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Assessment of Allergy Marker Leucocyte (Eosinophil) Count and other Blood Cells Parameters among Workers at Berber Cement Factory, Berber Governorate, River Nile State, Sudan, 2017

By Hisham AbdElhamid, Mosab Nouraldein Mohammed, Salima Abd Alrazig, Tagwa TajAlser, Eman Suliman, Kholood Abd Allah, Nihad Muzamil, Ensherah Said Ahmed, Manhal Mustafa Albakry & Zaineb Altaib

Elsheikh Abdallah Elbadri University

Abstract- Background: This study was conducted at Berber cement factory in barber to assess cement dust exposure and relationship to complete hemogram change and allergic condition among workers.

Rationale: Increasing of industrial activities in Berber governorate leading to many pathological conditions one of those phenomenons is allergy, certainly among whom worked in cement factories.

Objectives: To know the effect of exposure to the dust of cement on blood cells especially allergy Marker leucocytes.

Methodology: A total of 120 exposed and 30 non exposed workers were enrolled in this case control study.

Result: Allergy marker leucocyte (eosinophil) was increased (eosinophilia) other blood cell parameters were not affected.

Conclusion: There is intimate relation between exposure to cement dust and eosinophilia.

Recommendations: Furtherer studies are recommended with large sample size and taking the IgE measurement as a priority ofthe followingstudies.

Keywords: allergy marker, eosinophilia.

GJMR-C Classification: NLMC Code: QW 4

ASSESSMENT OF ALLERGY MARKER LEUCOCYTE EOSINOPHIL COUNT AND OTHER BLOOD CELLS PARAMETERS AMONG WORKERS AT BERBER CEMENT FACTORY, BERBER GOVERNORATE, RIVER NILE STATE, SUDAN, 2017

Strictly as per the compliance and regulations of:

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Hisham AbdElhamid [α], Mosab Nouraldein Mohammed [σ], Salima Abd Alrazig [ρ], Tagwa TajAlser [ω], Eman Suliman [¥], Kholood Abd Allah [§], Nihad Muzamil [χ], Ensherah Said Ahmed [ν], Manhal Mustafa Albakry [θ] & Zaineb Altaib [ζ]

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Keywords: *allergy marker, eosinophilia.*

I. Introduction

Allergy' is the term often used loosely to describe any intolerance of environmental factors irrespective of any objective evidence of immunological reactivity to an identified antigen.

Patients often present to an allergy clinic because of a popular public perception that they are 'allergic' in origin To Define These Mutually Exclusive Terms Allergy – is used to define those conditions in which antigen specific IgE or sensitized T cells play a definite role Atopy – is a state of disordered immunity in which Th2 lymphocytes drive an inherited tendency for hyper production of IgE antibodies after exposure to common environmental allergens Hypersensitivity –

refers to the Gell and Coombs classification for immunological diseases Intolerance is used to describe all abnormal but reproducible reactions to food when the causative mechanism is unknown Truly allergic diseases are common: about 20% of the population experience some form of allergy and this imposes a substantial physical and economic burden on the individual and society.[1]

II. Effect of Cement on Worker

Exposure to cement can occur through inhalation ingestion and eye or skin contact. Portland cement cause eye irritation and prolonged or repeated contact of the cement dust with skin cause dermatitis chronic exposure to cement dust may cause respiratory ailment in the form of cough, sputum, wheezing dyspnea, chronic bronchitis and adversely alter the pulmonary function indices.

Long term contact of skin with cement result in inflammatory changes or in some cases chemical burns. Chronic exposure to wet cement damages skin, leads to chemical burning rashes on skin and inflammation.[2]

III. Complete Hemogram

It includes blood Smear; Hemoglobin; Hematocrit; Red Blood Cell Count;White Blood Cell Count; WBC Differential; Platelet Count; Reticulocyte Count All content on Lab Tests Online has been reviewed and approved by our Editorial Review Board.[3]

CBC usually done to determine general health status; to screen for, diagnose, or monitor any one of a variety of diseases and conditions that affect blood cells, such as anemia, infection, inflammation, bleeding disorder or cancer.

They are produced and mature primarily in the bone marrow and, under normal circumstances, are released into the bloodstream as needed.

A standard CBC includes the following: Evaluation of white blood cells:

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WBC count; may or may not include a WBC differential Evaluation of red blood cells: RBC count, hemoglobin (Hb), hematocrit and RBC indices, which includes mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW).

The RBC evaluation may or may not include reticulocyte count Evaluation of platelets: platelet count; may or may not include mean platelet volume (MPV) and/or platelet distribution width (PDW) Significant abnormalities in one or more of the blood cell populations can indicate the presence of one or more conditions.

The three types of cells evaluated by the CBC include:

White Blood Cells there are five different types of WBCs, also called leukocytes which the body uses to maintain a healthy state and to fight infections or other causes of injury. They are neutrophils, lymphocytes, basophils, eosinophils, and monocytes. They are present in the blood at relatively stable numbers. These numbers may temporarily shift higher or lower depending on what is going on in the body. For instance, an infection can stimulate the body to produce a higher number of neutrophils to fight off bacterial infection. With allergies, there may be an increased number of eosinophils. An increased number of lymphocytes may be produced with a viral infection. In certain disease states, such as leukemia, abnormal (immature or mature) white cells rapidly multiply, increasing the WBC count.

Eosinophil: These cells are similar to neutrophils, except that the cytoplasmic granules are coarser and more deeply red staining and there are rarely more than three nuclear lobes.

Eosinophil myelocytes can be recognized but earlier stages are indistinguishable from neutrophil precursors. The blood transit time for eosinophil is longer than for neutrophils. They enter inflammatory exudates and have a special role in allergic response,defence against parasites and removal of fibrin formed during inflammation.Red Blood Cells also called erythrocytes, are produced in the bone marrow and released into the bloodstream as they mature. They contain hemoglobin, a protein that transports oxygen throughout the body. The typical lifespan of an RBC is 120 days; thus the bone marrow must continually produce new RBCs to replace those that age and disintegrate or are lost through bleeding.

A number of conditions can affect the production of new RBCs and/or their lifespan, in addition to those conditions that may result in significant bleeding. The CBC determines the number of RBCs and amount of hemoglobin present, the proportion of blood made up of RBCs (hematocrit), and whether the population of RBCs appears to be normal. RBCs normally are uniform with minimal variations in size and shape; however, significant variations can occur with conditions such as vitamin B12 and folate deficiencies, iron deficiency, and with a variety of other conditions. If the concentration of red blood cells and/or the amount of hemoglobin in the blood drops below normal, a person is said to have anemia and may have symptoms such as fatigue and weakness. Much less frequently, there may be too many RBCs in the blood (polycythemia).

Platelets also called thrombocytes, are special cell fragments that play an important role in normal blood clotting. A person who does not have enough platelets may be at an increased risk of excessive bleeding and bruising. An excess of platelets can cause excessive clotting or, if the platelets are not functioning properly, excessive bleeding. The CBC measures the number and size of platelets present.[3]

The human hematopoietic system is extremely sensitive to some environmental influences because of rapid synthesis and destruction of cells with consequent heavy metabolic demand.[4]

The CBC test is the most basic blood test used in assessing allergies in people. This test simply counts the number of red and white blood cells per cubic millimeter of blood. This test can be used to check for anemia or other blood conditions and is the most basic test to assess general health in individuals. If a person suffers from asthma, a physician may check for a high level of red blood cells, or polycythemia. Differential White Blood Cell Count A differential white blood cell count will provide physicians with a percentage of the different types of white blood cells in an individual. For example, if an individual suffers from asthma, a physician will look for a high eosinophil count, indicating a possible allergy. In extreme high levels, a person may be suffering a condition more serious than an allergy, such as a parasitic infection.

