0%
DRESS	1	1.0%
Total	102	100%

AGEP: Acute generalized exanthematous pustulosis

Table 3: Drugs causing cutaneous adverse drug reactions

	Females	Males	Total	Per-centage
Antibiotic	25	22	47	46.07%
NSAIDs	8	10	18	17.64%
Anticonvulsant	11	5	16	15.68%
DMARD	4		4	3.92%
Antidepressant	1	2	3	2.94%
Diuretic	2		2	1.96%
ATT		2	2	1.96%
Antipsychotic	1		1	0.98%
Antigout (Allopurinol)	1		1	0.98%
Mood stabilizer	1		1	0.98%
ATT	1		1	0.98%
Ayurvedic	1		1	0.98%
Proton pump inhibitor	1		1	0.98%
Urso deoxycholic Acid	1		1	0.98%
Anticoagulant	1		1	0.98%
Oral hypoglycemic agent		1	1	0.98%
Antifungal		1	1	0.98%
Total	59	43	102	100%

ATT: Anti tubercular treatment DMARD: Disease-modifying antirheumatic drug

The most common group of drugs involved in CADR was antibiotics. Among antibiotics, maximum frequency of drug reaction was seen with β lactam antibiotics, penicillin (36%) and cephalosporin (32%). Amoxycillin was the commonest penicillin and cefixime was the commonest cephalosporin causing CADRs. Overall, cefixime caused most of the CADRs. Other antibiotics were ciprofloxacin, levofloxacin, cotrimoxazole, doxycycline, metronidazole and ornidazole. Non steroidal anti-inflammatory drug (NSAID) was the second most common category of drug involved. Among the NSAIDs, the most of the drug reactions were noted with nimesulide, followed by diclofenac. The third common group of drug involved was anticonvulsant and the commonest anticonvulsant involved was phenytoin. Disease-modifying anti-rheumatic drugs (DMARDs) contributed to around 4% of CADR (Table 3).

The three most common types of drug reactions were exanthematous type (45%), fixed drug eruption (17%) and drug induced photosensitivity (8%). Other types of CADR were urticarial, Stevens-Johnson syndrome (SJS), hair loss, pitytriasis rosea, pruritus, pigmentation, purpura, acute generalized exanthematous pustulosis, acneiform eruptions, urticarial vasculitis, psoriasiform eruption, lichenoid eruption, erythroderma, toxic epidermal necrolysis (TEN) and DRESS syndrome. Among the severe types of drug reactions, maximum cases were of SJS (Table 2). The most common type of drug reactions observed in paediatric age group (<14 years age group) was exanthematous drug reaction followed by fixed drug eruption. Other types noted in the paediatric age group were urticarial drug eruption, pruritus, erythroderma and SJS (Table 4).

Table 4: Relationship between the drug and cutaneous adverse drug reaction

CADR type	≤ 14 years (n)	> 14 years (n)	Drug category	n
Exanthematous	4	42	Antibiotic	32
			Anticonvulsant	7
			NSAIDS	2
			Antipsychotic	1
			Antigout	1
			Mania	1
			DMARD	1
			ATT	1
Fixed drug eruption	2	15	NSAIDS	9
			Antibiotics	7
			Antidepressant	1
Photosensitivity		8	NSAIDS	5
			Antibiotics	2
			Diuretics	1
Urticaria	1	6	Antibiotics	5
			NSAIDS	2
Steven-Johnson syndrome	1	4	Anticonvulsant	3
			Antibiotic	1
			DMARD	1
Hair loss		4	Anticonvulsant	2
			DMARD	1
			Anticoagulant	1
Pityriasis rosea		2	Proton pump inhibitor	1
			Antifungal	1
Pigmentation		2	Antidepressant	2
Pruritus	1	1	Anticonvulsant	1
			ATT	1
AGEP		1	Diuretics	1

CADR type	≤ 14 years (n)	> 14 years (n)	Drug category	n
Purpura		1	Anticonvulsant	1
Acneiform		1	Anticonvulsant	1
Urticarial vasculitis		1	Ayurvedic	1
Psoriasiform		1	Antidiabetic	1
Lichenoid		1	ATT	1
Erythroderma	1		Anticonvulsant	1
Toxic epidermal necrolysis		1	Ursodeoxycholic acid acUDCA	1
DRESS Syndrome		1	DMARD: Leflunomide	1
Total	10	92		102

Antibiotic was the most common drug to cause exanthematous drug reaction. Other drugs that caused exanthematous reactions were anticonvulsants, NSAIDs, antipsychotic, drugs used in management of gout (Allopurinol), mood stabilizer (Lithium) and DMARD. Antibiotic was also the most common etiology for urticarial drug eruption. Fixed drug eruption was most commonly caused by NSAIDs. Antibiotic was the second most common cause for fixed drug eruption. Photosensitivity was most commonly caused by NSAIDs followed by antibiotics and diuretic (Hydrochlorthiazide). SJS was most frequently caused by anticonvulsants. Other drugs associated with SJS were antibiotics and DMARD. Hair loss was caused by anticonvulsant, DMARD and anticoagulant. Pityriasis rosea was induced by proton pump inhibitor and antifungal. There were two cases of pigmentation and those were caused by antidepressants. There were two cases of pruritus caused by anticonvulsant and antitubercular treatment (ATT), one each. There was one case each of acute generalized exanthematous pustulosis (AGEP), caused by diuretic (amiloride), purpura caused by anticonvulsant, acneiform eruption caused by anticonvulsant, psoriasiform caused by antidiabetic, lichenoid drug reaction induced by ATT, Erythroderma caused by anticonvulsant, TEN caused by Ursodeoxycholic acid , DRESS (Drug rash eosinophilia and systemic symptoms) syndrome caused by DMARD and urticarial vasculitis caused by Ayurvedic drug (Table 4).

DISCUSSION

Drug reactions can be classified into immunologic and nonimmunologic etiologies, the most being caused by predictable, nonimmunologic effects[3]. It is very important to diagnose CADR as early as possible to prevent the morbidity and mortality from the reactions. The serious reactions can be fatal at times. We had followed the WHO causality definitions to categorize the CADR, as it is a very simple and widely accepted method.

The mean age of our patient was 32 ±15.7 years, comparable to the finding of a Malaysian study[4] but younger than that was seen in French[5] and Italian studies[6]. A female preponderance was noticed in our study. Males were more commonly involved in several other studies[5,7-11].

Majority of our patients were in the age group of 21 to 40 years which is comparable with other studies[7,12]. Lesser number of CADRs were observed in pediatric age group (<14 years) in our study (10%), which is similar to the findings of Dimri D *et al*[12] and Gonzalez Martin G *et al*[13]. But some studies have suggested that CADR is more common in younger children and older adults, due to the dysfunctional immune system and inability to metabolize the drugs[14]. Old patients are involved in consumption of a large number of medications with increased incidence of drug reactions. Around seven percent of our patients were of more than 60 years of age.

A wide clinical spectrum of cutaneous adverse drug reactions was observed in our study. Altogether we observed 18 different types of cutaneous adverse drug reactions. Exanthematous type was the commonest type of reaction observed in our study which is in accordance to the finding of several other authors[4,8,12,15-17]. Fixed drug eruption was the second most common drug eruption seen in our study similar to the finding of Patel RM *et al*[18]. Pudukadan D *et al* have shown fixed drug eruption to be the most common type[19].

Antimicrobials was the commonest drug category of drug involved in CADR observed by several authors[8,12,16]. Antibiotic was the most common cause of CADR in our study. This is similar to the finding of other authors[7,9,17]. Cefixime was the most common drug incriminated in CADR in our study. Cotrimoxazole was the most common cause of drug eruptions in other studies[9,18,19]. In our study, antibiotics were associated with both non serious as well as serious CADR like SJS.

NSAIDs induced adverse events most frequently in a study of Kacalak-Rzepka A *et al*[20]. Anticonvulsants were responsible for the majority of CADRs in a study of Botelho LF *et al* in around 24% of cases[21]. Antibiotics were implicated in the majority of exanthematous drug reactions (69%) in our study. Sharma VK *et al* observed that anticonvulsant was the commonest group of drugs implicated in exanthematous reaction[8]. In our study, FDE was most frequently caused by NSAIDs. Sulfonamide was the commonest drug group implicated in FDE in the observation of Sharma VK *et al*[8].

The overall incidence of CADR in our study was 0.32%. This finding was similar to the finding of a French survey[5]. A higher incidence rate was reported in an Indian Study[7]. The reported incidence of CADR in Mid-Western Nepal was 1.6%. Serious CADRs were found in around 9% of our cases. It was 24% in Mid-Western Nepal[9]. Tuchinda P *et al* observed serious CADR in 16.5% patients[17]. A higher incidence of serious CADR has been reported by other authors[5,7]. We observed that SJS was the most common type of serious CADR, similar to the observation of other authors[9,22]. Anticonvulsant was the commonest drug group involved in serious CADR in our study. Anticonvulsants were also responsible for the serious CADR in other studies[8,21].

DRESS syndrome is one of the severe forms of CADR. The drugs incriminated in DRESS syndrome are anticonvulsants, allopurinol, sulphonamides and antibiotics[23]. We had observed one case of DRESS syndrome that was caused by Leflunomide. DRESS syndrome caused by Leflunomide was also reported by Parajuli S *et al*[24]. Dress syndrome was caused by allopurinol followed by carbamazepine in a study by Chiou CC *et al*[25]. Antibiotic was the main culprit drug for DRESS syndrome in a study by Skowron F *et al*[26]. We had observed one case of toxic epidermal necrolysis caused by ursodeoxycholic acid and one case of acute generalized exanthematous pustulosis (AGEP) caused by the diuretic, amiloride.

In our study, six patients (6%) had previous CADR with the drug of similar category, four had recurrent FDE, one had exanthematous drug reaction and one patient got TEN for second time with ursodeoxycholic acid. Hence, drug preventability was noted to be six percent. Drug preventability has been reported in 15% in other study[5].

CONCLUSIONS

The commonest type of CADR noted was exanthematous type. Antibiotic was the commonest drug group involved in CADR. Six percent of CADR were preventable.

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Loop-Mediated Isothermal Amplification Assay for Rapid and Reliable Detection of *Mycobacterium tuberculosis* in Sputum Samples

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ABSTRACT

Introduction: Tuberculosis (TB) remains a major global health problem. The most common method for diagnosing TB in developing countries is sputum smear microscopy; however, the sensitivity of this test is relatively lower. Detection of *Mycobacterium tuberculosis* using conventional culture and biochemical-based assays is time-consuming and laborious. Polymerase Chain Reaction (PCR) is also available for diagnosis of *Mycobacterium tuberculosis*. However, the PCR assay requires an expensive thermal cycler to amplify the DNA fragment in multiple temperature-dependent steps. Therefore, a simple and sensitive method for rapid detection has been anxiously awaited. The loop-mediated isothermal amplification (LAMP) assay is a diagnostic technique which can aid in the fight against TB in resource-poor countries. The LAMP assay can amplify a targeted sequence at a constant temperature. Therefore, a large and costly thermal cycler is not necessary for a LAMP assay.

Objectives: The objective of this study was to identify *Mycobacterium tuberculosis* directly from sputum by LAMP and to compare its efficacy over routinely used methods.

Methods: A total of 106 (53 fluorochrome staining positive and 53 fluorochrome staining negative) sputum samples were collected in this study. Mycobacterial DNA was extracted from concentrated sputum samples by freezing and boiling method. LAMP assay using a set of six specific primers targeting the *M. tuberculosis* 16S rRNA gene with high sensitivity was used to analyze sputum samples. The results were then compared with that of the culture method, which was considered as the gold standard method.

Results: Among total of 106 samples studied by microscopy and culture, 53 were positive by both, whole four were positive by culture but negative by microscopy. With reference to culture, the microscopy had sensitivity 92.98%, specificity 100%, and predictive value of positive

test 100%, predictive value of negative test 92.5%. Out of 106 samples subjected to culture and LAMP for the diagnosis of TB, 55 samples were positive by both tests and two were positive only in culture, while 48 were negative in both tests and one was negative only in culture. While comparing the LAMP with culture as a gold standard, the sensitivity of LAMP was 96.49%, specificity was 97.95%, predictive value of positive test was 98.21%, predictive value of negative test was 96%.

Conclusions: Comparative experiments showed that the LAMP assay is a rapid, sensitive, and specific method to detect *M. tuberculosis* infection. Indeed, an inexpensive LAMP assay would be potential as a diagnostic test for tuberculosis, especially in resource-limited settings.

INTRODUCTION

Tuberculosis (TB) remains a major global health problem. Although the worldwide incidence and prevalence of TB are gradually decreasing, approximately 1.5 million deaths a year are attributed to TB according to the World Bank and the World Health Organization[1]. The major challenge in combating TB is the lack of a rapid, reliable, and inexpensive diagnostic test for detection of *Mycobacterium tuberculosis*.

Early diagnosis is important for the control and prevention of TB. Currently, the most common method for TB diagnosis worldwide is sputum smear microscopy, the sensitivity of which is notoriously poor, particularly in human immunodeficiency virus (HIV) positive patients[2,3]. Culture methods can detect as few as 100 *Mycobacterium* cells per one ml specimen[4] and TB culture is still considered the gold standard for the detection of *Mycobacterium tuberculosis*. However, the culture methods require up to several weeks in sophisticated facilities, such as biosafety level 3, and delays in the diagnosis of TB may harm many patients due to the current prevalence of multidrug resistant TB (MDR-TB)[5]. To address the need for rapid and sensitive diagnosis of TB, a number of nucleic acid amplification assays have been invented [6,7,8]; however, they are still not routinely applied in developing countries due to their high cost, complicated procedures, insufficient laboratory facilities, and a shortage of skilled technologists [9,10,11].

To overcome the limitations of current molecular techniques, a new molecular-biological technique, known as loop mediated isothermal amplification (LAMP), was developed by Notomi *et al*[12]. As the LAMP technique uses strand-displacing DNA polymerases that have high activity under isothermal temperatures ranging from 60 to 68^{0}C, no expensive equipment is required. The LAMP reaction produces stem–loop DNA structures with several inverted repeats and cauliflower- like structures with multiple loops. The products of amplified LAMP reactions can be measured by a change in the turbidity of the reaction mixture because magnesium pyrophosphate accumulates in the reaction mixture as a byproduct of the amplification reaction[13]. The LAMP assay does not require the use of complicated procedures, equipment or machines. Therefore, the LAMP assay is considered more rapid, simpler and more economical than other TB diagnostic methods. An inexpensive LAMP has the potential to be adopted for the diagnosis of tuberculosis in peripheral laboratories and resource-limited settings. The aim of this study was to identify *Mycobacterium tuberculosis* directly from sputum by LAMP and to compare its efficacy over routinely used methods.

METHODS

Study design and clinical sputum specimens

This study was carried out from October 2006 to November 2007 at National TB Reference Lab German-Nepal Tuberculosis Project (GENETUP), Kalimati; Mycobacterial Research Laboratory, Anandaban Hospital; Everest International Clinic and Research Center (EICRC), Kathmandu in collaboration with Osaka Perfectural Institute of public health, Japan. A total of 106 sputum samples were collected and divided into two groups i.e. group A and group B. In group A, 53 sputum samples were collected from the new cases of pulmonary tuberculosis patients and Group B includes 53 acid fast bacelli negative sputum samples from patients who had complains of chest pain, cough and fever were collected at GENETUP. During sample and data collection, study objectives and expected outcomes were explained to

each study participant before receiving their consent. About four ml of muco-purulent early morning sample was collected, followed on the same day by microscopy and sample culture preparation.

Smear microscopy was performed according to the acid-fast bacillus stains, with auramine-O staining. Grading of microscopy was done as suggested by WHO, 1998a[14]. The remaining sample after treated with N-acetyl-L-cysteine - NaOH method to kill the undesirable microorganisms other than the AFB. Decontaminated sputum of 0.1 ml was inoculated into each of the two culture tubes containing 2% Ogawa medium and incubated at 37^0C. When colonies were observed during incubation, acid fastness of bacilli was determined by a smear test. When no colonies appeared after weekly observation for eight weeks, the result was considered negative. Grading of primary culture was done as suggested by WHO, 1998b[15].

Loop-Mediated Isothermal Amplification (LAMP)

Sample Treatment for LAMP:

Methods performed by Sapkotal *et al* was adopted to treat the concentrated sputum samples for LAMP. Briefly, an equal volume of 2% NaOH and 0.5% N-acetyl-L-cysteine (NaLC) was added to the concentrated sputum sample. These were mixed by inverting for several times and left for 10 minutes to thinning out the sputum. The sample was then centrifuged at 12,000 rpm for 15 minutes. The supernatant was discarded and the pellet was resuspended in 50 mM Tris HCl of pH 8.3 equivalent to not less than $1/20^{th}$ of the original thinned sputum. Then it was heat inactivated at 80°C for 20 minutes in a dry heat block. After cooling to room temperature, an equal volume of chloroform was added and mixed by vortex and then stored in refrigerator[16].

DNA extraction

The Mycobacterial DNA used for LAMP was extracted from concentrated sputum samples by freeze and boil method[17]. Briefly, 50 µl of sample was taken into an eppendorf tube. Then it was immersed into liquid nitrogen (-196°C) for one minute and it was heated to 100°C in the dry heat block for one minute. The alternate cooling and heating was repeated for five times to effect DNA extraction and the extracted DNA was stored in the refrigerator for LAMP and PCR. The concentration and quality of genomic DNA in PCR reaction were determined by spectrophotometric analysis at 260 and 280nm[18].