Allergen Specific IgE Antibody Test Immunoglobulin E, or IgE, is a protein related substance that is usually found in minute amounts in a person's blood. IgE is actually part of a normal individual's immune system and it helps to fight foreign substances that threaten one's health. As you would expect, elevated levels of IgE will alert physicians to the existence of allergies. This test can be used when a patient has symptoms of allergies to a variety of substances. Negative results to this antibody test probably mean you don't have an allergy, which involves an IgE response by a patient's immune system. However, results of this test should be interpreted cautiously because there is a small chance that an allergy may be present even if the test results were negative. If the test does show the presence of elevated IgE antibodies, an allergy is most likely present, but we need to qualify that statement.

You may never have an allergic reaction to that particular substance even though you tested positive. Also, the degree of IgE antibody measured does not indicate the severity of a supposed allergy. As you can see, blood allergy tests should be interpreted with caution. We should note that the conventional method of administering the IgE antibody test has been the RAST test. More recently, most medical laboratories test for specific IgE antibodies using a more modern, immunoassay method. The PRIST test is now the most common immunoassay method used for testing the amount of IgE present in a person's blood. Radio allegro-sorbent Test. In the 1960s, the radio allegro-sorbent test (RAST) was developed as an in-vitro blood allergy test.[5]

a) *Rationale*

Increasing of industrial activities in Berber governorate leading to many pathological conditions one of those phenomenons is allergy, certainly among whom worked in cement factories.

b) *Objectives*

To know the effect of exposure to the dust of cement on blood cells especially allergy Marker leucocytes.

IV. Methodology

a) *Study design*

Case control study.

b) *Study area*

Berber cement factory.

c) *Study population*

Workers at Berber cement factory.

d) *Inclusion criteria*

Work at Berber cement factory.

e) *Exclusion criteria*

Work out Berber cement factory.

f) *Sample size*

150 samples, 120 exposed to cement dust and 30 non exposed college students.

g) *Sample processing*

120 male samples was taken in subjects employed in barber cement factor, was collected from different area such as crusher, crane, backing, and 30 health selected subjects were randomly From college student.

Questionnaire 30 healthy male control include age, smoking habit, and 120 workers include age, smoking, job duration, exposure to dust, safety, working place, hours' work, allergic condition and cough.

Blood sample was collected from vein in 5cc disposable syringe of which 2.5 ml in EDTAas an anticoagulant for the analysis of different hematological parameters by CBC mandarin machine. The various haematological parameters, such as haemoglobin (Hb) concentration, total red blood cells (TRBC) count, total white blood cells (TWBC) count, different count of WBC, platelet count, packed cell volume (PCV), mean corpuscular volume MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), were estimated. And made thin blood film, fixed byAlcohol, stained by Leishman stain, and seen under microscope to estimate of eosinophil to assessment of allergic condition.

h) *Ethical consideration*

All participants were informed about the purpose of the study and they consent to enroll in it.

i) *Data Analysis*

The difference in means of the different groups was determined using the Student's t-test while the relationship between variables was determined using Pearson correlation coefficient. All the statistical analyses were done using the SPSS, version 17.0 and p-values less than0.05 were considered sign.

V. Result

Allergy marker (eosinophil) was increased, 53.3% of workers showed eosinophilia. Other parameters of hemogram were not affected.

VI. Discussion, Conclusion and Recommendations

This pioneer study in Berber governorate showed that cement dust effect on the health of workers inside the factory but the harmful effect of duct may affect huge number of people around the factory so control measures should be obtained and Furtherer studies are recommended with large sample size and taking the IgE measurement as a priority of the following studies.

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Study of Breast Lump, A Histopathological Audit of Five Years Specimen in a Medical College

By Raza AM, Ahmed Z, Khatun T & Islam MR

Jahurul Islam Medical College

Abstract- Background: Various types of lesion from inflammation to carcinoma can affect the breast. Some lesions are common in young females while others are more common in elderly age group. Early presentation and prompt diagnosis is essential to relieve anxiety of non-neoplastic conditions, and in casse of carcinoma, it can save the patient from metastases.

Methods: A Retrospective study was conducted for the period of five years in the Pathology Department of Jahurul Islam Medical College and Hospital. Histopathology samples were received, processed, reported and recorded in the Pathology laboratory. Data analysed from 2012 to 2016. Descriptive statistics was used to analyse the data.

Keywords: breast lump, carcinoma, fibroadenoma, biopsy.

GJMR-C Classification: NLMC Code: QZ 4

STUDY OF BREAST LUMP A HISTOPATHOLOGICAL AUDIT OF FIVE YEARS SPECIMEN IN A MEDICAL COLLEGE

Strictly as per the compliance and regulations of:

Study of Breast Lump, A Histopathological Audit of Five Years Specimen in a Medical College

Raza AM [α], Ahmed Z [σ], Khatun T [ρ] & Islam MR [ω]

Abstract- Background: Various types of lesion from inflammation to carcinoma can affect the breast. Some lesions are common in young females while others are more common in elderly age group. Early presentation and prompt diagnosis is essential to relieve anxiety of non-neoplastic conditions, and in casse of carcinoma, it can save the patient from metastases.

Methods: A Retrospective study was conducted for the period of five years in the Pathology Department of Jahurul Islam Medical College and Hospital. Histopathology samples were received, processed, reported and recorded in the Pathology laboratory. Data analysed from 2012 to 2016. Descriptive statistics was used to analyse the data.

Result: 228 sample of breast tissue sent for histopathology were studied. Peak incidence of benign lesion was in between 21-30 years and malignant lesions in between 31-50 years. No breast lesions were seen in the first decade of life. Cancer of the breast was seen in 12.28% of cases. Fibroadenoma and fibrocystic disease were the commonest benign lesion and infiltrating ductal carcinoma was the commonest malignant lesion.

Conclusion: Majority of the breast lumps are benign either fibroadenoma or fibrocystic disease. Benign lesions were common in second to fourth decade and malignancy in fourth and fifth decades.

Keywords: breast lump, carcinoma, fibroadenoma, biopsy.

I. Introduction

The human breast is paired mammary glands composed of specialized epithelium and stroma in which can occur both benign and malignant lesions. Benign breast diseases (BBD) however constitute the greater of the breast lesions [1]. These BBD are diverse, ranging from disorders of development, inflammatory lesions, proliferative diseases of the epithelium and stroma to different types of neoplasms[2]. Though most of the available literature show that breast lumps are mostly benign and nonproliferative epithelial lesions, it is known that certain benign breast diseases (BBD) are important risk factors for breast cancers which can develop in either breast later[3]. Breast cancer is one of the commonest cancers among women and commonly presents with a lump in breast to the physician. It is related to morbidity and mortality worldwide among women. In Asia, the incidence of breast cancer is increasing and may occur in younger age group. About 25% of breast cancer occurs in younger patients in developing Asian countries as compared to developed Asian or Western countries[4]. As breast lump can be the cause of different benign and malignant lesions, the management of the patients varies. Though clinical examination of the breast lump and the age of the patient can provide information about the nature of the lump, histopathological examination is necessary to establish the diagnosis. The aim of the present study is to see the spectrum of conditions/ lesions in breast lump specimens in Jahurul Islam Medical college Hospital.

II. Material and Methods

This is a retrospective cross sectional study of breast tissue specimen received from 2012 to 2016 at the Department of Pathology, Jahurul Islam Medical College and Hospital. The specimens were labelled, entered in the data system of the lab and kept for fixation in 10% Formalin overnight. After grossing, it was processed in the tissue processor, making blocks and cut into sections of 0.5 micron thickness. After staining with hematoxylin and eosin, slides were examined by pathologists. All the findings were recorded in the database. All the original request forms and histopathological reports on the breast specimens received within this study period with their slides were retrieved from the archives and reviewed. From the request forms and histopathological reports, information on the age, sex, nature of specimen, hospital numbers, laboratory numbers and histopathological diagnosis were extracted. New slides were made from formalin fixed, paraffin-embedded tissue blocks and stained with Haematoxylin and Eosin (H&E) where necessary for appropriate diagnosis and classification. Male breast tissues, cases of breast lesions with incomplete data and cases unable to trace slides or blocks were excluded from the study.

a) Statistical Analysis

Microsoft Excel software was used to generate tables. The descriptive statistics were used to infer results.

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III. Results

A total of 228 breast tissue specimen were examined in the five years period, which formed around 6.5% of the total specimens received for histopathological examination. The age of the cases ranged from 11 to 70 years. Most of the patients were in the age group 31 to 40 years group (38.6%) (Table-1).

Table 1: Age distribution of patients of breast tissue specimen (n=228)

Age group (in years)	Number of cases	Percentage
11-20	18	7.9%
21-30	66	28.9%
31-40	88	38.6%
41-50	38	16.7%
51-60	12	5.2%
61-70	06	2.6%
Total	228	100%

The presenting complain of the patients coming to the hospital was feeling of lump (34.2% cases), Pain in the breast (29.3% cases), Tenderness (17.5% cases), Feeling of heaviness in the breast (10.9% cases) (Table-2).

Table 2: Type of presenting complain of the patients (n=228)

Presenting complain to Physician	Total cases	Percentage
Lump	78	34.2%
Pain	67	29.3%
Tenderness	40	17.5%
Lumpiness with heaviness	25	10.9%
Skin redness with rash	10	4.4%
Pain in the axilla and hand	08	3.5%
Total	228	100%

The average age of presentation was analyzed and it was around 34 years. The benign lesions and malignant lesions were most common in the age group of 31-40 years and 41-50 years respectively. Benign breast lesion were 87.7% and malignant cases were 12.2%. The ratio between benign and malignant cases is 7:1 (Table-3).

Table 3: Distribution of benign and malignant breast lesion by age (n=228)

Age group (in years)	Benign lesions	Malignant lesions
11-20	18	0
21-30	64	02
31-40	80	08
41-50	28	10
51-60	08	04
61-70	02	04
Total	200 (87.7%)	28 (12.2%)

The histopathological diagnosis revealing benign lesions including ninety (39.4%) cases of fibroadenoma, forty two (18.4%) cases of fibrocystic disease, thirty six (15.9%) cases of breast abscess. Other benign lesions (30 cases) included duct ectasia 10 cases, granulomatous lesion 06 cases, fat necrosis 08 cases and intraductal papilloma in 06 cases. The average age for all benign breast disease was found to be 30 years. Intraductal papilloma was observed in six cases and periodical check up was advised to the patients. The carcinoma cases including in-situ carcinoma (DCIS) were found in 12.2% cases in the age range between 21 to 70 with 52 years as the average age of presentation. There were 18 cases of invasive ductal carcinoma, 04 cases of Invasive lobular carcinoma and 02 cases of medullary carcinoma (Table-4).

Table 4: Histopathological diagnosis of breast lump (n=228)

Histopathological Finding	Number of cases	Percentage
Fibroadenoma	90	39.4%
Fibrocystic disease	42	18.4%
Breast abscess	36	15.9%
Duct ectasia	10	4.3%
Granulomatous lesion	06	2.6%
Intraductal papilloma	06	2.6%
Fat necrosis	08	3.5%
In-situ carcinoma (DCIS)	06	2.6%
Invasive ductal carcinoma	18	7.9%
Invasive lobular carcinoma	04	1.7%
Medullary carcinoma	02	0.9%
Total	228	100%

IV. Discussion

The average number of breast tissue specimens received (6.5%) in our study is almost similar to that shown by Singh and Thakur (2.3%)[5]. The peak incidence of benign lump was found in 21 to 30 years age group and peak incidence of malignant lumps 31 to 50 years which is younger compared to the western observation[6]. No breast tumors were seen in the first decade of life. The youngest patient in this study was 14 years similar to that seen in other parts of Nepal[7]. The rarity of breast disease in the first decade of life is also reported by others[8]. Most common complain of the patients of breast tissue specimen was lump (34.2%), pain (29.3%) and tenderness (17.5%) similer to other study[9].

Fibroadenoma (39.4%) followed by fibrocystic disease (18.4%) formed the majority of breast lesions sent for histopathology, which is similar to that seen by Khanna et al. from Banaras- India[10]. Singh and Thakur in their study showed similar incidence as 28.28% and 21.71% respectively for fibroadenoma and fibrocystic changes[5]. The real incidence of fibrocystic disease is difficult to estimate and diagnosis depends a great deal

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on individual clinician or pathologist acumen. Ten (4.3%) cases of duct ectasia were present in this study. Duct ectasia of the breast (or mammary duct ectasia) is a condition in which there is an obstruction of the lactiferous duct. Mammary duct ectasia can mimic breast cancer. It is a disorder of premenopausal age. Signs of duct ectasia can include nipple retraction, inversion, pain, and sometimes bloody discharge[11]. Microglandular adenosis is widely known as a benign breast lesion that can produce a mass. The main importane of this lesion is that it is usually considered as a precursor for malignancy. Four (1.75%) of breast lesions in our study was diagnosed as microglandular adenosis[12]. The benign to malignant ratio was 3:1 in a study in Calcutta and 7:1 in our study. In that study the percentage of malignancy was higher (24.44%) as compared to our Study[4]. Benign lesions were common in the second to fourth decade and malignant lesion in fourth and fifth decades, which is similar to that seen in other parts of the world[13]. Eight cases of traumatic fat necrosis and six case of granulomatous lesion were also found in our study.

Cancer was seen in 12.28% of our cases. Singh and Thakur found the incidence of cancer as 18.42%[5]. The percentage of carcinoma in this study appears to be slightly closer to the west (10.5%) and lower than that of Africa (21%)[14]. Among the cases of breast carcinoma, Invasive ductal carcinoma was the commonest malignancy seen (7.89%) in our study. Singh and Thakur[5] in their study found invasive ductal carcinoma in 18.48% cases which is similar to that reported by Ali et al[15] and is higher then the present study. There was six cases of In-situ carcinoma (DCIS), four case of lobular carcinoma and two cases of medullary carcinoma in our study. Prakash et al. reported the incidence of malignancy as 2.5% for age group 30 years and below and 97.5% for age group above 30 years. She therefore pointed out the necessity of investigating all patients with breast lumps to rule out malignancy especially in women above 30 years[16].

V. Conclusion

Breast tissue specimen were 6.5% of the total specimens received for histopathology in the department of pathology. Majority of the breast lumps are benign either fibroadenoma or fibrocystic disease. Benign lesions were common in second to fourth decade and malignancy in fourth and fifth decades. Ductal carcinoma is the commonest subtype in this study. It is thus recommended that all women above the age group of 40 presenting with a palpable breast lump or a suspicious non-palpable abnormality on screening mammogram to have their lump excised. However, women below 30 years should also have the lump excised in the presence of risk factors such as a family history of breast cancer.

Competing Interests

The authors declare that they have no competing interests.

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Clinico-Hematological Study of Pancytopenia with Special Reference to Idiopathic Pancytopenia

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Material & Method: It was a prospective study conducted over a period of one year (Jan 2014-February 2015) at Department of Pathology in Institute of Medical sciences, Banaras Hindu University, Varanasi. Patients presenting with pancytopenia were included in the study. A provisional diagnosis was made on the clinical findings. Extensive laboratory work up (including LFT, RFT, Serology etc) was carried out to find the cause of pancytopenia in all the patients. Bone marrow aspiration was done in all the cases as a routine procedure. Bone marrow biopsy was done in 48 cases where indicated.