Primer for LAMP

For rapid diagnosis of tuberculosis, mycobacterial DNA extracted from the concentrated sputum sample was used for LAMP all together six primers were used during the study. These were: outer primers (F3 and B3), a forward inner primer (FIP), a backward inner primer (BIP), and loop primers (loop F and loop B). They recognize eight distinct regions of the 16SrRNA (Eiken Chemical)

FIP:
CACCCACGTGTTACTCATGCAAGTCGAACGGAAAGGTCT

BIP: TCGGGATAAGCCTGGACCACAAGACATGCATCCCGT

FL: GTTCGCCACTCGAGTATCTCCG

BL: GAAACTGGGTCTAATACCGG

F3: CTGGCTCAGGACGAACG

B3: GCTCATCCCACACCGC

LAMP assay:

LAMP reaction was performed in a total 25 µl reaction mixture containing 2.5 µl 10 x LAMP buffer (200 mM Tris-HCL [pH8.8], 10 mM KCl, 10 mM (NH4)2SO4, 1% Triton X-100, 14 mM dNTPs (Deoxynucleoside triphosphates), 0.8M betaine, 300 mM $MgSO_4$, primer mix (30 pmol each of inner primers FIP and BIP, 5 pmol each of outer primer FL and BL, 20 pmol each of loop primers F3 and B3), 8U Bst DNA polymerase (New England BIolabs) with specified amounts of bacterial DNA, and Loopamp Fluorescent Detection Reagent (Eiken Chemical Co Ltd., Tokyo, Japan). Loopamp Fluorescent Detection Reagent enables the direct detection of DNA amplification in reaction tubes by naked eye as green fluorescence under ultraviolet light.

Statistical analysis

We assumed statistical significance at p <0.05. The sensitivity, specificity, positive predictive value and negative predictive values were calculated with a 95% confidence interval (95% CI) using the standard formulas considering culture as the gold standard.

RESULTS

In order to evaluate the different diagnostic techniques for rapid diagnosis of tuberculosis, a comparative study of LAMP and microscopy to detect *M. tuberculosis* in sputum sample, against culture as standard method was performed. The sputum samples

from the patients belonging to two different study groups were collected in this study. Out of 106 samples, 53 were from smear positive TB patients and 53 were from smear negative patients respectively.

Distribution of total study cases (N=106) by age and sex

Among the studied 106 cases, 72.64% (n=77/106) were males and 27.36% (n=29/106) were females in the age group 14 to 81 years. This study showed that the highest number was seen in the age group of 21 - 40 years (36.79%) among males and in the age group 41 - 60 (9.43%) among females.

Table 1: Distribution of total study cases by age and sex

Age	Males		Females		Total	
Group (years)	No	%	No	%	No	%
<20	8	7.54	8	7.54	16	15.09
21 - 40	39	36.79	9	8.49	48	45.28
41 - 60	21	19.81	10	9.43	31	29.24
>60	9	8.49	2	1.89	11	10.37
Total	77	72.64	29	27.36	106	100

Age and sex distribution of culture, LAMP and PCR positive cases

Out of 106 sputum samples, 53.77% (n=57/106) were culture positive, 52.83% (n=56/106) were LAMP positive. Among total culture positive isolates, 73.68% (n=42/57) were males and 26.31% (n=15/57) were females. The highest number of culture positive cases were belonged to the age group 21-40. Out of 56 LAMP positive isolates, 71.42% (n=40/56) were males and 28.57% (n=16/56) were females.

Socio-demographic and disease characteristic of interviewed persons

106 patients were interviewed personally through structured questionnaire. Among 106 interviewed patients, 47.16% (n=50/106 were smokers; 52.83% (n=56/106) were non-smokers; 42.45% (n=45/106) had habit of taking alcohol and 54.71% (n=58/106) were non alcoholic; 27.35% (n=29/106) showed presence of TB in their family where 72.64% (n=77/106) showed absence of TB in their family members.

Out of total interviewed patients, 70.75% (n=75/106) were immunized by BCG vaccination, where as 29.25% (n=31/106) were non-vaccinated. While asking the patients about the symptoms of TB, 74.52% (n=79/106) had the symptoms of fever, 68.86% (n=73/106) had chest pain, 86.79% (n=92/106) had cough, 71.69% (n=76/106) had weight loss, and 34.90% (n=37/106) had hemoptysis respectively.

Table 2: Socio-demographic and disease characteristic of interviewed persons (n=106)

Socio-demographic characteristics	No. of patients	Percentage
Smoking		
Smoker	50	47.16%
Non-Smoker	56	52.83%
Alcohol		
Habit of drinking alcohol	45	42.45%
Not	58	54.71%
Family history of TB		
Present	29	27.35%
Absent	77	72.64%
BCG		
Vaccinated	75	70.75%
Non-Vaccinated	31	29.25%
Symptoms		
Fever	79	74.52%
Chest pain	73	68.86%
Cough	92	86.79%
Hemoptysis	37	34.90%
Weight loss	76	71.69%
X-Ray		
Positive	55	51.89%
Negative	51	48.11%

Evaluation of MTB-LAMP for the detection of *M. tuberculosis* in clinical specimens

Application of MTB-LAMP to clinical specimens was evaluated by comparing LAMP results with acid-fast smear tests and standard bacterial cultures as gold standard test of 106 patients in Nepal. Results are summarized in Table 3.

In the LAMP, all the positive extraction controls and positive LAMP controls gave expected green color changed throughout the experiment, thus, validating the

extraction procedure and LAMP reaction condition. Also all the negative extraction controls and the negative LAMP controls gave no bands and orange color respectively, hence, excluding any possibility of contamination.

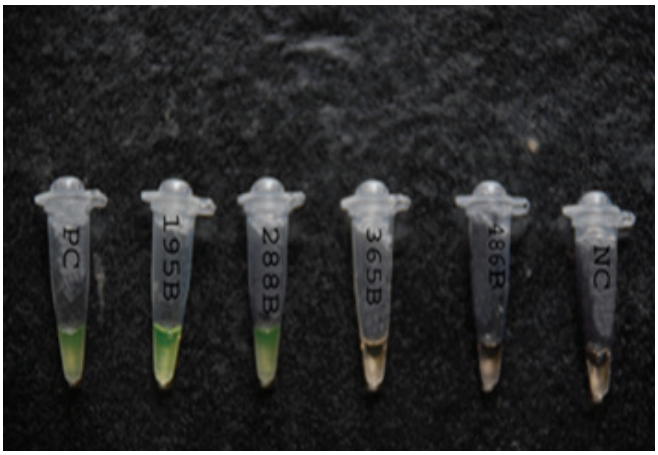
In order to minimize the risk of contamination with exogenous *M. tuberculosis* DNA, these procedures were performed using disposable tubes and pipettes. In addition, a sample blank was taken through all the preparation steps to detect possible contaminations. Furthermore, DNA extraction and LAMP assay were performed in different laboratories.

Table 3: Comparative results of stain, PCR and LAMP with culture

	Stain			LAMP		
	+ve	-ve	Total	+ve	-ve	Total
Culture +ve	53	4	57	55	2	57
Culture –ve	0	49	49	1	48	49
Total	53	53	106	56	50	106
Sensitivity	92.98%			96.49%		
Specificity	100%			97.95%		
PV +	100%			98.21%		
PV -	92.45%			96%		

PV+: predictive value of positive test, and PV-: predictive value of negative test

Fig 1. Visual inspection of LAMP products. The *M. tuberculosis* primers were used for this assay. Tubes: PC, positive control; NC, negative control (no DNA (Nucleic acid free water only)); 195B and 288B, positive samples; 365B and 486B, negative samples.



Among 53 smear positive cases; all were positive while culturing on Ogawa medium. However, among 53 smear negative cases, four were positive by culture. In this study, none of the fluorochrome staining positive samples showed negative results in culture, thus showing 100% specificity. This means that as long as the stain gives positive result, it is not necessary to do further test for the purpose of diagnosis. However, due to the increase of drug resistant bacilli, culture is recommended to test for the sensitivity of antituberculosis drugs.

While in smear negative cases, the sensitivity of microscopy in reference to culture was found to 92.98%. This indicates the sensitivity of microscopy was not good as its specificity. However, considering the availability and technical easiness, microscopy method could be still used as the method of choice in the first line diagnosis of tuberculosis.

Among 53 PTB patients with a positive acid fast stain on sputum smear and culture positive too, LAMP was positive for 51 samples while it was negative for the remaining two samples. While out of 53 smear negative samples from the patients of group B, LAMP was positive for five samples and it was negative for the rest of the samples. The finding reveals sensitivity of LAMP over microscopy. When the result of culture on Ogawa medium obtained, only four samples were found to be positive. These four culture positive samples were also positive by LAMP confirming the LAMP result with culture. These findings emphasized the sensitivity of the LAMP over culture. It is remarkable that LAMP positive sample was found to be negative by culture. 48 microscopy negative samples were found to be negative by both LAMP and culture. This finding highlighted the specificity of LAMP because all the samples that were negative for tubercle bacilli microscopically and culturally were also negative by LAMP. Thus, LAMP combines the rapidity of microscopy with specificity of culture.

While comparing the LAMP with culture as gold standard, the sensitivity specificity, predictive value of positive test, predictive value of negative test, percentage of false negative and percentage of false positive were 96.49%, 97.95%, 98.21%, 96%, 3.50% and 2.04% respectively. The findings of the present study was in agreement with the previous findings of Boehme *et al*[19] who reported the sensitivity of LAMP as 97.7% in comparison to culture as gold standard.

Discussion

Tuberculosis (TB) is a communicable disease caused by the bacterium *Mycobacterium tuberculosis* (MTB) and is a persistent problem in the developing countries. Early

and effective detection of *Mycobacterium tuberculosis*, particularly in smear-negative tuberculosis, is a priority for global TB control. Detection of *M. tuberculosis* using conventional microscopy, culture and biochemical-based assays is time-consuming and laborious. Therefore, a simple and sensitive method for rapid detection that can be performed in any standard laboratory has been anxiously awaited to facilitate the initiation of clinical TB treatment. LAMP can be used for this purpose, providing results within 1h with high sensitivity.

The objective of this study was to identify *M. tuberculosis* directly from sputum by LAMP and to compare its efficacy over routinely used methods. Sputum samples were collected from the patients visiting at the National TB Reference Lab German-Nepal Tuberculosis Project (GENETUP), Kalimati. Here in group A, a total of 53 sputum samples were collected from the new cases of pulmonary tuberculosis patients and in group B, another total of 53 sputum specimens obtained from patients who were suspected of having TB were subjected to culture confirmation, smear microscopy and the LAMP method. The clinical sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated and compared with the culture results.

In this study, males (72.64%) were found to be more infected than females (27.36%) and the result was found to be significant statistically. This findings was in agreement with the findings of the Ponticeellio *et al*[20], who reported 82.2% males and 17.8% of females among 90 active pulmonary TB cases. This does not however reflect an increase in the occurrence of disease in males, since in present study the attendance of females is lower than males. TB was not diagnosed in the pulmonary TB suspects below 14 years. On the other hand females make less report than males in health care facilities, because of social pressure or stigma and males have more exposure to external environment than females for their job and other activities. In the present study maximum number of culture positive was observed in the economically most productive age group of 21 – 40 years.

Among 53 pulmonary TB patients with a positive acid-fast stain on sputum smear, 94.33% (50/53) were positive by chest X-ray while the remaining were negative. The X-ray positive results are due to cavities usually develops because of immune response to the tubercle bacilli leading to destruction of lung tissue. The X-ray negative result may be due to the people who don't have fully functioning immune system like in HIV cases, where there is less tissue destruction and hence lung cavitations. Another reason might be due to the patients who were suffered from bronchial or tracheal tuberculosis in whom infectious agent might not be spread to the lungs.

Patients with negative acid fast staining on sputum samples were recruited into group B where 9.43% (5/53) were positive in chest X-ray and the rest were negative. Abnormalities on chest X-ray may be suggestive of, but are never diagnostic of TB because a number of other bacterial conditions (such as pneumonia or abscess) or non-bacterial processes (fungal diseases, carcinoma, sarcoidosis or pneumoconiosis) can produce similar images. The vast majority of patients (over 90%) with cavitary pulmonary TB are sputum smear positive (WHO, 1997)[21].

Among 53 smear positive cases; all were positive while culturing on Ogawa medium. However, among 53 smear negative cases, four were positive by culture. In this study, none of the fluorochrome staining positive samples showed negative results in culture, thus showing 100% specificity. This means that as long as the stain gives positive result, it is not necessary to do further test for the purpose of diagnosis. However, due to the increase of drug resistant bacilli, culture is recommended to test for the sensitivity of antituberculosis drugs[22].

While in smear negative cases, the sensitivity of microscopy in reference to culture was found to 92.98%. This indicates the sensitivity of microscopy was not good as its specificity. However, considering the availability and technical easiness, microscopy method could be still used as the method of choice in the first line diagnosis of tuberculosis[23].

Among 53 PTB patients with a positive acid fast stain on sputum smear and culture positive too, LAMP was positive for 51 samples while it was negative for the remaining two samples. While out of 53 smear negative samples from the patients of group B, LAMP was positive for five samples and it was negative for the rest of the samples. The finding reveals sensitivity of LAMP over microscopy. When the result of culture on Ogawa medium obtained, only four samples were found to be positive. These four culture positive samples were also positive by LAMP confirming the LAMP result with culture. These findings emphasized the sensitivity of the LAMP over culture. It is remarkable that LAMP positive sample was found to be negative by culture. Forty eight microscopy

negative samples were found to be negative by both LAMP and culture. This finding highlighted the specificity of LAMP because all the samples that were negative for tubercle bacilli microscopically and culturally were also negative by LAMP. Thus, LAMP combines the rapidity of microscopy with specificity of culture.

While comparing the LAMP with culture as gold standard, the sensitivity specificity, predictive value of positive test, predictive value of negative test, percentage of false negative and percentage of false positive were 96.49%, 98.21%, 98.21%, 96%, 3.50% and 2.04% respectively. This demonstrates a high sensitivity, specificity and predictive value of positive test and predictive value of negative test of LAMP as compared with that of culture. Percentage of false negative and percentage of false positive are also very low indicating the higher accuracy of the test.

LAMP-based assays targeting the gyrB (Iwamoto *et al*. 2003), 16S rRNA (Pandey *et al*. 2008)[23], IS6110 (Aryan *et al.* 2010)[24] and rimM (Zhu *et al*. 2009)[25] sequences have been developed for the detection of *M. tuberculosis.* A multicenter study of *Mycobacterium tuberculosis* detection showed the feasibility of using the LAMP method in developing countries by Boehme *et al*[19]. They had shown the sensitivity of MTB-LAMP 97.7% (173/177) in smear positive and culture positive sputum samples and 48.8% (21/43) in smear negative and culture positive sputum samples.

The findings of the present study was in agreement with the findings of Pandey *et al* on Nepalese patients demonstrated the feasibility of LAMP for the detection of *M. tuberculosis*. They had shown the sensitivity of LAMP 100% (96/96) in culture positive sputum sample, and the specificity was 94.2% (98/104) in culture negative sputum samples. Similarly, Cathrina *et al*[26] reported that the sensitivity of LAMP in smear and culture positive sputum specimen was 97.7% and sensitivity in smear negative and culture positive specimen was 48.8%. The specificity in culture negative sample was 99.0%.

CONCLUSIONS

In conclusion, this study showed that LAMP test is not necessary for the diagnosis of smear positive cases. However LAMP could be possible tool for confirmatory diagnosis of the smear negative cases, which show clinical symptoms of TB. Among the different diagnostic tools, LAMP assay is more advantageous than other techniques performed in this study due to its easy operation without sophisticated equipment, it will be simple enough to use in small-scale hospitals, primary care facilities, and clinical laboratories in developing countries if the remaining issues such as sample preparation, nucleic acid extraction, and cross-contamination controls are addressed.

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Study on the Relativity between Cytogenetics and Cytomorphology and its Prognosis Significance in Children with Acute Myelogenous Leukemia

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ABSTRACT

Objective: The main objective of this study was to retrospectively evaluate that the cytogenetic abnormalities is an important prognostic factor for the cure of acute myeloid leukemia (AML).

Methods: This retrospective study enrolled newly diagnosed 70 cases (37 males and 33 females, aged 10.1 months to 14.5 years) of pediatric patients with AML during 2010 January - 2016 February from the Second Affiliated Hospital of Anhui Medical University. Excluding criteria were cases secondary to treatment-related MDS and AML. Samples were obtained from bone marrow cells in patients after treatment on the anterior superior iliac spine, blood diseases laboratory by direct culture or 24/48 hour short-term culture, G -banding technique for testing. Follow-up of 1 - 60 months, the analysis of treatment response rates of different karyotypes, distribution ratios in various subtypes, normal karyotype and abnormal karyotype.

ISPSS17.0 software statistics was used for statistical analysis. Groups were compared using chi-square test; Survival rate was calculated by method of Kaplan Meier and survival difference between groups were compared with breslow test.

Results: Among 70 cases, 42 cases were detected for chromosomal abnormalities (i.e. 60% of the total number of cases), M3 abnormal karyotype distortion rate of 78.5%, M2 abnormal karyotype aberrations 63.3%, M4 60.0%, M1 50%, M5 lowest 38.9 %, M7 nuclear aberrations highest rate was 100%. Total chromosomal aberration rate was 60%. Acute myeloid leukemia cases, t (8; 21) at most , there are 15 cases, and the presence of abnormal karyotype 86.7% in the original part of differentiated myeloid leukemia (M2); t (15; 17) has 11 cases, exists only in acute promyelocytic cell leukemia (M3). After treatment, the remission rate of t (8; 21) was 80%; the remission rate of t (15; 17) was 90%; the remission rate of other abnormal karyotype abnormalities was 50%; the remission rate of total abnormal karyotype was 71.4%. The event free survival rate was significantly different between normal karyotype, t (8; 21), t (15; 17) and other abnormal karyotype groups (P<0.05).

Conclusions: Acute myeloid leukemia karyotype abnormalities among FAB subtypes are different; M3 is the highest rate of abnormal

Keywords
Abnormal karyotypes, Acute myeloid leukemia, Cytogenetics, Prognosis..

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karyotype aberrations, M2, M4 medium, M5 minimum. t (15; 17) seen in acute promyelocytic leukemia (APL), prognosis is good; t (8; 21) is more common in M2, prognosis is good, also found in M4 and M5, worse prognosis; +8 Abnormalities found in AML M2, M3, M4, M5 and M6 subtypes, prognosis medium; inv (16) high white blood cells, low platelet poor prognosis, AML patients with normal karyotype prognosis medium.

NTRODUCTION

Acute Leukemia belong to the group of hematopoietic stem cell mutation clone malignancy which is characterized by certain blood cells of hematopoietic tissue system hyperplasia, and infiltration to the bloodstream and then to various tissues and organs, which led to a series of clinical manifestations.