Keywords: *pancytopenia, aplastic anemia, myelodysplastic syndrome(MDS), idiopathic pancytopenia, idiopathic cytopenia of undetermined significance(ICUS).*

GJMR-C Classification: *NLMC Code: QV 180*

CLINICO-HEMATOLOGICAL STUDY OF PANCYTOPENIA WITH SPECIAL REFERENCE TO IDIOPATHIC PANCYTOPENIA

Strictly as per the compliance and regulations of:

Clinico-Hematological Study of Pancytopenia with Special Reference to Idiopathic Pancytopenia

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Result: A total of 140 patients presented with pancytopenia. Among the causes, Aplastic anemia was the most common cause (31.4%) followed by Megaloblastic anemia (22.1%) The third common cause was Myelodysplastic syndrome (MDS) (12.9%) followed by Acute leukemia (11.4%). Other causes were hypersplenism (4.3%), kala azar (2.1%), drug induced (2.1%), two cases each of HIV (1.4%), myelofibrosis (1.4%), lymphoma (1.4%) & multiple myeloma (1.4%). One case each of ITP (0.7%), SLE (0.7%) and Fanconi anemia (0.7%). Idiopathic pancytopenia constituted 5% (7 cases) of the total. On follow up of patients with idiopathic pancytopenia at 6 months, all the seven patients were having persistent pancytopenia. They were labeled as ICUS (Idiopathic cytopenia of undetermined significance (ICUS)). One patient was hospitalized with complains of generalized body weakness and few episodes of malena. Thorough work up was done to look for any cause of pancytopenia but no cause was identified and patient died due to complication of pancytopenia (due to hemorrhagic shock) in one month course and hence "Idiopathic fatal pancytopenia" term was coined for the patient. Remaining six patients were followed up at 12 months, 03 patients were having persisting pancytopenia without any specific complaints and remaining 03 patients died, for which cause is unknown.

Conclusion: Pancytopenia is a common haematological problem encountered in clinical practice.The natural history of patients with ICUS is largely unknown and appears to be highly variable. ICUS patients require long term follow up to assess the evolution. "Idiopathic fatal pancytopenia (IFP)" is an emerging new entity with a grave prognosis. Further research may elucidate the underlying pathology & potential drugs to halt the inevitable fatal outcome.

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I. Introduction

Pancytopenia is the simultaneous occurence of anemia, leucopenia and thrombocytopenia. Many disease processes involve the bone marrow primarily or secondarily resulting in pancytopenia. Pancytopenia can develop due to decrease in hematopoietic cell production as a result of destruction of marrow tissue by toxins, suppression of normal marrow growth and differentiation or due to replacement of bone marrow by abnormal or malignant tissue. The marrow may be hypocellular or hypercellular. Bone marrow examination usually provides the diagnosis in these cases. Few cases where exact diagnosis could not be made even after an exhaustive work up, these cases were regarded as Idiopathic pancytopenia. We followed up these cases at 6 month and 12 month. We particularly emphasized on these cases with the comparative study with other diagnosis. No study undertaken in this regard in India yet.

II. Material and Method

The present study was a prospective study. A total of 140 patients presenting with pancytopenia were enrolled in the study. Approval from Ethical Committee and patient consent were taken. These 140 patients were divided into two age groups : Children (<18 years) & Adult (≥18 years). The inclusion criteria for pancytopenia were hemoglobin (Hb) less than 10 gm/dL, total leukocyte count (TLC) less than 4000/cumm and platelet count less than 150000/cumm. A detailed clinical history and physical examination was undertaken in all the cases. A provisional diagnosis was made on these clinical findings. Peripheral smear examination and reticulocyte count was done. Samples of bone marrow aspiration were taken from the patients admitted in the Department of Medicine and Pediatrics of Sir Sunderlal Hospital, Banaras Hindu University, Varanasi. All the patients were checked for having any major clotting disorder before undergoing any procedure. BMA was performed by the standard

technique using Salah needle from the posterior iliac crest under local anesthesia with standard aseptic precautions. Leishman stain was used to stain all bone marrow smears. BM aspirate for cytogenetics was takrn into a sodium heparin tube. Excess aspirate was used to make particle clot preparations or placed in an EDTA tube for making additional smears. Special cytochemical stains were undertaken in cases of leukemia. rk-39 dipstick test was done in all cases of Kala-azar. Bone marrow biopsy was done in 48 cases where the diagnosis was doubtful on aspiration. Chromosomal breakage study was advised in a suspected case of Fanconi anemia. Immunohisto-chemistry (IHC) with CD34 was done on biopsy section in certain cases to enumerate the exact count of blast. We followed the algorithm presented in figure 1.

Algorithm for Work Up of Cases of Pancytopenia
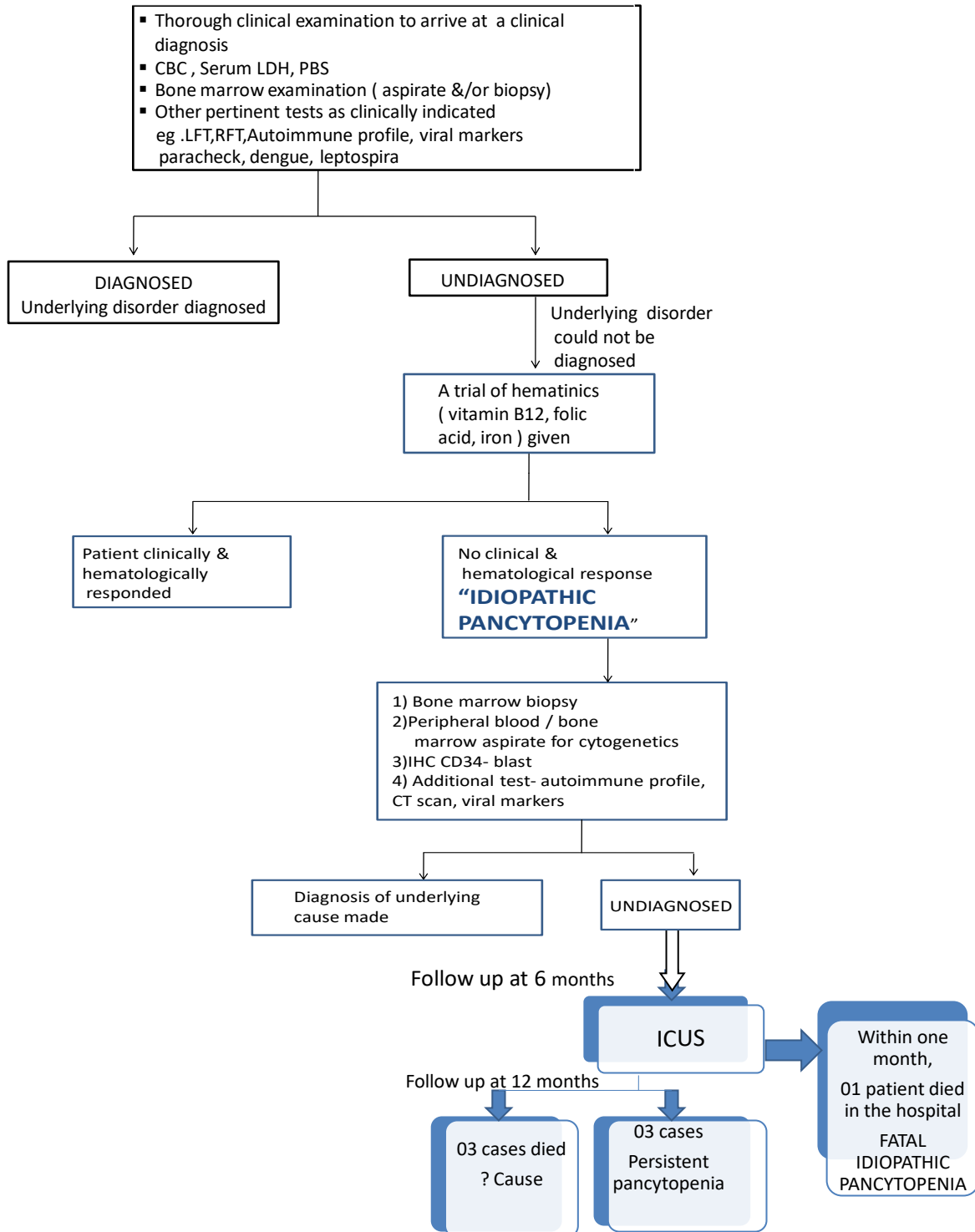
Figure 1: Algorithm for work up of cases of pancytopenia

III. RESULTS

A total of 140 patients of pancytopenia were enrolled in the study. The patient age ranged from 1 to 72 years. The maximum number of patients of pancytopenia were found in 11-20yr age group, followed by age group 21-30years. The overall male to female ratio (M:F) was 1.37:1. Out of 140 patients, 92 patients (66%) were adults and 48 (34%) were children.

The most common cause of pancytopenia in our study was Aplastic anemia in 44 cases (31.4%) followed by Megaloblastic anemia in 31 cases (22.1%). The incidence of Idiopathic pancytopenia in our study was 5%. Incidence of various causes of pancytopenia are tabulated in table 1.

Table 1: Incidence of various causes of pancytopenia (n=140)

Diagnosis	Number of cases	Incidence (%)
Aplastic anemia	44	31.4
Megaloblastic anemia	31	22.1
o without IDA	18	
o with IDA	13	
Myelodysplastic syndrome (MDS)	18	12.9
Acute leukemia	16	11.4
Hypersplenism	6	4.3
Kala-azar	3	2.1
HIV	2	1.4
Septicemia	1	0.7
Drug induced	3	2.1
Lymphoma	2	1.4
Myelofibrosis	2	1.4
Multiple myeloma	2	1.4
Fanconi anemia	1	0.7
SLE	1	0.7
ITP(Immune thrombocytopenia purpura)	1	0.7
IDIOPATHIC (?Cause)	7	5
TOTAL	140	100%

Table 2: Profile of Idiopathic pancytopenia (n=07) cases.

Case no	Age	Sex	Hb(gm%)	TLC(/UI)	Platelet(/cumm)	MCV(fl)	%Blast in PBS*	%blast in BM	BM** cellularity	BM fibrosis	CD34on BM cells	Cytogenetics
1	60	M	4.3	1100	13000	72	0	0	NC***	No	0	46,XY
2	17	F	4.9	3100	39000	84.1	0	0	NC	No	0	46,XX
3	65	M	4.5	2200	11000	106	0	0	NC	No	0	46,XY
4	11	M	10.6	2700	84000	90	0	0	NC	No	0	46,XY
5	17	M	4.4	2600	75000	90	0	0	NC	No	0	46,XY
6	55	M	9.5	3700	29000	86.2	0	0	NC	No	0	46,XY
7	38	M	7.4	3800	23000	100	0	0	NC	No	0	46,XY

*PBS – peripheral blood smear, **BM- bone marrow, ***NC- normocellular

The mean age of patients of Idiopathic pancytopenia was 37.6±22.7 years. There were 3 children and 4 adults. The male to female ratio was 6:1. The mean Hb (gm/dl) was 6.5±2.6(4.3-10.6), mean TLC(/cumm) was 2743±929(1100-3800), mean Platelet count (/cumm) was 39100±29260 (11000-86000) and mean MCV (fl) was 89.7±11(72-106).

In our study, 7 cases presented with pancytopenia with normocellular marrow. No signs of dysplasia or increase in blast count were noted. Bone marrow biopsy was undertaken for these patients. No bone marrow fibrosis was noted. No CD34 positive cells (blast) were seen in the bone marrow by IHC. Conventional cytogenetics was performed on bone marrow aspirate and it was normal in all the cases. These cases did not respond to Vitamin B12 and folic acid therapy. Serum biochemical parameters and coagulation profile was within normal limit.

Autoimmune profile was conducted for these patients and was within normal limit. Radiological investigations were done to rule out any specific pathology. On follow up at 6 months all seven cases were having persistent pancytopenia. These cases were diagnosed as ICUS (idiopathic cytopenia of undetermined significance). One patient presented with generalized body weakness and few episode of malena in the past. The patient was hospitalized and further work up was started to find the cause of malena and pancytopenia. In due course, patient had declining trend in the hematological parameters and started bleeding and went into hemorrhagic shock and died. No cause of pancytopenia could be identified and hence we coined the term Idiopathic fatal pancytopenia for this patient. Remaining six patients were followed up at 12 months, 3 patients were having persistent pancytopenia and three patients died, for those the cause is not known as they were not available for any work up.

Table 3: Table showing frequency of various symptoms and signs.

Symptoms & signs	Number of patients (out of 140)	Percentage %
Generalized body weakness	126	90
Pallor	137	97.8
Fever	86	61.4
Bleeding	57	40.7
Splenomegaly	26	18.6
Hepatomegaly	16	11.4
Lymph node	15	10.7
Pedal edema	10	7.14

The most common presenting symptom was generalized body weakness in 126 patients (90%). The most common sign was pallor in 137 cases (97.8%)

The various causes of pancytopenia were divided into five categories for further evaluation as Aplasticanemia, Megaloblastic anemia, Infiltrative disorders[including acute leukemia-myeloid(AML) and lymphoid(ALL), myelodysplastic syndrome(MDS), lymphoma, multiple myeloma], Others [including, Fanconi anemia, hypersplenism, kala-azar, HIV, septicemia, drug induced causes, SLE, myelofibrosis and immune mediated thrombocytopenic purpura(ITP)] and Unknown causes(idiopathic pancytopenia)

Table 4: Table showing relationship of MCV, RDW-CV, & MPV with different causes of pancytopenia.

Diagnosis	MCV(fl) Chi square(DF=8) 55.78 P value <0.05			RDW-CV (%) Chi square(DF=4) 31.26 P value <0.05		MPV(fl) Chi square(DF=4) 88.04 P value <0.05	
	< 83fl	83-99fl	>99fl	11.6-14%	>14%	6-13fl	>13fl
Aplastic anemia	3	34	7	31	13	44	0
Megaloblastic anemia	4	3	24	2	29	6	25
Infiltrative disorders	7	22	9	14	24	36	2
Others	8	11	1	8	12	18	2
Unknown cause	1	4	2	3	4	7	0
Number of cases	23	74	43	58	82	111	29

In our study, MCV was significantly increased in cases of pancytopenia due to megaloblastic anemia (24 out of 31 cases) where as it was within normal range in other causes of pancytopenia due to aplastic anemia and infiltrative causes. The correlation between megaloblastic anemia and macrocytic anemia was found to be significant in our study with p value <0.05. Aplastic anemia and other infiltrative conditions were associated with normocytic anemia which was found to be significant. Red cell distributon width RDW-CV was significantly increased in cases of pancytopenia due to megaloblastic anemia as compared to other causes which had normal RDW values. Mean platelet volume (MPV) was also significantly increased in cases of megaloblastic anemia as compared to other causes of pancytopenia (aplastic anemia, infiltrative lesion).

IV. Discussion

Pancytopenia is defined by reduction of all the three formed elements of blood below the normal reference range[1]. Pancytopenia is a common hematological finding with different clinical scenario.

In our study the male: female ratio was 1.37:1. This is in agreement with other studies shown in (table 5). The male preponderance may be partly explained by increased exposure of male to environmental agents like agricultural pesticide [2]. Few studies showed female preponderance [3,4]. The most common symptom was generalized body weakness (90%) and most

common sign was pallor (97.8%) comparable to many other Indian studies (table 5).

We compared our data with various International and Indian studies on pancytopenia (table 6). The most common cause in our study was Aplastic anemia. Many International studies[5,6,7,8,9,10,11,12] and national studies[4,13,14] were in agreement with our finding as aplastic anemia as the most common cause of pancytopenia. Whereas few studies reported aplastic anemia as the second most common cause[15,16,17,18,19,20,22]. Significant lymphocytosis was associated with aplastic anemia compared to other causes of pancytopenia. This finding was in agreement with SomaYadav et al., The pathophysiology of Aplastic anemia is believed to be immune mediated, with active destruction of blood forming cells by the lymphocytes[4]

Megaloblastic anemia was the 2nd common cause in our study whereas few Indian studies[18,19,20,21, 22, 23,24,25] and international studies[16,34] reported it as the first common cause. This may be due to the fact that ours is a tertiary care centre where many referral cases come which might have been treated with hematinic therapy previously.