In China, pediatric malignancies have the highest incidence of leukemia. With the continuous improvement of chemotherapy, the rapid development of immunology, cytogenetic and molecular genetics, it is no longer considered a fatal disease. Complete remission rate of initial treatment in children with acute myeloid leukemia has reached 80% and five year disease-free survival rate of about 40 - 60%.

Over the past years, blood disease research work caught committed to explore the pathogenesis of acute leukemia from cytogenetic and molecular level that the considerable number of leukemia is chromosomal changes because of genetic abnormalities. Oncogene activation, tumor suppressor gene deletion, causing the proliferation of hematopoietic cells, differentiation and regulation disorders, to malignant transformation.

With the advancement of cultivation method and banding techniques, currently age-related macular degeneration (AMD) chromosomal aberrations detected rate has risen from 50% in the 1960s and 1970s and the current to around 80% - 90%. Although the type of AML chromosomal aberrations up to 100 or more, But it can be summarized into two categories: one is FAB subtype-specific chromosomal rearrangements related, about 60%, including the t (8; 21) (q22; q22), t (15; 17) (q 22; q12 or 21), inv (16) (p13; q22) etc.

Where in t (8; 21) (q22; q22) is closely related to AML-M2, t (15; 17) (q22; q12 or 21) found only in AML-M3, inv (16) (p13; q22) more common in M4EO. Such karyotype on early diagnosis and classification of AML has a guide. FAB subtype and the other is not related to abnormal, most of the number of abnormal, common chromosome number has increased first 8, 21, 19, 11 and 22 there appears chromosome loss, Y chromosome 7, 5 and 12, common chromosome 16 deletion of the short arm missing 6 and No. 7, 5 and 1, 12, 3. Among them +8, -7, -5 are the most.

Over the years, more cooperative groups abroad, including SWOG (South West Oncology Group), MRC (Medical Research Council) analyzed by a large sample of cases, confirmed cytogenetic findings prognosis and significance for acute myeloid leukemia. In accordance with the prognosis of low-risk group divided cytogenetic risk groups and high-risk groups that differences in each group complete remission rate, overall survival, disease free survival time were significant, although each between the study groups of individual cytogenetic abnormalities prognostic significance remains controversial, but most of the prognostic significance of cytogenetic abnormalities results are consistent and clear.

Simple t (8; 21), and with t (15; 17), the general prognosis in patients with inv (16) change is good, with -7, -5, inv (3) (q21; q26) / t (3; 3) (q21; q26) or complex karyotype chromosome abnormalities, poor prognosis. Thus, by cytogenetic characteristics of prognostic evaluation, selection the appropriate treatment is essential to prolong the survival rate of patients.

In this study, 70 cases of hospitalized patients with recently diagnosed AML from January 2009-2015 February were enrolled with karyotype abnormalities, patients with acute myeloid leukemia were involved in the treatment to understand AML patients karyotype distribution, survival follow-up and outcome combined with the clinical diagnosis and treatment. The aim is to analyze the correlation between karyotype and prognosis.

METHODS

2.1 Study

Newly diagnosed 70 cases (37 males and 33 females, aged 10.1 months to 14.5 years) of pediatric patients with AML were enrolled from 2009 January – 2016 February from the Second Affiliated Hospital of Anhui Medical University.

Excluding criteria were cases secondary to treatment related MDS and AML.

By FAB classification, M1 subtype 2 cases, M2 subtype 30 cases, M3 subtype 14 cases, M4 subtype 5 cases, M5 subtype 18 cases, and M7 subtype, one case. Diagnosis and evaluation of the efficacy of all patients were done with reference to "blood disease diagnosis and treatment standards"[1].

2.2 Specimen collection

Patients admitted to hospital after chromosome analysis, samples were obtained from the bone marrow cells before and after treatment in patients from iliac spine. Five ml injection containing two ml broth, culture bottles were immediately taken for inspection.

2.3 Cytogenetic analysis

The samples were submitted to the Second Affiliated Hospital of Anhui Medical University, Laboratory of Hematology line karyotype. Direct method or short-term legal culture piece, karyotype analysis using G banding analysis of 20 metaphase cells, press "Human cytogenetic international nomenclature system (ISCN1995)" were to be carried out[2].

At least two cells having the same or increase in chromosome structural rearrangements, or three cells having the same chromosome loss, will be confirmed as an exception clone.

2.4 Treatment and efficacy evaluation

Patient with recently diagnosed M3 with all-trans retinoic acid (ATRA) 25mg / (m^2•d) and arsenic trioxide (As_2O_3) 0.2 mg / (kg•d) during induction therapy period, 28 days of oral. In consolidate the treatment period, patient were treated with cytarabine (Ara-C) 100 mg/ (m^2 •q12h) from one to seven days and DNR 40 mg/ (m^2•d) from one to three days, fowling with ATRA 25 mg / (m^2•d) and As2O3 0.2 mg/ (kg• d) for 28 days, and the very next treatment with Ara-C 100 mg/ (m^2 •q12h) from one to seven days and DNR 40 mg/ (m^2•d) from one to three days. After completing the consolidating the treatment, patient entered maintenance treatment with ATRA 25 mg/ (m^2•d) for 28 days, Ara-C 75 mg/ (m^2 •q12h) for five days, harringtonine 3 mg/ (m^2•d) for seven days, As2O3 0.2 mg/ (kg• d) for 14 days, Ara-C 75mg/ (m^2 •q12h) and 6-TG 75 mg/ (m^2 •d) for 7 days; five times totally. Efficacy criteria: Zhang Zhinan reference "blood disease diagnosis and treatment standards"[1]. Complete remission (CR); it means that the leukemic cells without clinical presentation caused by infiltration, or near normal life values = 1.5 × 10^9/L, platelets = 100 × 10^9/L. Peripheral blood leukocyte no leukemia cells. Bone marrow blasts = 5%, red blood cells and megakaryocytes normal series. Partial remission (PR): Bone marrow myeloblast type I + type II (original ten immature monocytes or ten original immature lymphocytes) equals to >5% and = 20%, or clinically, a standard blood counts by the end of complete remission No remission (NR): None of the above criteria. Recurrence was defined by one of the following three criteria including (1) bone marrow blasts >5% and = 20%, after a course of an effective anti-leukemic treatment, bone marrow failed to achieve complete remission. (2) Bone marrow blasts >20%; (3) myeloid leukemia cell infiltration. Overall survival is defined as the time of diagnosis until death or end of follow-up. Disease-free survival is defined as the time to achieve complete remission to relapse during this time, if more than one relapse and remission as per the cumulative basis.

2.5 Experimental Method

2.5.1 Reagent

(1) Medium: containing 20% fetal calf serum and 20 μ/ml heparin RPMI l640.

(2) Mitosis blockers: colchicines, formulated 5 μg/ml were used.

(3) Hypotonic: 0.075 mol/L potassium chloride solution.

(4) Fixative: 1: 3 glacial acetic acid and methanol.

(5) Banding solution: 0.1% trypsin, 0.02% EDTA solution, pH 6.8 phosphate buffer solution, Tris solution 3%.

(6) Giemsa staining solution: phosphate buffer solution concentration of 10%, pH 6.8 ~ 7.4.

2.5.2 Step

(1) Take specimens and inoculation culture: Sterile needle to extract bone marrow fluid 0.5 ~ 2 mL in heparin tube, add broth to 8 mL, washing and percussion, centrifugation (1200 rpm/min, 5 min), repeated 2 - 3 times to wash away the fat and other impurities. Per ml medium 1 ~ 3 × 106 cells were inoculated to a sterile culture medium containing 5 ml in to two flasks, shake gently and placed in the box 37 for 48 hours.

(2) Colchicines treatment: Sample bottle was added with colchicines 50 μl, using liquid 0.05 ml (final concentration 0.05 μg/mL) to continue to foster 50 min.

(3) Preparation of samples:

1) After termination of the culture, the culture was poured into the conical centrifuge tube, 6 minutes centrifugation (1200 rev/ min), the supernatant was discarded.
2) The prewarmed 8 ml hypotonic solutions added by repeatedly pipetting at 37°C with bubble method, water bath for 30 minutes.
3) PreFixed: Add fixative 1 ~ 5mL, percussion mix placed under room temperature 3 ~ 5 min.
4) Centrifugation: 800 to 1000 rev / min.
5) Fixed: The supernatant was discarded, freshly prepared fixative was added to 5 ~ 6 mL, mix by pipetting.
6) Centrifugation: 800 to 1000 rev / min, 10 minutes
7) Repeat 5 and 6 steps two times.
8) The supernatant was discarded, adding an appropriate amount of fixative gently pipetting the cell suspension to spare.
9) Drop sheet: Take number of slides that stored in clean ice cold wet sheets, slightly tilted, with a capillary dropper suction cell suspension, dropping from 10 cm height of one to two drops on the slide in the air to be dry.
10) Roasted pieces: slides placed in 70°C oven bake for two hours.

(4) G-banding:

1) The first, vat 25 mL (0.1%) mixed with trypsin 25 mL (0.02%) EDTA solution, Tris was adjusted to pH 7. Second, three vat 50 mL were added at pH 6.8 phosphate buffer. The fourth was added 50 mL (5%) Giemsa dye vat. The three dye vats were kept at 37°C water bath.
2) Slide into the first cylinder 20 - 30 seconds, remove successively rinsed in the second, three-cylinder, followed by the third set dye vat five to 10 minutes.
3) Tap water, dry equipment seized.
4) Each patient observed for at least 20 metaphase cells, abnormal karyotype press "Human Cytogenetic Nomenclature System (ISCN 1995)" are identified and described.

2.6 Statistical Methods

ISPSS17.0 software statistics was used for statistical analysis. Groups were compared using chi-square test; Survival rate was calculated by method of Kaplan Meier and survival difference between groups were compared with breslow test.

RESULTS

Among 70 cases, 42 cases were detected for chromosomal abnormalities, i.e. 60% of the total number of cases.

Table 1: Karyotype distribution

FAB sub-type	Number of cases	Normal karyotype	Abnormal karyotype	Distortion rate (%)
M1	2	1	1	50%
M2	30	11	19	63.3%
M3	14	3	11	78.5%
M4	5	2	3	60.0%
M5	18	11	7	38.9%
M7	1	0	1	100%
Total	**70**	**28**	**42**	**60%**

As can be seen from Table 1, all 70 patients with acute myeloid leukemia, M1 subtype one case of abnormal karyotype; M2 subtype 19 cases of abnormal karyotypes, M3 subtype 11 cases of abnormal karyotypes; M4 subtype three cases of abnormal karyotypes, M5 subtype seven cases of abnormal karyotypes; M7 subtypes one case of abnormal karyotype. M3 abnormal karyotype distortion rate of 78.5%, M2 abnormal karyotype aberrations 63.3%, M4 60.0%, M1 50%, M5 lowest, 38.9%, M7 nuclear aberrations highest rate was 100%. However, due to a number of cases investigated test is too small, it is no clear statistical significance between this groups. So this experiment shows M3 subtype highest distortion, total chromosomal aberration rate 60%.

Table 2: Abnormal karyotype in AML

Abnormal karyo-type	FAB Subtype						Total
	M1	M2	M3	M4	M5	M7	
t (8;21)		13			2		15
t (5;17)			11				11
Inv (16) (13; q22)				1	1		2
8q-		2					2
11p+		1					1
11q-		1					1
+8				1			1
+12				1			1
-7	1						1
-19		1					1

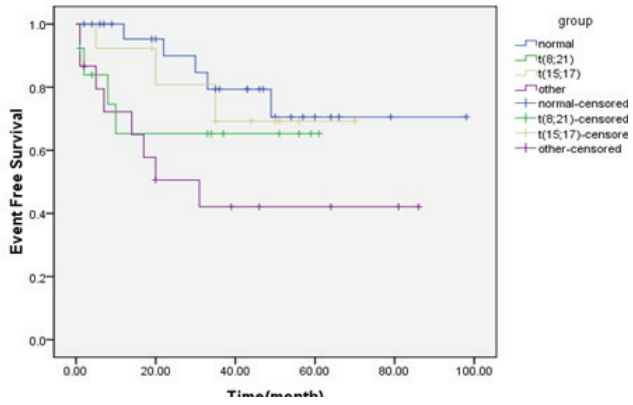
Hyperdiploidy		1			3		4
Complex abnormalities					1	1	2
Total	**1**	**19**	**11**	**3**	**7**	**1**	**42**

Table 2 shows that, in acute myeloid leukemia patients, there are 10 kinds of abnormal karyotype and complex chromosomal aberrations. t (8; 21) at most, 15 cases, and the presence of abnormal karyotypes was 86.7% in the original part of differentiated myeloid leukemia (M2); t (5; 17) 11 cases, exists only in acute promyelocytic cell leukemia (M3) , inv (16) (p13; q22) in two cases, respectively, in the presence of the M4 and M5, 8q- two cases exist in M2, 11p +1 case and 11 q-, are present in M2, the +8 and +12 in one case, all exist in M2, the -19 one case, exist in M2, four patients hyperdiploidy were present in M2 and M5, and the remaining two cases were complicated karyotype, they were present in the M5 and M7.

Table 3: Abnormal karyotype and efficacy in AML

Group	CR+PR	NR	Total	Response Rate (%)
t (8; 21)	12	3	15	80
t (5; 17)	10	1	11	90.1
Other abnormal karyotype	8	8	16	50
Total	**30**	**12**	**42**	**71.4**

Fig 1: The event free survival curves of different karyotype in AML



From table 2, table 3 and fig 1 can be seen, M2 subtype in 13 patients with abnormal karyotype t (8; 21), of which 12 cases of remission after treatment, two cases are not alleviated after treatment, remission rate 80%. M3 subtype in 11 patients with abnormal karyotype t (15; 17), 10 patients were in complete remission after treatment, one case was not alleviated, and remission rate was 90.1%. Other abnormal karyotypes total of 16 cases, 50% response rate. There are two cases which inv (16) (p13; q22), one case remission, and one died, two cases of 8 q- were relieved, one case of 11p +, follow-up death, one case of 11q- was remission. One case of +8 was died. One case of +12 was remission. One case of -7 patients was followed up in death. One case of -19 was remission. The event free survival rate was significantly different between normal karyotype, t (8; 21), t (15; 17) and other abnormal karyotype groups (P=0.028 <0.05). Further statistical analysis found that event free survival rate free survival rate between normal karyotype and other abnormal karyotype groups was statistically difference (P=0.004 <0.05), no difference between the rest of the groups.

DISCUSSION

With the continuous improvement of chromosome banding techniques, especially the rapid development of high-resolution technology, such chromosomal abnormalities relationship between certain types of leukemia and has become more and more closely, but also identified a number of subtypes iconic chromosome abnormal characteristics. Meanwhile, with disease progression or improvement of chromosomal abnormality occurs constantly changing, these changes we adjust has a significant role in the treatment and prognosis of the disease. In most patients, the application of cytogenetic methods can be found in clear cell clonal chromosomal abnormalities. The same chromosomal abnormalities are also seen in acute leukemia (AL) of each subtype, which means not only the diagnosis, but also prognostic value, the clinical characteristics of patients; help us to predict the patient's condition, because the merits of any karyotype changes are not absolute. Chromosomal abnormalities are important prognostic factor, such as t (8; 21) and t (15; 17) or inv (16) of better prognosis, and -5, -7, t (9; 22), and complex chromosomal abnormalities have poor prognosis[3-4].

The group of 70 children with acute myeloid leukemia, chromosome aberrations was 60%, suggesting that cytogenetic abnormalities are an important factor in the occurrence of acute myeloid leukemia. Highest karyotype aberration M3, 78.5%, M5 lowest, 38.9%, between the groups, not statistically significant. Considering the small sample volume chromosome aberration rate difference

between the AML subtypes may vary.

4.1 Acute promyelocytic leukemia (APL) and t (15; 17)

This group included 14 patients with 11 cases of abnormal karyotype APL, where, t (15; 17) 11 cases, accounting for 100%. Alan k Burnett[5] and other studies of 218 patients with APL, 187 cases of t (15; 17), accounting for 85.8%, there was no difference between the present studies. Kühnl A and Grimwade D[6] studied 1612 cases of AML patients, 932 cases had abnormal karyotype, t (15; 17) 198 cases, accounting for 21.2% response rate was 87%. The patients with t (15; 17) of the total 70 cases of abnormal karyotype AML 15.6% response rate was 100%, more consistent both with David Grimwade and other reports. 10 cases of t (15; 17) have been applied, all-trans retinoic acid treatment, 10 cases of remission, remission rate was 100%. M3 has t (15; 17) translocation PML (promyelocytic leukemia gene) on chromosome 15 and 17, retinoic acid receptor gene (RARα) chromosome formation PML-RARα fusion gene, which is M3 incidence and application of all-trans retinoic acid treatment effective molecular basis. t (15; 17) prognosis is good, has its foundation in molecular biology[6-7].

4.2 Acute myeloid leukemia part differentiation type (M2) and t (8; 21)

t (8; 21) is the first to be identified as chromosomal abnormalities in AML, seen in AML-M2, especially in childhood AML is more common, a few also found in AML-M4. In adults, t (8; 21) prognosis is good, but in children, the prognosis is poor. Foucar K[8] reported 11 cases of t (8; 21) patients, nine cases of M2, two cases of M4, M4's two cases were five and 11 years old, after allogeneic hematopoietic stem cell transplantation to achieve complete remission, nine cases M2, seven cases of complete remission. t (8; 21) showed no difference between the proportion of this group of patients. The patients in all AML t (8; 21) a total of 13 cases, 10 cases of remission, remission rate was 78.5%, 86.7% seen in M2. Prigogina EL *et al*[9] reported 88 cases of acute myeloid leukemia (AML) patients, 27 cases of t (8; 21), 20 cases of cases of M1, 3 cases of M2, 4 cases of M4, t (8; 21) in patients with abnormal karyotype prognosis than the normal karyotypes or other cytogenetic abnormalities better. John C. Byrd[10] analyzed 1213 cases of *de novo* AML patients, 81 cases of t (8; 21), the prognosis is good, and the remission rate of 91%, more in line with this group, and the combined second abnormal karyotype or complex karyotypes does not affect the prognosis. t (8; 21) rarely seen in M5, in Molero MT only one case reported[11]. The patients had abnormal karyotype +8 one case, accounting for 3.1% of the total AML, as M4, did not ease. Li X[12] reported 2308 Chinese children and adults with *de novo* acute myeloid leukemia; abnormal +8 (5.5%) +8, the prognosis is moderate, consistent with the results of this group. Vaskova J[13] reported of 10% and other reported of 12% and 8%. Sandra R. Wolman *et al*[14] reported 849 cases of AML patients, 108 cases of +8 (12.7%), 43 cases (5.1%) +8 as the only exception, the exception has +8 poor prognosis.