Out of 31 cases of Megaloblastic anemia, 26 had elevated LDH levels. All the cases improved with Vitamin B12 and folic acid therapy. R Para et al., and Evazi-ziaei et al., also observed increased LDH in megaloblastic anemia[25,26]. Lactate dehydrogenase enzymes is released during the The expected increase in LDH activity is the result of an accelerated turnover of bone marrow cells implying release of this enzyme from dividing or decaying cells.[27]

In MDS, most of the patients (75%) were under the age 50years and 27% of cases were below 20 years. Usually MDS is considered to be disease of elderly, but in the Indian series by S Nigam and Sudha Rani et al.,overall 75.5% of individual (20/33) were of <50 years of age, 8 (21.6%) of 33 patients were less than 20 years of age [28]. This may be due to the fact that the incidence of MDS appears to be increasing over the past decade due to recognition of the syndrome by the physician and hematopathologist.

Acute leukemia is the 3rd common cause in our study. Many Indian studies are in agreement with ours study. Sarod R etal., reported acute leukemia as the 2nd common cause.[29]

Various International studies reported few cases of pancytopenia with normal bone marrow (refer table 6). The frequency varies from 3.38% to 10.5%. No follow up was mentioned in them. No study has reported the phenomenon of Idiopathic pancytopenia. In our study we reported 5% of these cases (pancytopenia with normal marrow and normal karyotype). We followed up these cases at 6 months and 12 months.

ICUS is a recently proposed, provisional diagnostic category that recognizes patients who present with cytopenias of undetermined etiology[30,31]. The proposed criteria for diagnosing ICUS[30]:

a) Persistent cytopenia (for 6 months): hemoglobin <11 g/dl, neutrophils <1.5×109L, and platelets <100×109/L;
b) No morphologic features of myelodysplasia
c) Normal chromosome analysis and
d) A detailed clinical history and investigation that excludes other secondary causes of cytopenias.

The natural history of patients with ICUS is largely unknown and appears to be highly variable [32]. Small studies indicate that some patients will go on to develop frank MDS or a related myeloid malignancy such as AML [33]. Others may follow a more indolent course [34]. ICUS cases require long term follow up to assess the evolution. In our study, all the seven cases fulfilled the criteria for ICUS. One patient was hospitalized for an extensive work up but within one month patient died due to hemorrhagic shock and no cause could be identified. The course of the patient was fatal and hence the patient was termed as Idiopathic fatal pancytopenia. Out of remaining six patients on follow up at 12 months, three patients were having persistent pancytopenia and another three patients were expired due to unknown cause. Hence the course of ICUS in our study was variable.

The incidence of hypersplenism was 4.3%. All cases were caused by portal hypertension secondary to liver cirrhosis. The incidence of pancytopenia caused by hypersplenism among international studies varied from 0 % to 19%[14,29] as well as among Indian studies varied from 0 %-11.5%. Our incidence of 4.3% was within the range reported by various workers.

Other infections included Septicemia and HIV. The incidence was 2.1% in our study. There was a single case of septicemia presenting as pancytopenia in children. Two cases of HIV in adults were reported. The incidence of septicemia in various studies varied from 1.6% to 17.2%[4,19,25,35] and our data was within the aforementioned range.

There was a single case of ITP in a 17 year old female with fever, pallor and petechial rashes. The incidence of ITP in our study was 0.7%. In other studies, the incidence varied from 1.7-7.8%.[23,25,13]

We also compared our data with various studies in children (table 7). Overall in adult(51.1%) and children (58.4%), aplastic anemia and megaloblastic anemia were the two most common causes of pancytopenia in our study.

MCV, RDW-CV & MPV was significantly increased in cases of pancytopenia due to megaloblastic anemia where as it was within normal range in other causes of pancytopenia due to aplastic anemia and infiltrative causes. Soma Yadav et al., & Gupta et al., also assessed the role of MCV, RDW, MPV in

cases of pancytopenia and they are in agreement with our study.[4,38]

V. Conclusion

Pancytopenia is a common hematological problem encountered in clinical practice. The most common cause of pancytopenia is Aplastic anemia followed by Megaloblastic anemia.ICUS cases require long term follow up. "Idiopathic fatal pancytopenia (IFP)" is an emerging new entity with a grave prognosis. We wish to sensitize the medical community & the scientists to this rapidly fatal condition of unknown etiology.Further research may elucidate the underlying pathology & potential drugs to halt the inevitable fatal outcome.

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Table 5: Comparison of clinical profile of pancytopenia patients.

Study	Age group	M:F ratio	Most common presentation	Most common sign
Deepak B.Kr *et al., 3*	10-70yr	1.0:1.8	Generalisedweakness (70.83%)	Pallor (45.83%)
Soma Yadav *et al., 4*	All ages, <30yr- 73.3%	1:1.2	Fever	Pallor
JigneshKumar *et al., 21*	1-95yr	1.6:1	Easy fatigability (79%)	Pallor (100%)
Nigam RK *et al., 22*	2-80yr	1.12:1	Generalized weakness, fever	Pallor , splenomegaly
BhaskarBThakkar *et al., 23*	13-86yr	1.08:1	Generalisedweakness (97%) and fever (70%)	Pallor (100%)
A Chhabra *et al., 24*	6month – 14yr	-	Bleeding manifestation (70.3%) Fever (63.7%)	Pallor (64.8%)
Rajesh Para &Shailajapara *et al., 25*	3-90 yr	1.1:1	Generalized weakness (51.7%)	Pallor (25.9%)
ArvindJain *et al., 39*	2month-95yr	2.6:1	-	-
Present study	1-72yr	1.37:1	Generalisedweakness (90%)	Pallor (97.8%)

Table 6: International & National Studies on Pancytopenia.

Study	Country	No. of cases	Commonest cause of pancytopenia	Second common cause	Third common
International agranulocytosis and aplastic anemia study 5	Israel Europe 1987	389	Aplastic anemia (52.7%)	MDS (10.5%)	-
Keisu M and Ost A *et al., 15*	Israel Europe 1990	100	Neoplastic disease, Radiation therapy (32%)	Aplastic anemia (19%)	MDS (14%)
Hossain MA *et al., 6*	Bangladesh1992	50	Aplastic anemia	Chronic malaria, Kala-azar	Hypersplenism, Acute leukemia
David G, Savage 16	Zimbabwe 1997	134	Megaloblastic anemia 48 (35.8%)	Aplastic anemia 35 (26.1%)	AIDS, Acute leukemia (11.82%)
Mussarrat Niazi Fazl-i-Razig7	Pakistan 2000	89	Aplastic anemia (38.3%)	Megaloblastic anemia (24.7%)	-
Ishtiaq O Bagai HZ40	Pakistan 2001	100	Megaloblastic anemia 39 (39%)	Hypersplenism secondary to cirrhosis 19 (19%)	-
Khudairabbas *et al., 17*	Iraq 2004	105	Acute leukemia (30.47%)	Aplastic anemia (17.14%)	Non hodgkinslymphoma (14.47%), megaloblastic anemia (13.33%)
Jha *et al.,8*	Nepal 2008	148	Hypoplastic anemia (29%)	Megaloblastic anemia (23.64%)	Hematological malignancies(21.62%), erythroid hyperplasia (19.6%) and **normal marrow in 3.38% cases**
Lakhey *et al., 9*	Nepal 2010	54	Hypoplasticanemia (29.6%)	Hematological malignancies (27.78%)	Megaloblastic anemia (24.1%), erythroid hyperplasia (11.11%), **normocellular marrow in 7.41% cases**
Tajali khan *et al.,10*	Pakistan 2011	160	Aplastic anemia (37.5%)	Megaloblastic anemia (13.75%)	Acute leukemia (13.75%), hypersplenism (10%)
Pudasaini *et al., 41*	Nepal 2012	57	Erythroid hyperplasia (21%)	Megaloblastic anemia (12.3%)	Acute leukemia (12.3%), infective pathology (12.3%), ITP (10.5%), microcytic anemia (7%), hypoplastic anemia (5.3%), MDS(3.5%), Multiple myeloma (3.5%), leishmaniasis(1.8%) and **normal marrow in 10.5%**
Anwar jebjan *et al., 11*	Pakistan 2012	205	Aplastic anemia (28.3%)	Hematological malignancies (23.9%)	Megaloblastic anemia (19.5%), ITP (7.8%), Iron deficiency anemia (4.4%)
Pathak *et al.,12*	Nepal 2013	102	Hypoplastic anemia (32.3%)	Hematological malignancies (19%)	Megaloblastic anemia (11.7%), erythroid hyperplasia (20%), Leishmaniasis, plasmacytosis, gaucher diseases, relative myeloid hyperplasia, eosinophilia, **normocellular marrow in 5.8% cases and 5.8% cases remain inconclusive**
N Verma and S Dash 13	India 1992	202	Aplastic anemia (40.6%)	Megaloblastic anemia (23.26%)	Acute leukemia (17.75%)
Vijai Tilak, Raini Jain 18	India 1999	77	Megaloblastic anemia (68%)	Aplastic anemia (7.7%)	
Kumar R, Kalra SR 14	India 1997	166	Aplastic anemia- (29.5%)	Megaloblastic anemia (22.28%)	Aleukemic leukemia (12%)
Kishor Khodke and S. Marawah 19	India 1999	50	Megaloblasticanemia (44%)	Aplastic anemia (14%) and Kalazar (14%)	
Sarod R, Garelwal 29	India 2001	139	Aplastic anemia 38%	Acute leukemia	
Khunger JM, S Arulsclvi 20	India, 2002	200	Megaloblastic anemia (72%)	Aplastic anemia (14%)	Subleukaemic leukemia (5%)
Jignesh kumar et al 21	India, 2011	100	Megaloblastic anemia (45%)	Malaria (14%)	Aplastic anemia (11%)
Nigam RK *et al., 22*	India, 2012	155	Megaloblastic anemia (43.2%)	Hypoplastic anemia (12.9%)	Dimorphic anemia (8.38%), hypersplenism (3.22%), aplsatic anemia (2.58%), ITP, MDS, chediakhigashi syndrome,CDA,PRCA, Erythroid hyperplasia, gaucherds

Deepak B.Kr etal3	India, 2012 (18month)	48	Hypoplastic anemia (33.3%)	Normoblasticerythroid hyperplasia (27.8%)	Megaloblasticanemia (18.75%), MDS (8.33%), Normal marrow and dry tap (12.5%)
Soma yadav et al.,4	India, 2012	60	Aplastic anemia (38.3%)	Megaloblasticanemia (21.7%)	Leukemia, non-Hodkings lymphoma, infilteraion, MDS
Bhaskar b thakkar et al.,23	India, 2012	100	Megaloblasticanemia (37%)	Malaria(19%)	Hypersplenism (14%), and aplastic anemia (6%), TB (5%)
A chhabra et al.,24	India, 2012	111	Megaloblastic anemia (31.8%)	Hematological malignancies (25.2%)	Infectious diseases (19.7%) Aplastic anemia (18.8%)
Rajesh Para & Shailajapara et al., 25	India, 2013	58	Megaloblastic anemia (46.6%)	HIV(17.2%)	Malaria(8.6%), aplastic anemia (8.6%), dengue (8.6%), subleukemic leukemia (3.4%), ITP, Iron deficiency anemia
Arvindjain et al.,39	India, 2013	250	Hypersplenism (29.2%)	Infection (25.6%)	Myelosuppresants (16.8%) Megaloblastosis (13.2%)
Present Study	India 2014-2015	140	Aplastic anemia (31.4%)	Megaloblastic anemia (22.1%)	Myelodysplasic syndrome (12.9%0), Acute leukemia (11.4%), Hypersplenism (4.3%), kala-azar (2.1%), other infection(2.1%), **normocellular marrow in 5% cases(IDIOPATHIC PANCYTOPENIA)**

Table 7: Comparison of Various Studies in Children

Study	Country	Number of cases	AGE	Most common cause	2nd Most common cause	3rd Most common cause
Gupta V et al.,36	India 2008	105	1.5-18yr	Aplastic anemia (43.8%)	Acute leukemia (25.7%)	Kala azar (9.5%)
Chabbara A et al.,24	India 2012	111	6 month - 14 yr	Megaloblastic anemia (31.8%)	Malignancies (25.2%)	Infectious diseases (19.7%)
Jan AZ et al.,11	Pakistan 2013	205	6 month- 14 year	Aplastic anemia (28.3%)	Hematological malignancies (25.2%)	Megaloblastic anemia (19.5%)
Pathak et al.,12	Nepal 2013	6(out of 48)	<15yr	Hypoplastic anemia (3cases)	Hematological malignancies (2 cases)	Megaloblastic anemia (1 case)
Present Study	India 2015	48(out of 140)	<18yr	Aplastic anemia (41.7%)	Megaloblastic anemia (16.7%)	Acute leukemia (12.5%) MDS (8.3%)

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Adult Osteomyelitis in a Developing Community

By Wilson I. B. Onuigbo

Abstract- Osteomyelitis, which means bone marrow inflammation, has been known since antiquity. However, it is still a current challenge. Accordingly, its epidemiology has been studied worldwide. For example, from USA has come a case series. In like manner, the present series comes from a developing community consisting of the Ibos or Igbos, an ethnic group domiciled mostly in South-eastern Nigeria. The study was stimulated by the affirmation of a Birmingham (UK) group that the establishment of a histopathology data pool facilitates epidemiological analysis. The present pool is a Reference Pathology Laboratory. It was striking that, among the 24 patients documented, the males were more often involved than females. Other parameters featured singly such as that the 3rd Decade was the commonest for both sexes.

Keywords: bone, inflammation, osteomyelitis, epidemiology, developing community.

GJMR-C Classification: NLMC Code: WE 251

Strictly as per the compliance and regulations of:

Adult Osteomyelitis in a Developing Community

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I. Introduction

In the words of Lew and Waldrogel (1), "Known since antiquity, osteomyelitis is a difficult-to-treat infection characterized by the progressive destruction and new apposition of bone." From Brazil (2), there is the report that, over the last 30 years, "the pathogenesis of osteomyelitis has almost been totally elucidated, and many factors responsible for the persistence of this infection have been identified." Indeed, the view from Columbia (3) is the same over the past four decades. In general, the establishment of the histopathology data pool has facilitated epidemiological analysis (4). Therefore, with regard to an Ethnic Group, the Ibos or Igbos (5), who are domiciled in South-eastern Nigeria, the present paper deals with their own analysis.

II. Investigation

From the end of the Nigerian Civil war in 1970, physicians began to send to me numerous formalin-fixed specimens. They were submitted with standard clinical details until the 1990s. Accordingly, the osteomyelitis data were carefully assembled with reference to epidemiological analysis, especially as regards the adults.

III. Results

Table 1 summarize the local data. At first glance, only the earliest 2 cases were from outside the capital city, Enugu. Unfortunately, the named doctor was a German who died after performing the autopsy on an unrecognized case of Lassa fever. Incidentally, he lived long enough to have sent to me many cases of teenage appendicitis (6). The rest of the doctors operated in the National Orthopedic Hospital, Enugu, where Dr. Osisioma held the pride of place as the saying goes!

Table 2 shows that the males preponderated over the females in the ratio of almost 3 to 1. It also shows that the 3rd decade was the commonest for both sexes.

Table 3 reveals the sites affected. Clearly, the bones of the lower extremities were most commonly affected.

Regarding the duration of the illness before the attendance at the hospital, two patients boldly attested to the duration of 50 and 54 years respectively. Three old people generalized that the lesions started during childhood. Up to 9 patients admitted to the duration of only up to 1 year. For 8 patients, it was up to 2 years.

IV. Discussion

For years, it has become clear that the prevention of bone infections is most important, including the prevention of infection after surgery (6). Of course, case series are needed (7). In this context, retrospective study is well worth it as in that of Gallaher's group (8). As they concluded, "Daptomycin appears to be an effective therapeutic choice with an acceptable safety profile in the management of osteomyelitis that does not involve hardware." Of course, these comments pertain readily to developed communities. The ball should roll pertinently to the developing communities, seeing that what I receive is not clinical management information but the osteomyelitis specimens themselves.