4.3 Clinical relationship of karyotypes inv (16) (p13; q22)

The patients of inv (16) (p13; q22) there are two cases, one case exists in M4, achieve remission, one case of M5, did not achieve remission. The patients were males, aged four, WBC 56.2 × 10^9/L, platelets 12 × 10^9/L; there are many adverse prognostic factors age, low platelets, and its poor prognosis. Eghtedar A, *et al*[15] summarized 110 cases of inv (16) patients, CR 93%, high white blood cell (>120 × 109/L) and low platelets (<30 × 10^9 / L) difficult to achieve CR. 65.3%, the prognosis is moderate.

4.4 Clinical relationship of normal karyotype

This group of patients with normal karyotype had 28 patients, seven cases of NR, response rate was 75%, means for survival time is 78.5 ±7.5 month. Torstein Haferlach[16] and other reports analyzed 453 cases of newly diagnosed AML patients. It showed normal karyotype and abnormal +8, prognosis medium.

CONCLUSIONS

This article summarizes 70 cases of children with acute myeloid leukemia untreated cases, the application of short-term culture and/ or direct method to do G-banding karyotype analysis, all patients of acute myeloid leukemia were given standard regimen and observed cytogenetic karyotype relations and efficacy, the following conclusions occur:

(1) Acute myeloid leukemia cell has its genetic basis, diagnosis of chromosomal studies in acute myeloid leukemia are important in treatment and prognosis.

(2) Acute myeloid leukemia karyotype abnormalities among FAB subtypes are different; M3 is the highest rate of abnormal karyotype aberrations, M2, M4 medium, M5 minimum.

(3) t (15; 17) seen in acute promyelocytic leukemia (APL), prognosis is good.

(4) t (8; 21) is more common in M2, prognosis is good, also found in M4 and M5, worse prognosis;

(5) +8 Abnormalities found in AML M2, M3, M4, M5, M6 subtypes, prognosis medium;

(6) inv (16) high white blood cells, low platelet poor prognosis;

(7) AML patients with normal karyotype prognosis are better than other groups.

This study belongs to the single center study with small sample, only one case of some subtypes, so, we will join with other centers to expand the samples and further improve this study.

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Problem Based Learning: An Experience of B.P. Koirala Institute of Health Sciences, Nepal

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Keywords
Attitude, Medical education, Problem-based learning, Students.

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ABSTRACT

Introduction: Problem-based learning (PBL) is a kind of teaching-learning method in which students' role is prime and they learn about a subject by active participation in problem solving.

Methods: A total of 96 students studying in second year MBBS program were enrolled into present study. A questionnaire was given to students and they were asked to choose the option they felt about PBL methods. The questionnaire comprised of ten likert items out of which three were negative and seven were positive items.

Results: Mean score for each statement in the questionnaire and the overall mean score were calculated. Overall mean score was 3.47.

Conclusions: It was concluded that students of second year MBBS at BPKIHS, had a positive attitude towards PBL method of teaching-learning activity.

INTRODUCTION

Problem-based learning (PBL) is a learning method in which students' role is prime and they learn about a subject by active participation in problem solving.

They develop collaboration skills among colleagues, intrinsic motivation and self directed learning effectively[1]. There is active transfer of knowledge from teacher to student in PBL. The tutor's role is important in facilitating learning by being as the guide, supporting and monitoring the learning process[2]. PBL differs from the traditional learning methods which were more lecture based[3].

To conduct PBL, students are divided into smaller groups. Each group contains six to eight students. One facilitator is appointed for the group. Usually junior faculty or residents are appointed as facilitator or tutor. The facilitator or tutor monitors or supervises the PBL.

Medical problems (triggers) inform of case history (patient's complaint, history of present illness, required personal, medical, family history) physical and systemic examination, investigation results are provided to students. Students discuss and interact themselves in PBL session. Students are suggested to consult standard textbooks for searching the information they deem needful but the facilitator or tutor does not teach them in PBL session. The session lasts two hours in a day. Following dealing all the problems or triggers from a chapter, students present a seminar.

Rationale for the study

B. P. Koirala Institute of Health Sciences (BPKIHS) of Nepal is running the PBL program for MBBS since18 years but so far what was the attitude MBBS students toward it had not been assessed; thus the study was undertaken.

METHODS

One hundred students studying in the second year MBBS program were enrolled into research study. Four students were absent on the day of the study so total number of subjects for the study was 96. A questionnaire with 10 likert items was given to students and they were asked to choose the option as they felt right about PBL methods. Students were asked not to disclose their name so that actual or realistic information could be obtained for the study.

The questionnaire comprised of ten likert items out of which three were negative and seven were positive items.

Table 1: Questionnaire

1	**PBL is effective in stimulating students for self-directed learning** a. Strongly agree b. Agree c. Can't say d. Disagree e. Strongly disagree
2	**PBL is a better way of learning method** a. Strongly agree b. Agree c. Can't say d. Disagree e. Strongly disagree
3	**PBL classes are interesting sessions at BPKIHS** a. Strongly agree b. Agree c. Can't say d. Disagree e. Strongly disagree
4	**PBL involves all students in interaction and discussion thus every student enjoys learning** a. Strongly agree b. Agree c. Can't say d. Disagree e. Strongly disagree
5	**PBL induces thirst for knowledge** a. Strongly agree b. Agree c. Can't say d. Disagree e. Strongly disagree
6	**PBL is not useful for understanding the subject** a. Strongly agree b. Agree c. Can't say d. Disagree e. Strongly disagree
7	**Conventional lecture classes are better than PBL tutorial classes for teaching –learning activity** a. Strongly agree b. Agree c. Can't say d. Disagree e. Strongly disagree
8	**PBL is a preferable method of learning** a. Strongly agree b. Agree c. Can't say d. Disagree e. Strongly disagree
9	**There is not much difference between SIS and PBL.** a. Strongly agree b. Agree c. Can't say d. Disagree e. Strongly disagree
10	**Students are happy to learn in small groups in PBL tutorial sessions** a. Strongly agree b. Agree c. Can't say d. Disagree e. Strongly disagree

Analysis

Mean score for each statement in the questionnaire and the overall mean score were calculated.

After receiving the completed questionnaire sheets from the students the score was given for each statement as follows:

For negative item statements like 6, 7 and 9, score 1 = for strongly agree; 2 = agree; 3 = can't say; 4 = disagree; 5 = strongly disagree

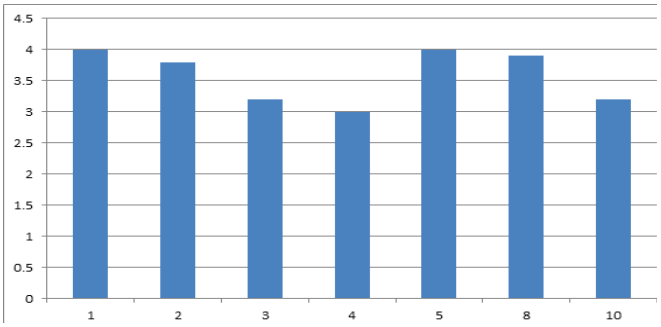
For positive item statements like 1, 2, 3, 4, 5, 8 and 10, score 5 = for strongly agree; 4 = agree; 3 = can't say; 2 = disagree; 1 = strongly disagree.

Out of the ten questions in the questionnaire, seven questions (1, 2, 3, 4, 5, 8, 10) were positive statements which were scored from 1 to 5 and, score 5 = for strongly agree; 4 =agree; 3 = can't say; 2 = disagree; 1 = strongly disagree.

RESULTS

The mean score for these questions is as follows

Fig 1: Mean scores of responses for all the positive statements



This figure shows that most responses are in agreement towards the benefit of PBL, since the mean score of most questions is more than 3 which is an equivocal response.

Table 2: Average of all the mean scores for positive statements

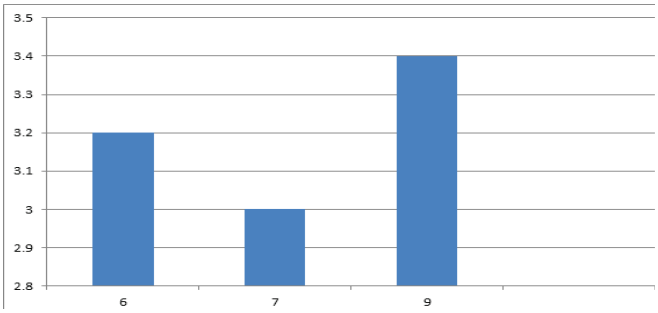
Question no	Mean score
1	4.0
2	3.8
3	3.2
4	3.0
5	4.0
8	3.9
10	3.2
Average score	3.59

Average score for all the positive statements = 3.59 which is inclining more towards a positive attitude of the students towards problem based learning.

Response to the negative statements: Questions 6, 7, 9 are negative statements which were scored from 1 to 5,

score1= strongly agree; 2 = agree; 3 = can't say; 4 = disagree; 5 = strongly disagree

Fig 2: Mean scores for the negative statements



The mean score for all the responses to the negative statements are in disagreement with the claim that PBL is not helpful, hence there is conclusive evidence that majority of the students have a explicitly good attitude towards PBL

Table 3: Average score for negative statements

Question no:	Mean score
6	3.2
7	3.0
9	3.4
Average score:	3.2

Average score for the responses for the negative statements = 3.2, which is a score indicating the positive influence of PBL.

Lastly the mean score for each statement was calculated which is as follows:

Table 4: Mean score for each statement

Sl No	Statements	Mean score
1.	PBL is effective in stimulating students for self-directed learning	4.0
2.	PBL is a better way of learning method	3.8
3.	PBL classes are interesting sessions at BPKIHS	3.2
4.	PBL involves all students in interaction and discussion thus every student enjoys learning	3.0
5.	PBL induces thirst for knowledge	4.0
6.	PBL is not useful for understanding the subject	3.2
7.	Conventional lecture classes are better than PBL tutorial classes for teaching–learning activity	3.0
8.	PBL is a preferable method of learning	3.9
9.	There is not much difference between SIS and PBL	3.4
10.	Students are happy to learn in small groups in PBL tutorial sessions	3.2
	Over all mean score	3.47

Overall mean score was 3.47 which suggested that students had a positive attitude towards PBL method of teaching-learning activity.

DISCUSSION

BPKIHS follows the Barrows' PBL model where in students are active in learning, learning in small student groups, ideally consisting of 6-10 students, and tutors or facilitators to guide the students rather than teaching[4,5].

The response acquired from the students at BPKIHS has been a positive one towards the benefits that can be procured from adopting PBL. The average of the scores for the positive statements was 3.59 and out of the seven positive statements in the questionnaire, all the questions had a mean score of 3.59 or more which is a response in agreement to the benefits of PBL. The average score for the negative statement in the questionnaire was 3.2 and out of the three questions, the responses generated to all three were three or more which is in disagreement to the statements that PBL may not be beneficial. Overall mean score was 3.47 which suggested that students had a positive attitude towards PBL method of teaching-learning activity.

The present study conducted at BPKIHS proved that this learning method is well accepted by students as it enhances their power of critical thinking[6,7]. The another study also found that teaching methods based on inquiry greatly reduced the achievement gap (underachievement)[8].

In line with BPKIHS findings a systematic review of the effects of PBL has found communication skills and coping with uncertainties are enhanced. Research resulting from 10 years of data from the University of Missouri, School of Medicine showed that PBL has a positive effect on the students' competency[9].

BPKIHS has similar experience with another study from Sloveniathat students who were exposed to PBL were better at solving more difficult problems, however, no significant difference was found with regard to their attitude regarding mathematics[10].

Like BPKIHS other universities have also successfully implemented and benefited from adopting PBL as a prime method in their teaching programs to enhance learning. Monash University, Maastricht University, Lake Erie and Libyan University were among the many who successfully implemented this technique as a prime teaching modality. More than 80% of all the medical schools in the United States currently have some form of PBL in their respective programs[9].

CONCLUSIONS

Overall mean score of 3.47 suggested that the students of second year MBBS at BPKIHS had a positive attitude towards PBL method of teaching-learning activity. Thus this type of teaching-learning method is preferable to students.

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Prevalence and Associated Factors with Respiratory Problems among Industrial Workers

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Keywords
Industrial workers, Prevalence, Respiratory problems.

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ABSTRACT

Background: In food processing industry, hazardous contaminants are generated. Flour dust has been introduced as one of the effective factors in the prevalence of respiratory disorders among the workers of the flour production factories.

Objectives: To assess the prevalence and associated factors with respiratory problems among industrial workers.

Methods: A cross-sectional descriptive study was used for the study. Non- probability purposive sampling technique was applied to choose flour industry and consecutive sampling technique was applied to select the sample from the industry. Hundred fifty respondents were taken as sample and self-administered structured interview questionnaire was used to collect the data.

Results: The study revealed that more than half 103 (68.70%) of the respondents had work-related respiratory problems. The prevalence of cough, phlegm, breathlessness, chest tightness and whistling problems were 100 (66.67%), 58 (38.67%), 65 (43.33%), 9 (6.0%) and 4 (2.67%). Among different demographic variables, association of respiratory problems was found with religion (p value = 0.015), income per months of respondents (p value = 0.034) and the information they had about work-related respiratory problems (p value = 0.000).

Conclusions: The study shows that there is higher prevalence of respiratory problems among industrial workers. Also, it shows there is association of respiratory problem with exacerbating factors and health checkup facility in the industry.

INTRODUCTION

"Occupational safety and health is vital to the dignity of work" - ILO Director-General Juan Somavia.

Industrialization is one of the important foundation stone for the development of a country. For the development of industrialization, it is essential to have enhanced productivity, which can be achievable with safe working environment. In most of the occupational establishments, adequate attention is not paid in making the work and the workplace safe, which may lead to the progress of various work related diseases and accidents[1].

Nepal is a developing country. It has not been able to manufacture much industrial goods. Manufacturing is still at the developmental stage. Major industries are woolen carpel, garments, textiles, agro-based, leather products, paper and cement. Other products are steel utensils, cigarettes, beverages, sugar, biscuits, noodles etc. There are mostly cottage and medium-scale factories. There are very few large scale industries[2].

In food processing industry, hazardous contaminants are generated when handling dry, bulk materials or smoke resulting from cooking emissions which can put employers into health risks[3]. As a result, workers are

at high risk for having asthma and Chronic Obstructive Pulmonary Disease (COPD)[4]. Additionally, flour dust has been introduced as one of the effective factors in the prevalence of respiratory disorders among the workers of the flour production factories[5].

Respiratory problems can be prevented by identifying harmful substances and measuring the degree of exposure to the work environment, control of exposure including maintenance of ventilation, use of personal protective equipment (PPE) and providing education on occupational disease to develop awareness[6].

According to the Global Estimates of occupational accidents and fatal work-related diseases in 2014, it is estimated that work-related diseases include seven percent of respiratory diseases[7]. According to WHO Global Plan of Action on Worker's Health (2008 - 2017), the top priority occupational disease of country is occupational respiratory diseases by 59.80%. Respiratory diseases are considered as one of the leading occupational diseases in most countries[8].

In Nigeria, Calabay, the study which was conducted among flour mill workers revealed that 14.17% productive cough, 27.06% unproductive cough, 13.02% chest pain, 3.15% dyspnea, 9.16% sneezing, 25.34% catarrh and 8.10% wheezing among test group whereas than control group showed 47.99% productive cough, 17.45% unproductive cough, 6% chest pain, 1.68% dyspnea, 3.42% sneezing, 19.62% catarrh and 3.84% wheezing. This study concluded that the high incidence of respiratory symptoms in the wheat flour mill workers may not only be attributed to chronic exposure to organic dust, but may be due to exposure to poisonous gases emitted from the flour mill[9].

Based on different related study, researcher shows that sex, age, education level, long work experience in dusty working environments, smoking habit, exposure to dust and previously identified chronic respiratory symptoms increases the risks of developing chronic respiratory symptoms. While higher education, effective dust control measures and training on occupational health and safety related to respiratory health problems were important determinant factors for maintaining the respiratory health of workers engaged in dusty[10].

Thus, occupational respiratory problem is one of the most important causes for the mortality and morbidity among occupational workers. Moreover, associated factors are responsible for the occurrence of respiratory symptoms among industrial workers.

OBJECTIVES

- To estimate the prevalence of respiratory problems among industrial workers.
- To explore associated factors with respiratory problems.

METHODS

A cross sectional descriptive method was used to determine the prevalence and associated factors with respiratory problems among 150 industry workers of Pokhara Industrial Estate in Kaski district. It was established on 2 October 1974 AD (16th Ashwin, 2031 BS). Non probability purposive technique was used to select flour industry. Non probability consecutive sampling technique was used to select sample. Semi-structured interview schedule was used. The interview schedule was divided into three parts:

Part I: Socio demographic performa

Part II: Prevalence of respiratory problems

Part III: Questions related to associated factors with respiratory problems

Validity of the instrument was established by developing the instruments on the basis of literature review, opinion of subject experts and research advisors. Reliability of the instrument was calculated by using Karl Pearson's correlation coefficient test by adopting Split Half technique. Pre-testing of instrument was conducted among 10% of the total sample size.

Data was collected after getting approval from Pokhara University, obtained written official permission from the head of industry; for Shree Gauri Shankar Foods Pvt. Ltd, Shree Fewa Pauroti Pvt. Ltd., Shree Taja Pauroti Pvt. Ltd., and Shree Himshree Food Pvt. Ltd. to give their permission for data collection. Privacy and confidentiality was maintained.

Ethical clearance was taken from Institutional Review Committee board of Pokhara University. The researcher obtained written official permission from the authority of industry.

After collection of data, editing was done manually on the same day of data collection. The collected data was coded and entered in SPSS 16.

RESULTS

Organization and presentation of related data

Section I: Socio-demographic characteristics of respondents

Table 1: Socio-demographic characteristics of respondents (n = 150)

Variables	Frequency (f)	Percent (%)
Age (in years)		
20 - 29	57	38.00
30 - 39	48	32.00
40 - 49	37	24.70
50 - 59	08	5.30
Sex		
Males	66	44.00
Females	84	56.00
Religion		
Hindu	135	90.00
Buddhists	05	3.30
Christians	09	6.00
Muslim	01	7.00
Ethnicity		
Dalit	06	4.00
Disadvantages non dalit terai people	16	10.70
Religious minorities	29	19.30
Relatively disadvantaged janajati	32	21.30
Relatively advantaged janajati	19	12.70
Upper caste group	48	32.00
Types of family		
Nuclear	70	46.70
Joint	78	52.00
Extended	02	1.30
Place of residence (Temporary)		
Urban	150	100.0
Rural	00	0.00
Educational status		
Illiterate	04	2.70
Informal education	23	15.30
Primary	39	26.00
Secondary	69	26.00
Higher secondary	13	8.70
Bachelor and above	02	1.30
Income per month (Rs.)		
Less than 5,000	03	2.00
5,001 - 10,000	46	30.70
10,001 - 15,000	100	66.70
More than 15,000	01	7.00
Information about work-related problems		
Yes	94	62.70
No	56	37.30
Sources of information (n=94)		
Different medias	05	5.30
Health personnel	05	5.30
Family members/ Friends	16	17.0
Work place	68	72.3

The data shows that out of 150 respondents, most 57 (38%) of the respondents were within 20 - 29 years majority were 84 (56.0%) females. Majority 135 (90%) of respondents were Hindus. Maximum 48 (32.0%) were from upper caste group. Likewise, there were maximum 78 (52.0%) respondents having joint family.

With regards to their temporary residence, 100% were from urban area. In terms of their educational status, majority 69 (46.0%) had secondary level education. In terms of income per months, 100 (66.7%) respondents earned Rs 10.001 - 15,000.

Most 94 (62.7%) of the respondents knew about the work related respiratory problems and in terms of source of information about work-related respiratory problems, majority 68 (72.3%) said their work place where as rest of them 16 (17.0%), 5 (5.3%) and 5 (5.3%) said family members/friends.

Section II: Distribution of respondents according to the presence of respiratory problems within 6 months

The data represents more than half 103 (68.70%) of the respondents had work-related respiratory problems and 100 (66.67%) respondents had only cough. Among 100, 79 (79.0%), 16 (16.0%) and five (5.0%) respondents suffered from cough for less than seven days, more than seven days and equal to seven days respectively. Among 150 respondents, 58 (38.67%) respondents had presence of only phlegm. Among them 43 (74.1%), 14 (24.1%) and 1 (1.7%) respondents had clear color, yellow color, and red color of phlegm respectively. Also, among 103 respondents, there was presence of breathlessness, chest tightness and whistling problems among 65 (43.33%), nine (6.0%) and four (2.67%) respondents.

Section III: Distribution of respondents according to biological factors

Total four (2.7%) had respiratory problems among their family members. Among four respondents, there were presence of respiratory problems only in three (75%) siblings and two (50%) grandparents. Asthma was present only in two (50%) and pneumonia only in two (50%).

Distribution of respondents according to their personnel habits

Total 15 (10%) respondents had personal habits of smoking. Among 15 respondents, majority eight (53.3%) respondents used one to five cigarettes per day where as rest three (20%) respondents used less than one cigarette per day. With regards to the type of fuel used, maximum 145 (96.7%) respondents used LPG gas.

Distribution of respondents according to exposure related factor

Majority 48 (32.0%) were working for two to five years, 47 (31.3%) were working for above 10 years, 28 (18.7%) were working for six to ten years and 27 (18.0%) were working for up to one year. With regards to work per day, majority 142 (94.2%) of respondents working for more than eight hours whereas six (4.0%) respondents worked for equal to eight hours and two (1.3%) respondents worked for less than eight hours.

With regards to the most common exacerbating factors of their respiratory problems, most 63 (42.0%) of the respondents said prolonged working hours. With regards to the most common alleviating factors of their respiratory problems, most 52 (34.70%) of the respondents said that rest. With regards to the most common ways of managing their respiratory problems, most 83 (55.3%) respondents said health check ups.

Distribution of respondents according to occupational safety

Data shows that with regards to occupational safety, most 88 (58.7%) of respondents said there is facility of health checkup when it required and majority 135 (90%) said that there is insurance facility.

Distribution of respondents according to physical environment of industry

Data shows that with regard to the physical environment, 100% respondents gave their positive response towards available of adequate space, ventilation and light.

Majority 101 (67.3%) respondents said most common technique of waste management is Municipality tipper whereas 38 (25.3%) said burning, seven (4.7%) said decomposition and four (2.7%) said dumping.

Section IV

Table 2: Association between demographic factors and respiratory problems (n=150)

Variables	Respiratory problems		χ^2	*p*-value	DF
	Yes	No			
Age (Years)					
20 - 29	41 (71.9%)	16 (28.1%)			
30 - 39	31 (64.6%)	17 (35.4%)	2.221	0.528NS	3
40 - 49	24 (64.9%)	13 (35.1%)			
50 - 59	7 (87.5%)	1 (12.5%)			
Sex					
Male	42 (63.6%)	24 (36.4%)	1.386	0.239NS	1
Female	61 (72.6%)	23 (27.4%)			
Religion					
Hindu	89 (65.9%)	46 (34.1%)	5.963	0.015#	1
Others	14 (93.3%)	1 (6.7%)			
Ethnicity					
Others	71 (69.6%)	31 (30.4%)	0.131	0.717NS	1
Upper caste group	32 (66.7%)	16 (33.3%)			
Types of family					
Nuclear	50 (71.4%)	20 (28.6%)	0.465	0.495NS	1
Joint and extended	53 (66.2%)	27 (33.8%)			
Educational status					
Illiterate	2 (50.0%)	2 (50.0%)	0.666	0.415NS	1
Others	101 (69.2%)	45 (30.8%)			
Income per month (Rs.)					
Less than and equal to 10,000	28 (57.1%)	21 (42.9%)	4.492	0.034#	1
More than 10,000	75 (74.3%)	26 (25.7%)			
Information about work-related problems					
Yes	82 (87.2%)	12 (12.8%)	4.344	0.000#	1
No	21 (37.5%)	35 (62.5%)			

= significant, NS: Non significant, χ^2 value at 1 df is 3.84, *p value* is considered significant

Table 2 shows there is association of respiratory problems only with religion (p value = 0.015), income per months of respondents (p value = 0.034) and the information they had about work-related respiratory problem (p value = 0.000).

Section V

Table 3: Association between respiratory problems with the associated factors (n=150)

Associated factors	Respiratory problems		χ^2	*p*-value	DF
	Yes	No			
Respiratory problems among family					
Yes	2 (50.0%)	2 (50.0%)	0.666	0.415 NS	1
No	101 (69.2%)	45 (30.8%)			
Smoking habit					
Yes	13 (86.7%)	2 (13.3%)	2.510	0.113 NS	1
No	90 (66.7%)	45 (33.3%)			
Fuel used					
LPG gas	101 (69.0%)	45 (30.8%)	0.618	0.432 NS	1
Others	2 (50.0%)	2 (50.0%)			
Working year					
Up to 1 year	17 (63.0%)	10 (37.0%)			
2-5 years	32 (66.7%)	16 (33.3%)			
6-10 years	24 (85.7%)	4 (14.3%)	4.791	0.188 NS	3
Above 10 years	30 (63.8%)	17 (36.2%)			
Work hour per day					
Less than and equal to 8 hour	5 (62.5%)	3 (37.5%)	0.149	0.699 NS	1
More than 8 hour	98 (69.0%)	44 (31.0%)			
Exacerbating factors					
Prolong working hour	52 (82.5%)	11 (17.5%)			
Lack of health services	26 (52.0%)	24 (48.0%)			
Not using protective devices	11(64.7%)	6 (35.3%)	12.231	0.007#	3
Negligence of health services utilization	14 (70.0%)	6 (30.0%)			
Management					
Ignorance	6 60.0%)	4 (40.0%)			
Self-limitation	19 (65.5%)	10 (34.5%)	1.005	0.800 NS	
Health check up	57 (68.7%)	26 (31.3%)			3
Self-medication	21 (75.0%)	7 (25.0%)			
Check up					
Yes	69 (78.4%)	19 (21.6%)	9.392	0.002#	1
No	34 (54.8%)	28 (45.2%)			

= significant, NS: Non significant, χ^2 value at 1 df is 3.84, *p-value* is considered significant

The Table 3 shows there is association of respiratory problems only with exacerbating factors (p value = 0.007) and available of health check up facility in their industry (p value = 0.002).

DISCUSSION

Prevalence of respiratory problems

Similar study was conducted among wheat flour mill workers in Ibadan, Nigeria among 30 internal controls and 121 external controls revealed that 54% of the flour-millers reported at least one respiratory symptom[11]. Study conducted among 200 exposed and 200 non-exposed groups of flour mill workers in Southern Egypt revealed 87.5% productive cough, 60% dyspnea and 7% chest pain among exposed group which is somewhat similar to our study[12].

Associated factors of respiratory problems

A similar study was conducted among 229 participants to determine the prevalence and factors associated with sensitization to wheat flour and α-amylase in bakers in Douala, Cameroon, Central Africa shows that most of respondents (54.6%) were working for more than 8 hours per day and majority (71.9%) were not smokers[13].

Association of respiratory problems with demographic variables and its associated factors

A cross-sectional study was conducted among 79 cases and 73 controls in Tanzanian primary coffee-processing factories, Kilimanjaro shows the differences between prevalence between exposed and unexposed groups were not statistically significant but it was found to be higher among exposed groups which recommended that interventions for reduction of dust levels and provision of respiratory protective equipment are necessary[14].

CONCLUSIONS

The result of the study showed that most 103 (68.70%) of the respondents had work-related respiratory problems. the prevalence of cough, phlegm, breathlessness, chest tightness and whistling problems were 100 (66.67%), 58 (38.67%), 65 (43.33%), 9 (6.0%) and 4 (2.67%). Among different demographic variables, association of respiratory problems was found with religion (p value = 0.015), income per months of respondents and the information they had about work-related respiratory problem. The result shows there is association of respiratory problems only with exacerbating factors and available of health checkup facility in their industry.

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Knowledge and Attitude on Legalization of Abortion among Undergraduate Students

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ABSTRACT

Background: Abortion is public health concern in many parts of the world and is also contentious issue with religious, moral, cultural and political dimensions. Awareness program has most essential role to prevent the unusual death of mothers in the world.

Objectives: To assess the knowledge and attitude on legalization of abortion among undergraduate students.

Methods: A cross-sectional descriptive research design was used for this study. Proportionate stratified sampling technique and simple random sampling technique was used to select the subjects. Total 90 students were chosen as a sample and structured self-administered questionnaire was used to collect data.

Results: The mean age of the respondents was 19.82. Most 58 (64.40%) of the respondents were females; majority 76 (84.40%) of the respondents were of upper caste group. Most 85 (94.4%) of the respondents had adequate knowledge on legalization of abortion and majority 83 (94.30%) of the respondents had positive attitude towards legalization of abortion. The study shows that the most knowledgeable area was on concept of abortion (88.55%) and the least knowledgeable (58.00%) area was on method of abortion. Factors like age, gender, residence and educational faculty were not found to be associated with knowledge on legalization of abortion.

Conclusions: Undergraduate students had good level of knowledge and attitude on legalization of abortion. Highest knowledge was present in concept of abortion and lowest in methods of abortion. More awareness program should be accessible to undergraduates in order to increase the level of knowledge.

Keywords
Attitude, Abortion, Knowledge, Legalization, Undergraduates.

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INTRODUCTION

"Abortion: The guilt hurts more than labor" - Joshua Michael Levinson.

Abortion means ending of pregnancy by removal or forcing out from the womb or fetus or embryo before the age of viability[1]. Induced abortion is the deliberate termination of pregnancy before the fetus attained viability i.e. become capable of independent extra-uterine life. Abortion is a public health concern in many parts of the world and is also contentious issue with religious, moral, cultural and political dimensions[2]. Abortion can occur spontaneously in which case it is often called a miscarriage. It can also be purposely caused which in case it is known as Induced

abortion. The term abortion most commonly refers to the induced abortion of human pregnancy[1].

Abortion was legalized in Nepal in 2002, the procedural under was passed in 2003 and the first CAC services was started at the Maternity Hospital, Kathmandu in March 2004. Two years later (April 2006) there were 122 approved facilities (76 Governmental organizations) located across the districts[3]. The abortion law allows women to terminate their pregnancy under the following conditions: Pregnancies of 12 weeks gestation or less for any woman on her own decision, pregnancies of 18 weeks gestation if the pregnancy is a result of rape or incest, and pregnancies of any duration with the recommendation of an authorized medical practitioner if the life of the mother is at risk, if her physical or mental health is at risk, or if the fetus is deformed. However, the law prohibits abortions done without the consent of the woman, sex selective abortions, and abortions performed outside the legally permissible criteria. If the girl is minor and when mother is mentally incompetent the consent of legal guardians is required to abort the pregnancy[4].

Out of estimated 46 million pregnancies around the world that are terminated through induced abortion, about 19 million of them are outside the legal system according to World Health Organization (WHO). Most abortions occur in developing countries 33 million annually compared with seven million in developed countries, a disparity that largely reflects the relative population distribution[5].

In developing countries as a whole it is estimated that five million women are admitted to Hospital for treatment of complications from induced abortions each year. This equates to an average rate of five to seven per 1000 women per year in all developing countries, excluding China[6]. Research studies conducted in United States have reported that higher rates of abortion occur among college aged women with 60% of unintended pregnancies among 20 - 24 years old[7].

It is estimated that among the total pregnancies each year in South and South-East Asia, about one third are unplanned pregnancies which leads to abortion. About 30% of pregnancies in Bangladesh, 21% in India and 35% in Nepal are unplanned that ultimately increases the rate of abortion[8].

Even after the legalization of abortion in Nepal, unsafe abortion practices are prevalent in society. Sixty six percent of women receiving post abortion services in the Tertiary Hospitals choose unsafe abortion methods. Forty four percent of women do not have knowledge regarding the legalization of abortion[9].

Conclusions: youth participation in creating awareness is an important implementation strategy of adolescent health. In this respect, undergraduate students in the University is the best place for providing the information related to their health where maximum number of adolescents are representing different areas as well background. Thus, providing information about abortion among the undergraduate students (most vulnerable group) is the best step for creating public awareness. In the context of Nepal, to access the youth the educational institution is regarded as a best source where target age group can be easily accessed.

OBJECTIVES

- To assess level of knowledge on legalization of abortion among undergraduate students.
- To determine the attitude towards legalization of abortion among undergraduate students.
- To find out the association between level of knowledge regarding legalization of abortion and selected demographic variables of undergraduate students.

METHODS

Descriptive cross sectional research design was employed to explore the knowledge and attitude regarding legalization of abortion among 90 undergraduate students of Health and Allied Sciences of Pokhara University. Proportionate stratified sampling technique was used in this study. The total population was divided into four strata and students were divided into proportion of 70% from each faculty. Lottery method of simple random sampling was used to select the samples. Data was collected using self-administered structured questionnaire. The research instrument was divided into three sections.

Section I: Socio demographics

Section II: Knowledge regarding abortion

Section III: Attitude towards abortion

For the validity of research pretesting of the instrument was done in 10 respondents in LA Grande International College, Reliability was maintained by using split half technique and obtained 0.93. Official written permission was obtained from concerned authorities and Pokhara

University before data collection. The data collection period was extended from 3 May 2014 (2071/1/20 BS) to 14 May 2014 AD (2071/1/31 BS). Written consent was taken voluntarily from the participant with assured confidentiality and anonymity. Self-administered structured schedule was administered to assess the knowledge on legalization of abortion among undergraduates. Ethical clearance is obtained from Research Committee. Formal permission and approval sheet was obtained from college. SPSS version 16.0 was used for entering and tabulating data. Frequency and percentage was used to analyze socio-demographic characteristics. Descriptive statistics as mean, standard deviation and mean percentage was used to assess the level of knowledge on postnatal care. The association of level of knowledge with their selected demographic variables was analyzed by using Chi-square test.

RESULTS

Organization and presentation of related data

Section I: Description of demographic variables of undergraduates.

Section II: Level of knowledge on legalization of abortion among undergraduates.

Section III: Attitude level on legalization of abortion among undergraduates.

Section IV: Association of the level of knowledge on legalization of abortion with their selected demographic variables.

Section I: Description of demographic variables of undergraduates

Table 1: Socio-demographic characteristics of respondents (n=90)

Variables	Frequency (f)	Percentage (%)
Age		
≤20 years	73	81.10%
>20 years	17	18.90%
Gender		
Males	32	35.60%
Females	58	64.40%
Ethnicity		
Upper caste religion	76	84.40%
Others	14	15.60%
Residence		
Rural	16	17.80%
Urban	74	82.20%
Type of family		
Nuclear	80	88.80%
Extended	10	11.20%
Family income		
≤35000	59	65.60%
≥35000	31	34.40%
Marital status		
Unmarried	89	98.90%
Married	01	01.10%
Education Faculty		
BPH	30	33.30%
BSc Nursing	14	15.60%
BMLT	17	18.90%
B. Pharm	29	32.20%
Source of information		
Radio/television	40	44.40%
Family members	06	06.70%
Friends	22	24.40%
Health service providers	06	06.70%
Others	16	17.80%

Others=Dalit, Religious minorities, relatively disadvantages Janajati, Relatively advantages Janajati

The data depicted in Table 1 shows that most 73 (81.10%) of the respondents were ≤20 years old and the mean age of the respondents was 19.82. Most 58 (64.40%) of the respondents were females; majority 76 (84.40%) of the respondents were of upper caste group whereas with regard to residence most 74 (82.20%) of the respondents belong to urban area and majority 80 (88.80%) of the respondents had nuclear type of family. With regards to family income (Rs/month), most 59 (65.60%) of the respondents had family income of ≤35000, majority 89 (98.90%) of the respondents were unmarried and in terms of education faculty 30 (33.30%) were of BPH, 29 (32.20%) of B. Pharma, 17 (18.90%) from BMLT and 14 (15.60%) were from BSc Nursing students. Regarding the source of information most 40 (44.4%) of the respondents got information from radio/TV.

Distribution of respondents based on knowledge on concept of abortion shows that most 56 (62.2%) respondents had knowledge about abortion. Ninety (100%) answered the correct meaning of safe abortion, 85 (94.4%) mentioned the correct definition of illegal abortion. Majority 89 (98.9%) of them had idea about the correct term for spontaneous abortion. Majority 79

(87.80%) knew that abortion is legal up to 12 weeks of gestation and majority 86 (95.60%) had knowledge that abortion followed by sex selection of baby is illegal. Majority 85 (36.5%) mentioned unmarried pregnancy is the most common cause of abortion.

Distribution of respondents based on knowledge regarding criteria required for abortion shows that majority 88 (97.80%) mentioned that Government approved Hospitals/clinics are the best place for providing abortion services. Most 90 (41.90%) said there should be trained health care providers. most 75 (33.8%) mentioned knowledge on right to informed consent and right to privacy equally. Similarly, most 43 (47.8%) had correct knowledge about abortion in ectopic pregnancy.

Distribution of respondents according to knowledge on laws regarding abortion: Majority 75 (83.30%) had correct knowledge that a pregnant woman can terminate pregnancy on her own will up to 12 weeks of gestation. Seventy four (82.2%) had knowledge that consent of women herself is only required for the termination of pregnancy up to 12 weeks of gestation and 66 (73.3%) mentioned that consent of legal parents is required to undergo abortion in less than 16 years old. Majority 85 (94.4%) are familiar that abortion can be done irrespective to gestational age, 41 (45.60%) had mentioned correct knowledge about the time period for termination of pregnancy due to rape/incest. Similarly, majority 87 (96.7%) had knowledge that consent of nearest guardian is required to procure abortion when pregnant women is mentally incompetent. Majority 79 (87.8%) illustrates that without the will of pregnant women Government restrict abortion within 12 weeks of gestation.

Distribution of respondents according to knowledge on method of abortion: Data shows that, 60 (66.7%) of respondents and 47 (52.2%) of respondents had knowledge about method of abortion up to nine weeks and after nine weeks respectively. Similarly, 50 (55.6%) of respondents correctly mentioned that six weeks is earliest period of gestation at which medical abortion can be done.

Distribution of respondents based on knowledge of complications of abortion: Data depicts that majority 89 (98.9%) of the respondents correctly mentioned that heavy bleeding is the most common complication of unsafe abortion and most 53 (58.9%) are not familiar with the complication of medical abortion.

Distribution of respondents based on knowledge on advices given after abortion procedure: The data shows that most 45 (50%) of the respondents illustrates that after abortion ovulation will return back within one month time period, majority 70 (77.8%) mentioned that IUCD cannot be used immediately after medical abortion, with regards to follow up after surgical abortion, 55 (61.1%) correctly mentioned two weeks period for follow up. With regards to counseling to be provided to the women after abortion all of the respondents have some sort of knowledge, 84 (41.4%) respondents said safe sexual practices.

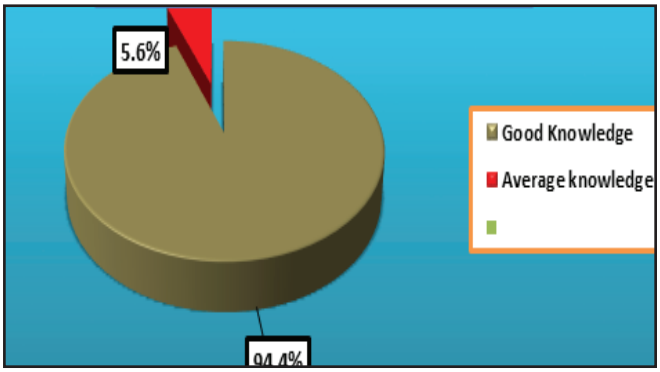
Distribution of respondents based on knowledge regarding other questions related to abortion shows that with regards to advantages of legalization of abortion, most 85 (42.30%) mentioned that unwanted pregnancy within criteria can be legally aborted, most 77 (36.70%) with regards to disadvantages of legalization of abortion illustrates that they may ignore contraception as abortion is available for termination of pregnancy. Regarding the ways to make abortion safe, 82 (36.1%) mentioned training health personnel to provide safe abortion services.

Knowledge aspect of respondents regarding legalization of abortion: Data shows that highest knowledge was in the area of concept of abortion with the mean percentage of 88.55, with mean and SD of 7.97 $\pm$0.99. In the area of method for abortion, the mean percentage was 58.00 with mean and SD of 1.74 $\pm$0.84. Similarly, mean percentage in the area of laws regarding abortion was 80.42 with mean and SD 5.63 $\pm$1.16.mean percentage in the area of criteria required for abortion procedure, the mean percentage was 80.12 with mean and SD 6.41 $\pm$1.03. In the area of other questions related to abortion the mean percentage was 79.66 with mean and SD 7.17 $\pm$1.53. Similarly, about abortion procedure the mean percentage is 64.33 with mean and SD 3.86 $\pm$1.06. Moreover, in the area of complication of abortion the mean percentage is 62.00 with mean and SD 1.24 $\pm$0.45.

Attitude of respondents towards legalization of abortion: Data shows the frequency and percentage of respondent's attitude towards each statement. With regards to positive statements majority (83.3%) respondents agreed that abortion is a reproductive right of women, (90.0%) on any pregnancy can be legally aborted within 12 weeks of gestation, (83.3%) on, if the girl is minor the consent of legal guardian is required to abort the pregnancy. Majority (63.3%) agreed that legalization of abortion controls the population. Moreover, (70.0%) agreed that abortion can be done irrespective to the gestational age when pregnancy risks the health of mother. With regards to negative statements most (64.4%)

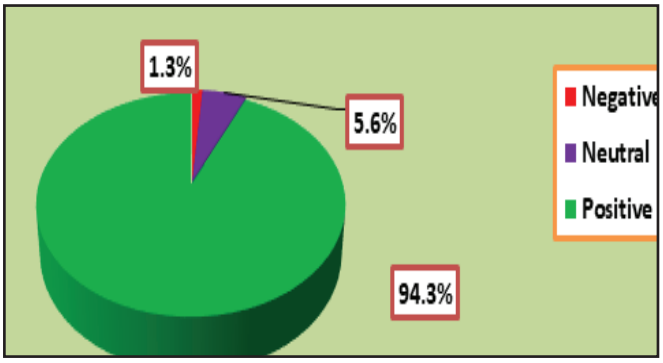
disagreed that abortion can hinder women empowerment, 37.8% agreed that legalization of abortion allows youth to freely engage in sexual activities and 57.8% agreed that termination of pregnancy due to rape/incest shouldn't be limited up to 18 weeks. Likewise, 83.3% disagreed that complications of the induced abortion shouldn't be shared with other and 47.8% disagreed that if pregnancy risks the mother, abortion can be done without the consent of women.

Fig 1: Knowledge level of respondents on legalization of abortion



The data in Fig 1 illustrates that 85 (94.4%) of the respondents had good level of knowledge on legalization of abortion, 05 (05.60%) had average knowledge while nobody had poor knowledge on legalization of abortion.

Fig 2: Attitude level of respondents towards legalization of abortion



The data depicted in figure 2 explains that majority of the respondents 83 (94.30%) had positive attitude towards legalization of abortion, 05 (05.60%) had neutral and 02 (01.30%) of respondents had negative attitude towards legalization of abortion.

Section IV: Association of level of knowledge with their selected demographic variables

Table 2: Association of level of knowledge with their selected demographic variables (n=90)

SN	Variables	Total scores		χ^2 value	DF	p-value
		Median ≤33	**Median >33**			
1	**Age in years**					
	≤20 years	36	37			
	>20 years	12	05	2.507	1	0.113 NS
2	**Gender**					
	Males	20	12			
	Females	28	30	1.676	1	0.195 NS
3	**Ethnicity**					
	Upper caste	42	34			
	group	06	08	0.731	1	0.393 NS
	Others					
4						
	Residence	09	07			
	Rural	39	35	0.067	1	0.796 NS
	Urban					
5						
	Type of family	41	36			
	Nuclear	07	06	1.256	1	0.262 NS
	Extended					
6						
	Education	17	13			
	faculty	06	08	6.137	3	0.197 NS
	BPH	12	05			
	BSc Nursing	19	10			
	BMLT					
	B. Pharm					

NS=Non significant; χ^2 2.706 at 1 DF, 6.251 at 3 DF

Data represented on Table 2 reveals that there is no significant association between selected variables like age, gender, and ethnicity, type of family and education faculty.

DISCUSSION

In this study 85 (94.4%) of the respondents had adequate level of knowledge on legalization of abortion. The result of our study was supported by the study conducted in Indian medical students in 27 different colleges in Maharashtra, India to assess the knowledge and attitude regarding abortion. The study reveals that 95.7% of students had very good knowledge regarding abortion[11].

In present study majority 83 (94.30%) of the respondents had positive attitude towards legalization of abortion. This finding was supported by the study conducted in Nepal to assess the knowledge and attitude about abortion among the adolescent students of higher secondary school. The study result shows that 60% of the respondents have positive attitude towards safe abortion and its legalization[10].

CONCLUSIONS

The results of the study showed that most 85 (94.4%) of the respondents had adequate knowledge on legalization of abortion. The total score for knowledge level was 44 with the mean score of 34.05. Majority 83 (94.30%) of the respondents had positive attitude towards legalization of abortion. The total score of attitude was 30 with the mean score of 24.48. Factors like age, gender, residence and educational faculty were not found to be associated with knowledge on legalization of abortion. The study shows that the most knowledgeable area (88.5%) was on concept of abortion and the least knowledgeable area (58.50%) was on method of abortion.

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Cytokine Induced Killer (CIK) Cells Based Adoptive Immunotherapy

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Cancer, Cytokine induced killer (CIK) cells, Immunotherapy, Viral infection.

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ABSTRACT

Cytokine induced killer (CIK) cells has been increasingly used in adoptive immunotherapy against various cancers and viral infections. This review summarizes the basic overview of CIK cells as a therapeutic immunocyte. Herein, the basic concepts on CIK cells, their general characteristics, approaches in enhancing their functions, cytotoxic mechanism of CIK cells and their therapeutic benefits in tumors and viral infections are explored.

INTRODUCTION

Immunotherapy has emerged as a promising treatment option to boost immune response to defense against diseases and infections. The two immunotherapeutic options include active and passive immunotherapy. Active immunotherapy mainly refers to vaccines, immune adjuvants and cytokines which can activate endogenous immune system. On the other hand, the latter consists of immune modulating antibody-based therapy and adoptive immunotherapy which provides or strengthen immune reaction in patients by infusing antibodies or effector cells produced *in vitro*[1].

ADOPTIVE IMMUNOTHERAPY

Over the past two decades, adoptive transfer of immunocytes has been increasingly used in treatment of cancers and viral infections[2]. The immunocytes for adoptive immunotherapy falls into two distinct categories. The first included lymphokine activated killer cells (LAK cells), cytokine induced killer cell (CIK cells) and natural killer (NK cells) that recognizes antigens in major histocompatibility complex (MHC) unrestricted manner. The other group included tumor infiltrating lymphocytes (TIL) and cytotoxic T lymphocytes (CTL) that recognize antigens presented by MHC molecules[1]. Previously, TIL and LAK used to be the best candidate for adoptive immunotherapy. However, limitations to obtain sufficient number of immune effectors cells, higher alloreactivity and inability to show antitumor property effectively attracted the researchers to seek for additional and better-tolerated strategy[3]. With the initial report of Schmidt-Wolf *et al*[4] (1991) CIK cells has been identified as a promising candidate of adoptive immunotherapy that holds all the desired properties.

CYTOKINE INDUCED KILLER CELLS AND THEIR GENERAL CHARACTERISTICS

CIK cells are *ex-vivo* expanded T cells that display phenotypic and functional characteristics of both NK cells and cytotoxic T cells[5]. As these cells were generated under the influence of cytokines and mediate the potent MHC-unrestricted cytotoxicity against various types of cancer,

they were named as "cytokine induced killer" cells. Briefly, CIK cells were generated by culturing interferon gamma (IFN-γ) activated human peripheral blood mononuclear cells (PBMC) in presence of cluster of differentiation 3 (CD3) antibody, interleukin 1 (IL-1) and IL-2 for 21 days[4,6,7]. These *ex-vivo* expanded cells were used for targeted treatment of human disease[1,8].

The CIK cells consisted of heterogeneous subsets based on surface expression of CD3 and CD56 molecules. The major population positive for both CD3 and CD56 ($CD3^+CD56^+$) (40 - 80%) exhibited MHC unrestricted antitumor activity against malignant cells lines and believed to be the type II NK-T cells[9-12]. The remaining other populations included; $CD3^+CD56^-$ (T cells, 20 - 60%), and a small fraction of $CD3^-CD56^+$ (NK cells, <10%) cells[11].

$CD3^+CD56^+$ cells are rare (1% to 5%) in human PBMC. However, enhanced increase in this major effector population was achieved during *ex-vivo* expansion[13]. Moreover, the degree of expansion of $CD3^+CD56^+$ cells vary among individual patients (mostly from three to thousand folds)[4,6,9,14]. Additionally, the $CD3^+CD56^+$ cells were derived from $CD8^+$ T cells suggesting their cytotoxic nature[9].

SOURCE FOR CIK CELLS

PBMC is given the first priority to generate these cells in humans[4,15]. However, expansion of human CIK cells from cord blood had been achieved[16,17]. Moreover, CIK cells were generated from splenocytes[18] thymus[13,19] lymph nodes[20] and bone marrow[19] of murine models.

IMPROVING CIK CELL PROLIFERATION AND FUNCTION

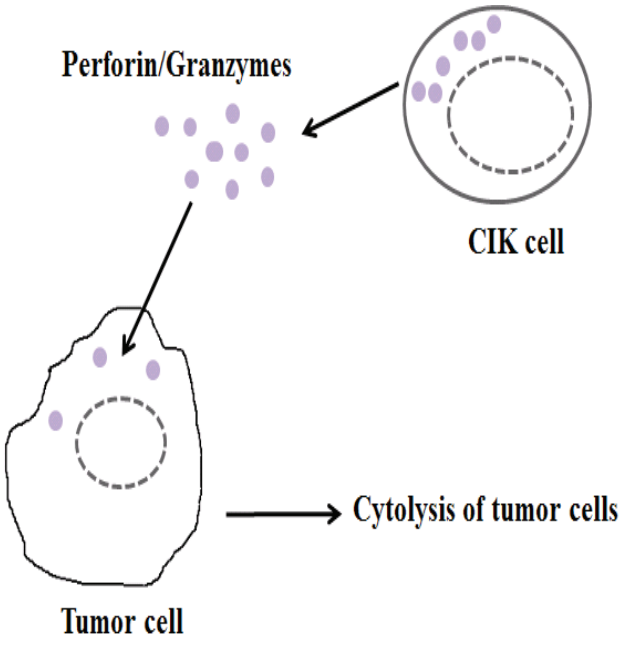
Since the first report of CIK cells by Schmidt-Wolf *et al*[4] in 1991, various groups of researchers focused on improving both the expansion and anti-tumor cytotoxicity of CIK cells. The use of cytokines other than IL-2 or the co-culture of CIK cells with dendritic cells (DCs) and even the suppression of regulatory T cells (Tregs) within the CIK cell culture are the major modification being performed[21-24]. As such, use of various cytokines and antibodies like IL-6[25] IL-15[26,27], IL-21[28], and anti-CD28[13,29]. These modifications enhanced the expansion and anti-tumor activity of CIK cells.

CIK cells primed with dendritic cells (DC-CIK cells) have been shown to promote their functions. As such, metastatic non-small cell lung cancer[30] cervical cancer[31] advanced renal cell carcinoma[32] and colorectal cancer[33] patients receiving DC-CIK cells improved the immune function, reduced the recurrence rate and prolonged the survival time. This might be due to reduced expansion of Treg cells[34]; however the detailed underlying mechanism is still unknown.

CYTOTOXIC MECHANISM OF CIK CELLS

CIK cells are endowed with potent MHC-unrestricted cytotoxicity against both syngeneic and allogenic hematological and solid malignancies. The antitumor activity is mainly associated with $CD3^+CD56^+$ fraction as this fraction of cells has higher proportion of $CD8^+$ cells. The exact mechanism involved in tumor recognition and killing by CIK cells is not completely known. However, cytotoxic granules like perforin and granzymes were proposed to be a mediator of cytolysis[35] as shown in Fig 1. It is believed that CIK cells approach the target cells by chemotaxis, and then release a number of toxins and chemicals into the medium to induce the apoptosis, necrosis and lysis of target cells. That's why in the early stage (5 hours) CIK cells induce apoptosis while in later stage (14 hours) they induce necrosis for cell lysis[36].

Fig 1: Mechanism of cytolysis of tumor cells by CIK cells (Perforin and granzymes released by CIK cells in tumor environment cause lysis of the tumor cells)



CIK CELLS IN IMMUNOTHERAPY

The dual T_H1 and NK properties that can kill abnormal cells such as cancerous cells have promoted these CIK cells as a unique immunotherapeutic approach[3,18,37]. There are

numerous research groups who have proven the efficacy and safety of CIK cells to treat a variety of cancers through clinical studies. Outcome of some of those clinical trials is as shown in Table 1.

Table 1: CIK cell based oncotherapy in phase-I clinical trials

Cancer type	Cases	Clinical Response (Cases)	Ref
Colon carcinoma; Follicular lymphoma; Renal cell carcinoma (RCC)	10	Complete response	[38]
Hepatocellular carcinoma (HCC)	13	Reduced tumor volume; Improved symptoms; Decreased HBV-DNA load	[14]
Relapsed Hodgkin's disease (HD); Non-Hodgkin's lymphoma (NHL)	9	Partial response (2); Stable disease (3)	[39]
Relapsed acute myeloid leukemia (AML); Chronic myeloid leukemia (CML); HD; Acute lymphoblastic leukemia (ALL); Myelodisplastic syndrome (MDS)	11	Complete response (3); Stable disease (1)	[40]
HCC	85	Decreased recurrence rate	[41]
Resected HCC	127	Increased DFS	[42]
Metastatic RCC; HCC	12	Complete response (3); Partial response (1); Stable disease (2)	[43]
AML; ALL	5	Partial response (1)	[44]
B-cell NHL; AML; Multiple myeloma; ALL; MDS; HD; Chronic lymphoid leukemia	18	Complete response (5)	[45]
B-cell NHL	9	Complete response	[46]
Multiple myeloma	1	Complete response	[47]

Moreover, a few studies have explored the therapeutic benefit of CIK cells against viral infection that cause mortality and morbidity in immunecompromised individuals. As such, CIK cell therapy has been shown to be effective in targeted lysis of cells infected with human immunodeficiency virus[48]. Epstein–Barr virus[49] or cytomegalovirus[50]. This antiviral activity could be due to the production of IFN-γ, TNF-α, perforin and granzyme B by CIK cells.

CONTRAINDICATIONS OF CIK CELL IMMUNOTHERAPY

There are many clinical studies using the CIK cells as immunotherapy. Till date, no severe side effects have been reported after CIK treatment. The most common side-effects were fever, chills, headache, rash, nausea, and vomiting occurring during or after transfusion. These could easily be treated with symptomatic therapy in case they did not resolve on their own within 24 hours[51,52]. This indicates that CIK therapy is safe for clinical application although larger population cohorts should be investigated.

CONCLUSIONS

CIK cells were used as an adoptive immunotherapy against various cancers and some viral infections. However, the effectiveness varies with different clinical settings, mainly due to different approaches followed in their generation. Hence, further studies are warranted to enhance the expansion and cytolytic function of the CIK cells. Moreover, the beneficial use of CIK cell in various viral infections needs to be investigated.

Competing interests

We declare no competing interest.

Financial disclosure

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Mechanics of Question Paper Setting

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ABSTRACT

The goal of medical education is to produce the physician we would like to see if we are sick - Melinkoff

In all educational developments, one of the most troublesome questions is that of evaluation. Written examination is the most widely used tool in evaluation and assessment of the competency of the medical students. At present, questions are prepared casually just before the examination and are not subjected to any quality check, which may lead to confusion or wrong understanding of the questions by the students. This article is aimed at providing guidelines and a scientific method to frame good question papers to improve the quality of evaluation and assessment of medical students.

INTRODUCTION

Written examination is still the widely used tool of evaluation both in formative and summative evaluation. It cannot be replaced entirely by any other method. In contrast to practical and oral examinations, written examination leaves a record for revaluation in case of a controversy. The instrument used in the written examination is the question paper.

Assessment is usually done at two levels: Internal assessment periodically, usually at the end of each term conducted by the departments known as formative assessment, and final examination, usually at the end of each year conducted by the University, known as summative assessment[1]. For these examinations question paper setting is an important task. At present, questions are prepared casually just before the examination and are not subjected to any quality check, which may lead to confusion or wrong understanding of the questions by the students. Moreover, the question paper quite often lacks validity, reliability, relevance and objectivity. The quality of question paper can be improved by adequate preparation[1-3].

Therefore this article is aimed at providing guidelines and a scientific method to frame good question papers to improve the quality of evaluation and assessment of medical students.

QUESTION PAPER SETTING/PREPARATION

The steps involved in question paper setting are[4-6]:

1. Decision on the design of question paper
2. Preparation of a blue-print of the question paper
3. Preparation of a model question paper
4. Preparation of a marking scheme
5. Refining the question paper

6. Editing the question paper
7. Review of the question paper

1. DESIGN OF A QUESTION PAPER

The decision of the design of question paper is a policy decision, which has to be made by the Dean/Principal of the college, in consultation with the Medical Education Unit. The recommendations of Nepal Medical Council and affiliated Universities are to be followed. The points to be decided are:

1. Weightage to be given to different forms of questions i.e. how many essays, short answer type questions, very short answer type questions, or multiple choice questions should be decided to make a balanced question paper. This also involves decisions on the number of question papers in a subject, number of marks, total number of questions, and time allotted for the paper. The number of sections in each question paper also requires to be decided.
2. Weightage to be given to different learning objectives and to different topics or areas of the subject. If the educational objectives were already divided into must know, desirable to know and nice to know categories, the same weightage can be adapted in the question paper. Since the terminal examinations are for certification of the competency of a learner in terms of knowledge and skills that he is supposed to acquire at the end of a course, the question paper should not be made very difficult. On the contrary, the question paper should aim at finding out whether the minimal skills/knowledge required has been achieved by the student or not.
3. Guidelines regarding the use of options, nature of sections and difficulty level of the paper are also required to be delineated.

   Once the above decisions are made, it is advisable to write them in clear and simple terms. Such a document will help at a later stage to write the instructions to the Question Paper setters.

2. BLUE-PRINT OF THE QUESTION PAPER

Blue-print of a question paper, also known as table of specifications, is a two-dimensional chart giving placement of different questions (in terms of marks and number of questions) in respect of objectives tested by the item(s), content area under which item is framed, and the form of question (Table 1).

Table 1: Blue-print of a question paper

Objectives/ content area	Knowledge (Recall)			Understanding (Interpretation)			Application (Problem solving)			Skill	Total
	O	SA	LA	O	SA	LA	O	SA	LA		
Total											

O = objective; SA = short answer; LA = long answer

3. PREPARATION OF A MODEL QUESTION PAPER

The model question paper is written on an item card. A model item card is given below.

Table 2: Model item card

Objective:		Marks:
Content area/Topic:		Estimated difficulty level:
Form of Question:		Estimated time:
Question		
Model answer	Points of answer	Marks for the points

4. PREPARATION OF A MARKING SCHEME

Once a model paper has been prepared, the next step is to evolve a marking scheme. The purpose of marking scheme is to assign proportions of marks to different parts of the answer. There are two types of marking schemes – Analytical (Objective type and short answer type) and Global (Long answer type).

5. REFINING THE QUESTION PAPER

After the model question paper and the marking scheme has been made, a second critical look at the questions is recommended. While reviewing questions to refine them, the following questions need to be asked:

1. Does the question test an important learning outcome?
2. Is it based on a predetermined objective?

3. Is the scope of the well defined as regards to:

- Clarity of directions?
- Language of the questions?
- Length of answer?
- Marking scheme?
- Appropriate difficulty level?

A question should be relevant to the set objectives of the course. The emphasis should be on the professional skill and competence. Questions such as "Describe important clinical and metabolic changes that occur in space flight" may be avoided. The questions should not relate to trivial or insignificant, vague and diffuse topics. The following questions given as short notes in Family Medicine are examples: Peripheral blood smear, Referral service, Family medicine, Social pathology, Physical examinations and health. Questions on a rare phenomenon or entity does not represent higher learning and does not necessarily judge the practical or professional ability of the student and hence must be avoided as far as possible.

The length of the question paper should be such that it should be reasonably feasible for an average student to answer it within the stipulated time. The mark allotted to each question is usually predetermined, and it should be proportional to the length and difficulty level of the question. At the same time the questions sampled should be such that uniform coverage of the entire curriculum is done.

The language should be clear, and unambiguous. The language should be understandable by an average student. Questions such as "Describe clinical paradigms of thrombotic thrombocytopenic purpura and hemolytic uremic syndrome" should be avoided. This phrase may not be familiar to many students. The dictionary meaning of 'paradigm' is 'example' or 'model'.

Special care may be taken to avoid spelling or grammatical errors which may cause confusion or even alter the meaning.

"Open ended" questions are apt to encourage rambling by a student and it may be difficult to ascertain substance amidst the verbiage. Short answer questions without a 'stem' or verb do not indicate precisely what the examiner wants. A question carrying two marks in a University examination paper reads as "brucellosis".

6. EDITING OF THE QUESTION PAPER

Editing is the next step in the preparation of the question paper. While editing the question papers, the following points are to be checked:

1. Grouping questions according to objectives, form of questions, content area, etc.
2. Numbering questions
3. Instructions for administration

7. REVIEW OF THE QUESTION PAPER

The question paper may be reviewed with the help of the following check list:

Table 3: Check-list for reviewing question paper

1. Has the paper covered the syllabus in a fair way without giving undue emphasis to one part or another?
2. Does the paper test the full range of abilities as defined by objectives in the syllabus?
3. Has the weightage to the various topics, different forms of questions and objectives adhered to the scheme shown in the blue-print?
4. Has the paper been set at an appropriate level of difficulty (i.e., the questions neither too easy nor too difficult to average students)?
5. Will the paper provide an adequate adequate discrimination between performance of candidates of different abilities?
6. Does the question paper have comparability of options in terms of objective, content, form and difficulty?
7. Are the questions precise and unambiguous?
8. Is there any excessive overlap between questions?
9. Can the paper be satisfactorily answered in the time allowed?
10. Is the question paper comparable in standard with those set in previous years?
11. Does the paper avoid repetition of questions set in previous years?

The final step is to ensure confidentiality by sending rough sheets to the University or destroying them as per the instructions.

CONCLUSIONS

In conclusion, a systematic approach will improve question paper setting in our examinations. The steps involved are plan the design, prepare the blue-print, prepare a model paper, prepare a marking scheme, refine the questions, edit the questions, review the question paper, and final typing/writing and dispatch.

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A Case of Myocardial Rupture on the Background of Coronary Artery Thrombosis

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ABSTRACT

Myocardial infarction infrequently complicates with the rupture of myocardial free wall in 6.2% of cases. It represents the second cause of death after cardiogenic shock, and accounts for as much as 15% of in-hospital mortality. The authors here report a case of an 80 year old male with the cause of death as myocardial rupture on the background of coronary artery thrombosis.

INTRODUCTION

Myocardial infarction occurs when there is severe stenosis or complete occlusion of a coronary artery so that the blood supply is insufficient to maintain the oxygenation of the myocardium. However, if there is adequate collateral circulation, myocardium can still be viable. These ischemic myocardium can be further weakened by the process of cellular death and the inflammatory response to these necrotic cells. The area of the myocardial infarct is weakest between three days and one week after the clinical onset of the infarct and it is at this time that the weakened area of myocardium may rupture and cause sudden death from a haemopericardium and cardiac tamponade. The rupture occasionally occurs through the interventricular septum, resulting in a left–right shunt. If a papillary muscle is infarcted, it may rupture, which will allow part of the mitral valve to prolapse, which may be associated with sudden death or may present as a sudden onset of valve insufficiency. Usually an infarct heals by fibrosis. This can be the pavement sites for physiological or electrical dysfunction of the cardiac cells. Cardiac aneurysms may form at sites of infarction; they may calcify and they may rupture[4]. Coronary artery disease accounts for approximately 75% of all sudden deaths handled by most medical examiners. About 50% die suddenly; 25% die without any preceding history or warning. Mechanism of death usually a lethal cardiac arrhythmia; ventricular arrhythmia in 80% of cases and sudden asystole or bradyarrhythmia in about 20% of cases. For death to occur, the degree of stenosis is usually 75% or greater[5]. Davies and Popple (1979) consider that 85% stenosis is the minimum criteria of stenosis reasonably associated with sudden death[6]. Usually 75% stenosis of the lumen is required to be designated significant occlusion. Myocardial perforation most commonly occurs during the first week, usually between four and seven days before connective tissue has had a chance to form

adequately. By the end of two weeks, much of the necrotic muscle has been replaced by fibrous tissue[7]. Rupture of the free wall of the heart has been reported to be the cause of death in 4 - 13% of fatal cases of acute myocardial infarction[8]. Although cardiac rupture has an incidence of only 1% to 4% following a heart attack, it accounts for up to 20% of mortalities associated with infarction[9].

CASE PRESENTATION

This case was of an 80 years old male who was found dead in his apartment on 2069/12/26. On the same day, the body was brought to Forensic Medicine Department, Teaching Hospital where the postmortem examination was performed. History relating to the deceased's medical, surgical, personal etc could not be extracted as the deceased was living alone. On external examination of the body, there was no any obvious fresh injury present over the body. On internal examination of the heart including pericardial cavity, the pericardial cavity contained an estimated 500 ml of blood with blood clots. Pericardium was intact. Multiple ruptures of myocardial wall were present over anterior aspect of inter ventricular septum and inferior wall of left ventricle. Proximal portion of left anterior descending artery was calcified with 80% occlusion. Proximal two-third of the left circumflex artery was also calcified with 80% to 90% occlusion with atheromatous plaque. Additionally, a thrombus was present in the proximal portion of left circumflex artery. Right coronary artery appeared normal. On sectioning, diffuse fibrotic lesions were present over the interventricular muscles. Hemorrhagic patches were seen over the myocardium of posterior wall of left ventricle. The stomach contained an estimated 100 ml of semi-digested egg chunks. Besides this, other systemic examinations of central nervous system, respiratory system, genitourinary and gastrointestinal systems appeared normal.

DISCUSSION

London and London found in a study of 1000 cases of fatal myocardial infarction that 50% of ruptures occurred within three days and 89% within 14 days. The anterior wall of the left ventricle is involved more commonly than the posterior wall[8]. Rupture of the free wall of the heart caused death within a very short time following rupture[10]. Clinical features which raise the suspicion of rupture of the heart include an abrupt decline of the arterial blood pressure and rapidly increasing venous distention. Van Torsel and Edwards, in reviewing 40 cases of cardiac rupture complicating acute myocardial infarction, presented evidence suggesting that the symptomatology in several patients was consistent with a gradual evolution of cardiac rupture[11]. Rupture of the midlateral wall was most common (32%) and usually occurred in the setting of an inferoposterolateral infarction related to an acute left circumflex artery occlusion in a retrospective and prospective study of 70 patients with rupture done by Oliva et al.[12] Among 26 cases of myocardial infarction with cardiac rupture (found in 6791 necropsies), 12 cases (63.0 percent) were due to occlusion of the left descending branch of the left coronary artery; three cases (16%) were due to the occlusion of the circumflex branch of the left coronary artery; and four cases (21%) were due to the right coronary artery[13].

CONCLUSION

Myocardial infarction infrequently complicates with myocardial rupture. In such cases it leads to a higher risk of mortality due to hemodynamic compromise due to pericardial tamponade.

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Demography of Dhampus: A Community Health Diagnosis Field Visit Report

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Community health diagnosis, Dhampus, Demography.

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ABSTRACT

Community health diagnosis is a comprehensive assessment of health status of the community in relation to its social, physical and biological environment. The purpose of community health diagnosis is to define existing problems, determine available resources and set priorities for planning, implementing and evaluation health action, by and for the community. A cross sectional descriptive study was done at Dhampus VDC from 1 Sept 2016 AD (16th Bhadra, 2073 BS) to 16 Sept 2016 AD (31st Bhadra, 2073). The data was collected by questionnaires, anthropometric measurements, interviews and secondary data. The community health diagnosis field visit gave us the opportunity to build our team spirit and taught us the arts of problem solving. It taught us the importance of correct conduct and building a good rapport with the people who were unfamiliar to us.

INTRODUCTION

Demography is the scientific study of human population based on population composition, its distribution in space and changes in population size. Health in a group depends on dynamic relationship between these factors. Hence demography is of vital importance in community health diagnosis (CHD).

The term demography is derived from two Greek words: Demos= People, Graphian= to draw or describe. It deals with three observable human phenomena:

a) Changes in population (Growth or decline)

b) Composition of population

c) Distribution of population in space

And five demographic processes namely fertility, mortality, marriage, migration and divorce. Besides disability, morbidity, literacy etc can also be noted. It gives general idea about the community for planning and formulating health policies and programs and to measure health status by the authorities.

OBJECTIVES

a) To determine the demographic and socioeconomic indicators to assess the health status of the community.

b) To present our findings to the community and receive feedback.

c) To prepare a report on the survey and submit it to the Department of Community Medicine, Gandaki Medical College, Lekhnath, Kaski, Nepal.

METHODS

Study Area: Dhampus VDC

Study design: Cross-sectional descriptive study. In a cross-sectional study, measurement of exposure and effect are made at the same time.

Study period: 1 Sept 2016 AD (16th Bhadra 2073 BS) to 16 Sept 2017 AD (31st Bhadra 2073 BS)

Process: Household survey

Survey unit: 410 households out of 620

Survey Duration: 15 days

Survey techniques: Every available household was covered in survey but there was limitation as many houses were inhabited due to migration, earthquake and Teej festival.

Table 1: Survey techniques, tools and respondents

Techniques	Tools	Respondents
Household survey	Questionnaires	Household heads, mothers, eligible couples
Anthropometric measurements	Tailor's tape, weighing machine	1- 5 year old children
Observation	Observation check-list	-
In-depth Interview	Semi structured interview guidelines	FCHVs

FINDINGS OF THE COMMUNITY

Population size and its composition

Population is defined as total number of people living in a particular place in a particular time. According to the data collected from the VDC office of Dhampus, VDC consists of 620 households with total population of 2537.

According to the data collected from survey, we surveyed 410 households with total population of 1732.

a) **Sex ratio**: It was found to be 92.01 being the population of males slightly higher than the females.

b) **Dependency Ratio:** It was found to be 52.19 i.e. 100 economically active (15 - 59 years) have to support about 53 dependent people (children below 15 years and elders above 59 years).

c) **Median age:** Median age was found to be 28.

d) **Fertility:** Total live births in last one year were found to be 38. CBR was calculated to be 22.5 per thousand populations per year

e) **Mortality:** Total deaths in last one year were found to be 10. CDR was calculated to be 5.8 per thousand population per year. IMR was found to be zero.

f) **Morbidity:** Total number of diseased persons in last one month was found to be 6.29% of total population.

g) **Disability:** Total number of disabled people was found to be four.

Table 2: Population size and its composition of Dhampus VDC

Indices	Figures obtained	National data (2011)
Sex ratio	92.01	94.2
Median age (years)	28	22.1
Average family size	4.23	4.88
Literacy rate	61.87%	65.9%
Dependency ratio	52.19 %	75.55 %
Disability rate	0.23%	1.94%
Fertility:		
A) Crude birth rate	22.5 per 1000	21.85 per 1000
Mortality:		
A) Crude death rate	5.8 per 1000	6.75 per 1000
B) Infant mortality rate	0	43.13 deaths per 1000 live births

IMR was found to be zero, probably because people didn't want to disclose about their family matters as it may revive the emotional trauma of the family and especially mother would be more affected.

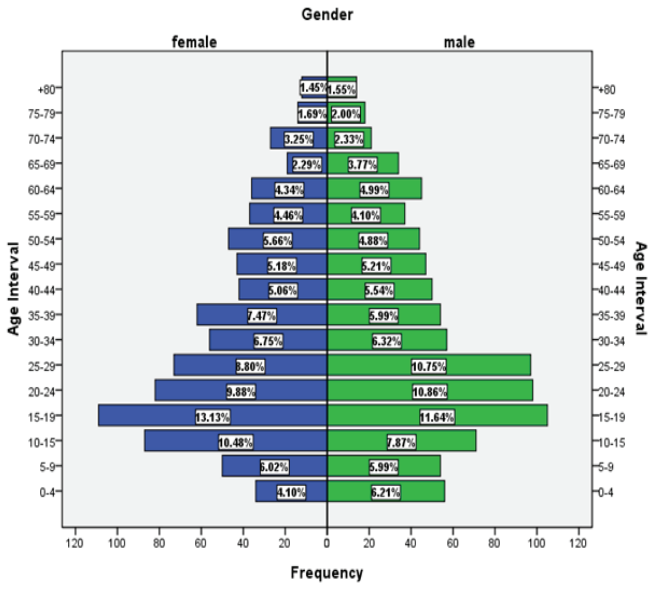
Age-sex composition

Age and sex is a vital component of population which have direct effect on the structure, social and economic aspects of population. The age-sex composition of Dhampus VDC is given in following Table

Table 3: Age and sex composition of Dhampus VDC

Age (Years)	Males	Females
0 - 4	56	34
5 - 9	54	50
10 - 14	71	87
15 - 19	105	109
20 - 24	98	82
25 - 29	97	73
30 - 34	57	56
35 - 39	54	62
40 - 44	50	42
45 - 49	47	43
50 - 54	44	47
55 - 59	37	37
60 - 64	45	36
65 - 69	34	19
70 - 74	21	27
75 - 79	18	14
80+	14	12

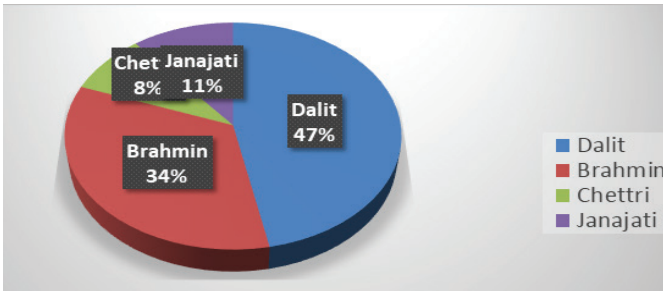
Fig 1: Age and sex composition of Dhampus VDC



Population distribution by ethnicity

Out of 410 households, most of the people of Dhampus were Dalits (47%) followed by Brahmins (34%), Janjatis (11%), Chettris (8%).

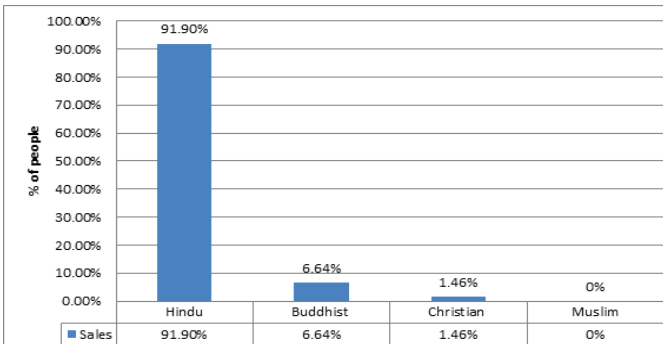
Fig 2: Population distribution of Dhampus VDC by ethnicity



Population distribution by religion

Most of the people of Dhampus were found to be Hindus (91.9%) followed by Buddhists (6.64%), Christians (1.46%), no religion (0.24%) and none were found to be Muslims.

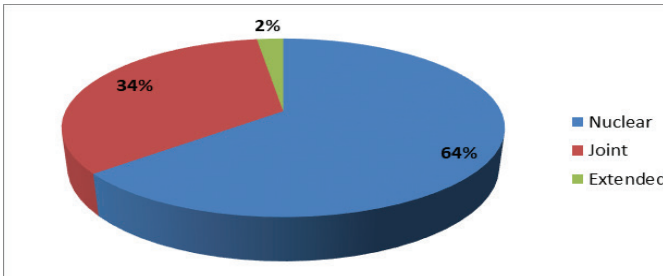
Table 3: Population distribution of Dhampus VDC by religion



Type of family

Most of the families in Dhampus VDC were found to be nuclear (64%) followed by joint (34%) and extended (2%).

Fig 3: Type of families in Dhampus VDC

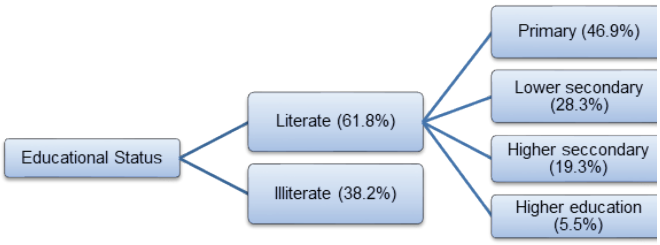


Educational status

Out of 1642 people with age above five years, most of the

people of Dhampus were found to be literate (62%). The educational status is given in the chart below.

Fig 4: Educational status of Dhampus VDC



Occupation

Out of 1166 people of age above 20 years, most of the people of Dhampus were engaged in agriculture (71%) followed by foreign employment (17%), business (7%), Government service (5%).

Fig 5: Occupational status of people of Dhampus VDC

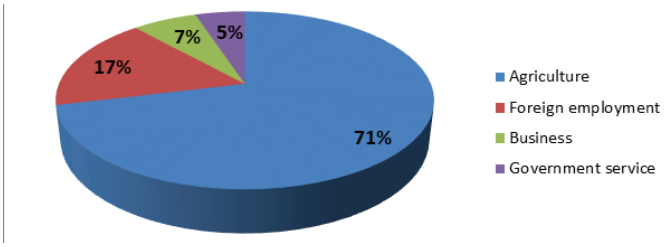


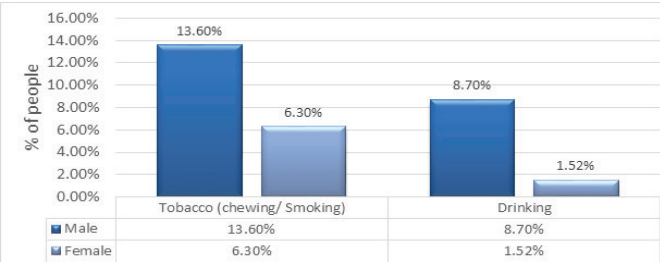
Table 4: Occupational status of people of Dhampus VDC

Occupational status	Percentage
Employed	55.66%
Unemployed	44.34%

Smoking and drinking habit

Out of total 1380 people of age above 15 years, 275 were found to be smoking and 141 were found to be drinking. The smoking and drinking habit of people of Dhampus VDC is shown in following bar diagram.

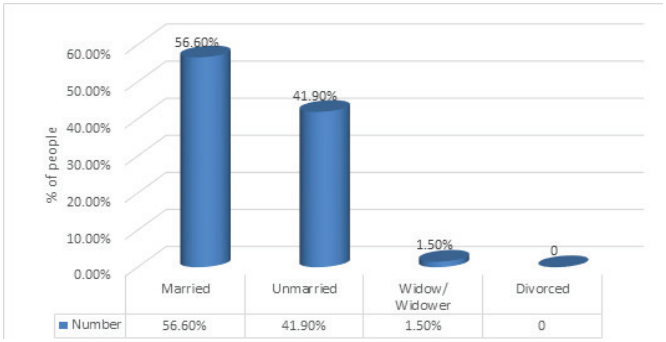
Fig 6: Smoking and drinking habit of people of Dhampus VDC



Marital status

The marital status of people of Dhampus VDC is given in the given pie chart.

Fig 7: Marital Status of people of Dhampus VDC

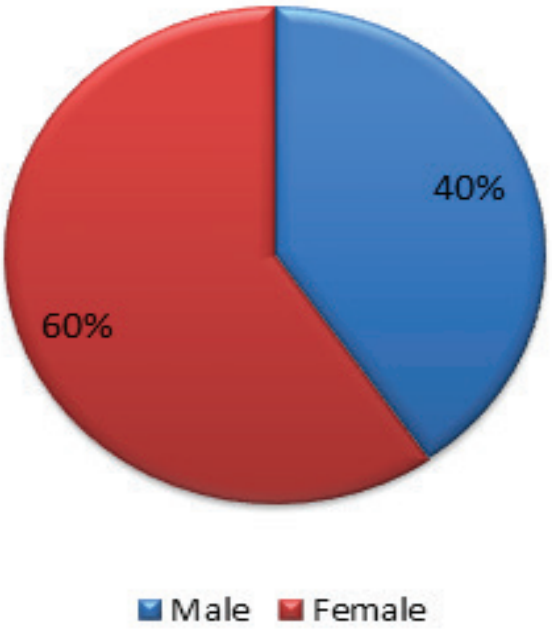


MORBIDITY

Morbidity refers to the diseases and illness, injury and disability occurrence in the population. By the knowledge of the disease one can know about the frequency and distribution of disease and also the causes and ways to control the spread of disease.

During our study in Dhampus, we came to know that, out of total population 6.29% people were diseased within 15 days. Among total diseased, 40.3% were males and 59.7% were females. The sex wise distribution of disease is given in following diagram.

Fig 8: Age wise distribution of disease among Dhampus population



Most of the people visited health institution for the treatment. The way of treatment adopted by people during disease is given in following diagram.

Table 5: Ways of treatment adopted for disease among Dhampus population

Ways of treatment adopted	No. of households
Self-medication	0.92%
Home remedy	6.48%
Traditional healer	0
Health institution	92.60%

Disability

Disability refers to the deviation from normal physical and mental functioning of the body. During our study in Dhampus we encountered four disability cases. Out of total study population, 0.23% were disabled. Among total cases of disability four were physical and none were mental.

Table 6: Functional distribution of disability

Physical	Mental
100%	0%

Birth

During our study in Dhampus, we found 39 live births within one year. Out of total live births within one year, 15 were females and 24 were males as shown in following table.

Table 7: Sex wise distribution of live birth within one year

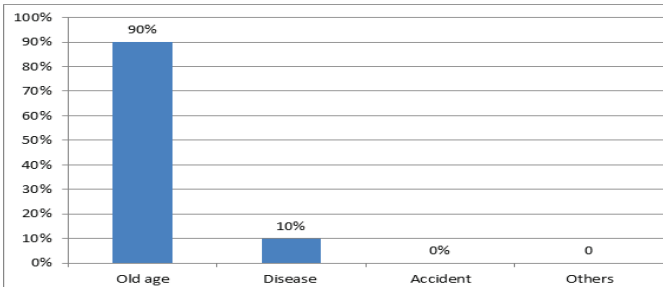
Males	Females
61.5%	38.5%

Mortality

Death is a permanent disappearance of evidence of life at any time after birth has taken place. It is a vital event that affects structure, size and growth of population.

During our study in Dhampus VDC we found 10 cases (0.57%) of death within one year among which six (0.35%) were males and four (0.22%) were females. Most of the people died because of old age as shown in following diagram.

Fig 9: Cause wise distribution of mortality



Migration

Migration is the geographical movement of individual from any usual place of residence for the purpose of establishing new permanent or semi-permanent residence. During our study in Dhampus we found three cases of migration among total population.

Vital Event Registration

It refers to the legal collection of data of vital events i.e. live birth, death, marriage and divorce of the specific population.

During our study in Dhampus, we found that most of the people have done vital events registration which is shown in following diagram.

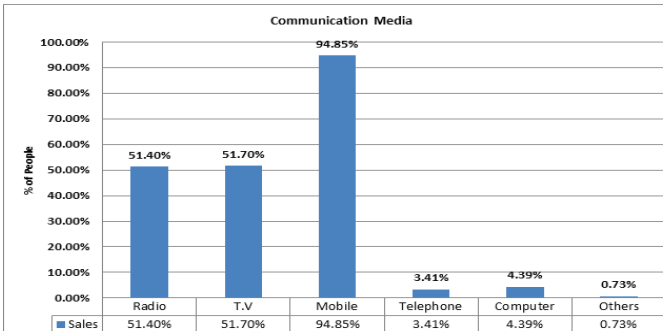
Table 8: Vital events registration

Vital Events Registration	Number
Yes	82%
No	18%

Communication Media

During our study in Dhampus, we found that most of the people uses mobile followed by TV, radio, telephone, computer and others which is shown in following pie chart.

Fig 10: Communication Media



CONCLUSIONS

The herculean task we thought earlier was made possible by the regular support and fascilitaion of the helping hands which are even now sparking in our eyes. Those unforgetable experiences, skills and learning that we learned in such a short period of time make us indebted to them. Our accomplishment was not only regarding the community, but also huge experience of the team work, cooperation and mutual partnership in solving the problems. Our small efforts though can not bring the dastric change; the fire has been ignited towards the approach of healthy living and well being. Yes Dhampus had taught us millions of knowledge!!!

Community field daignosis worked as the platform to use our theoritical knowledge.To list out we learnt the following things from the field visit:

- To work in a group as a teamwork and with proper co-ordination.
- To build good rapport with the community people and remain with them as their helpers as one family.
- To listen to others problems and analyse their views on several health related issues.
- To convince people on our program and collect maximum information required
- Learnt about group dynamics and conflict management.
- Got acquainted with various religious and cultural aspects of community.
- Accomplishing community based learning
- Planning, implementation and evaluation of MHP
- To face any hardships or problems during the stay and try to solve accordingly.

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Journal of Gandaki Medical College-Nepal

(J-GMC-N)

AIMS & SCOPE OF THE JOURNAL

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- Adequacy of experimental techniques
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- Relevance of discussion
- Clarity of presentation and organization of the article
- English composition

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Manuscripts must be clearly typed double-spaced on one side only on A4 size white paper with Arial Font, size of 12 points, with a margin not less than 25 mm. The pages should be numbered consecutively, beginning with the title page. Uniformity in language is required, with preference to American English.

Numbers less than 10 should be written in words. Words not numbers should begin a sentence. Numbers less than 1, begin with a zero. Use one space between a number and its unit. Generic drug names should be used.

The text of the article should be divided into sections with the headings, and should commence on a new page in the following sequence: title page, abstract, key words, introduction, materials and methods, results, discussion, conclusions, acknowledgement, references, tables and figures.

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The title page should carry

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3. Short, running title (should not be more than 45 characters).
4. Author(s) names with highest academic degree(s), designation, name of the department and institution affiliated (where the research was carried out), postal and email address, phone numbers.

Abstract

The abstract should contain the essence of the whole paper. Be clear and concise without any cited references and avoid unnecessary detail. Abstract must not exceed 250 words and should be presented in prescribed structured format: Background, Aims & objectives (hypothesis), Methods, Results, and Conclusions. Provide three to six key words below the abstract arranged alphabetically. The abstract need not be structured for a review article or case report. Universally accepted standard abbreviations used in standard text books can only be used.

Introduction

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Materials and Methods

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

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9. Protocols followed, if any.
10. Statistical analysis and computer software used.

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Ethics committee approval (for both human as well as animal studies) from respective institution is obligatory for manuscript submission. A statement on ethics committee permission and ethical practices must be included under the 'Materials and Methods' section.

Written informed consent must be obtained from the patient (or parent or guardian) for publication of any details or photographs that might identify an individual.

Results

The main outcome of the study and data obtained should be summarized in the Results section, in logical sequence in the text, tables and graphs. Remember that data and results are not the same thing. Results should be presented in a concise manner avoiding data that are already given in tables and figures. The tables and figures used in the manuscript should be precisely incorporated in sequential order in the result section. In this section, generally the minimum, maximum and mean values of the parameters should be mentioned. Likewise, statistical values should also be mentioned.

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In this section, at first the findings of the research should be elaborated giving citation of previous works supporting the hypothesis and present findings. Compare and contrast the results with other relevant studies. Describe the new and important aspects of the study. Do not repeat the data or other information given in the introduction or results section. State the limitations of the study.

Conclusions

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

Acknowledgements

This section should state person/s and/or institution/s or funding agencies to whom the author has to acknowledge, and should specify the nature of support.

Source of Financial support

Grants, funds, honoraria sanctioned for research, if any.

Conflicts of Interest

Potential conflicts of interest (e.g. employment, affiliation, consultancy, honoraria, grants or other funding etc.) should be disclosed.

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Review article must incorporate various aspects of topic chosen, and should also incorporate latest research and findings. It should not merely be a collection of quotes from text books or very old articles of journals that does not contribute anything new to the scientific literature base already available. The ideal review should be topical, up to date, balanced, accurate, authoritative, quotable, provocative and a good read. The ideal contents of review should contain the problem, historical background, basic science, methodology (Describing the methods used for locating, selecting, extracting, and synthesizing data), human studies, discussion, conclusions, recommendations, and the future. Of course with an abstract (need not be structured).

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Baral G. An assessment of the safe delivery incentive program at a tertiary level hospital in Nepal. *J Nepal Health Res Counc*. 2012 Jan; 10(20):118-124.

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Book

Reddy KR. Text book of Immunology. Delhi, India: AITBS Publishers; 2016.

Chapter

Shapiro BM. Awaking of the invertebrate egg at fertilization. In: Mastoianni L, Biggers JD, eds. Fertilization and embryonic development *in vitro*: New York, Plenum Press, 1981: 232-235.

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- Soundness of conclusions and interpretations
- Relevance of discussion
- Clarity of presentation and organization of the article
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The reviewer should make a recommendation regarding an article as follows:

- Rejected due to poor quality, or out of scope
- Accept without revision
- Accept but needs revision (either major or minor)

In the latter case, clearly identify what revision is required, and indicate to Editor-in-Chief whether or not you would be happy to review the revised articles.