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Table 1: Epidemiologic data concerning adult osteomyelitis

S/No	Lab. No	Initials	Gender	Age	Site	Town	Doctors
1	B 946/73	OA	M	55	Rib	Onitsha	Mandrella
2	B 601/73	JC	M	26	Femur	Onitsha	Mandrella
3	261/77	UA	M	51	Tibia	Enugu	Igwe
4	61/78	EM	F	45	Fibula	Enugu	Nwozo
5	H 21/78	IS	M	43	Femur	Enugu	Igwe
6	H 10/80	IJ	M	40	Humerus	Enugu	Igwe
7	H 478/80	OC	F	26	Femur	Enugu	Osisioma
8	H 72/81	ER	M	25	Ilium	Enugu	Ukegbu
9	H 771/81	EM	M	65	Femur	Enugu	Osisioma
10	H 154/82	IC	M	25	Humerus	Enugu	Osisioma
11	H 43/85	EM	F	59	Tibia	Enugu	Iregbulem
12	H 2313/86	ND	M	59	Humerus	Enugu	Osisioma
13	UH 1737/86	OP	M	22	Rob	Enugu	Aghaji
14	UH 929/87	OJ	M	43	Spine	Enugu	Okonkwo
15	UH 1765/87	IC	M	26	Maleolus	Enugu	Amamilo
16	H 156/88	NE	M	24	Humerus	Enugu	Osisioma
17	493/88	IP	M	30	Tibia	Enugu	Amamilo
18	UH 731/89	NJ	F	22	Tibia	Enugu	Okonkwo
19	H 10/90	IJ	F	66	Spine	Enugu	Ukegbu
20	H 19/90	IB	M	55	Tibia	Enugu	Nwozo
21	H 81/90	ON	F	25	Fibula	Enugu	Osisioma
22	H 165/91	OJ	M	54	Hip	Enugu	Osisioma
23	H 202/91	OU	M	33	Finger	Enugu	Osisioma
24	9301132	AP	F	70	Tibia	Enugu	Odukwe

Table 2: Sex pattern of lesions

	Male	Female	Total
21 – 30	7	3	10
31 – 40	2	0	2
41 – 50	3	1	4
51 – 60	4	2	6
61+	1	1	2
Total	17	7	24

Table 3: Site preference

Site	No.
Tibia	6
Femur	4
Humerus	4
Fibula	2
Ilium	2
Spine	2
Rib	2
Maleolus	1
Finger	1
Total	24

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Seroprevalence of Toxoplasma Gondii Infection among Pregnant Women in River Nile State, Sudan, from April to June 2017

By Mosab NM Hamad, Alaa M M Mustafa, Mona M Alkheir, Abd Alnasir Suliman, Tagwa Tarig, Basil Morsi, Anwer Mohialdeen, Mohammed Ismail & Abdallah Atayib

Elsheikh Abdallah Elbadri University

Abstract- Background: Toxoplasmosis is worldwide distribution disease, about 20% to 90% of the adult population in the world are reported with toxoplasmosis.

Objectives: To know the prevalence of toxoplasmosis among selected group of pregnant women from Atbara and Aldamer towns, River Nile State ,Sudan by applying Latex agglutination and ELISA serological methods and to compare between these two serological methods

Methodology: Blood specimen were collected from 50 pregnant women participated in this studies and then specimens were processed and examined by Latex agglutination and ELISA.

Result: 24% were seropositive and 76% were seronegative, 24% positive with latex agglutination and 18% positive with ELISA.

Discussion, conclusion and recommendations: ELISA is more specific than latex agglutination method, further studies are required with large sample size and more diagnostic methods.

GJMR-C Classification: NLMC Code: QX 140

SEROPREVALENCE OF TOXOPLASMA GONDII INFECTION AMONG PREGNANT WOMEN IN RIVER NILE STATE, SUDAN, FROM APRIL TO JUNE 2017

Strictly as per the compliance and regulations of:

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Mosab NM Hamad α, Alaa M M Mustafa σ, Mona M Alkheir ρ, Abd Alnasir Suliman ω, Tagwa Tarig ¥, Basil Morsi §, Anwer Mohialdeen χ, Mohammed Ismail ν & Abdallah Atayib ϴ

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I. Introduction

Toxoplasmosis is worldwide distribution disease, about 20% to 90% of the adult population in the world are reported with toxoplasmosis[1].

It is third leading infections cause of food–borne death after salmonellosis and listerosis.

In Sudan first report of human toxoplasmosis was dated back to 1996 with different prevalence rates[2]

Toxoplasma gondii is an obligate intracellular protozoan parasite that infects most species of warm blooded animals including humans and causing toxoplasmosis [3]

a) Morphology

During different period of its life cycle,individual parasites convert into various cellular stages with each stage characterized by a distinct cellular morphology.

This stages include tachyzoites, merozoites, bradyzoites and sporozoites.

b) Life cycle

The life cycle of T.gondii can be broadly summarized into two components, sexual part that occur only within cats.

The second part isasexual, it is occur within virtually all warm blooded animals include humans, cats and birds [4]. because T.gondii can sexually reproduce only within cats, they are called definitive host and other hosts in asexual reproduction are defined as intermediate hosts.

c) Sexual reproduction

When the cat is infected with T.gondii (example by consuming on infected mouse carrying the parasites tissue cyst), the parasite survive passage through the stomach, eventually infecting epithelial cell of the cat's small intestine.[4]

Inside their intestine cells the parasite undergo sexual development and reproduction, producing millions of thick walled zygote containing cyst called as oocyst.

Epithelial cells rupture and release oocysts into intestine's lumen, then shed in cat's feces. Oocysts can spread to soil, water, food and it can survive and remain infective for many months in cold dry climate [5], Ingestion ofoocysts by human or other warm-blooded animals is one of the common routes of infection. [6]

Other infected stages are tachyzoites of rapid division, and bradyzoites of slow division within tissue cysts, Tissue cysts in brain and muscle tissue formabout 7-10 days after initial infection.[7]

d) Asexual reproduction

Inside host cells the tachyzoites replicate inside specialized vacuoles called parasitophorus vacuoles, and multiply inside it until host cells dye and rupture releasing and spreading the tachyzoites via blood stream to all organs and tissues including brain. tachyzoites convert into any organ.

e) Modes of transmission

- Ingestion of undercooked, contaminated meat with infective stage of T.gondii.
- Drinking water contaminated with T.gondii or contact with contaminated soil.
- Accidently swallowing the parasite through contact with cat's feces that contain toxoplasma.
- Vertical (Transplacental) transmission.
- Organs transplantation.
- Sexual transmission.
- Inhalation of infective stage.

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f) Symptoms

Most people of toxoplasmosis are asymptomatic, some of them may feel as they have flu especial pregnant women with low fever, malaise, lymph adenopathy, muscle aches and pains for month or more.

Severe toxoplasmosis causing damage to the brain lead to encephalitis and damage in eye and other organs .most severe cases are individuals who have weak immune system.

Infant who are infected while still in womb have no symptoms at birth, but they may develop symptoms later in life.

II. Stages Toxoplasmosis Infection

a) Chronic stage

Tissue cysts can be maintained in host tissue for the life time of the animal. However the presence of cysts appear to be due to periodic process of cyst rupturing and re encysting rather than a perpetual life span of individual cysts or bradyzoites.[8]

It can passed between intermediate hosts via cycle of consumption of tissue cyst in meat, however parasite's life cycle begins and completes only when passed to host.

b) Acute stage

Infection of T.gondii during third trimester of pregnancy have high risk of congenital transmission, it causing severe damage to the fetus or abortion. Also can cause manifestation such as hydrocephalus, cerebral calcification and chorioretinitisin the new born.[9, 10]

c) Diagnosis

T.gondii infection can be identified with serologic testing or aminocentesis or presence of abnormal ultra sound findings.

Several serological tests are available for detection of T.gondii antibodies such as Sabin field man dye test, Indirect Immune fluorescent test "IFAT", Modified agglutination test "MAT", latex agglutination test and Enzyme –linked Immuno sorbent assay "ELISA".

Serologic testing is the first step in diagnosis by using IgG and IgM antibodies ,the diagnostic challenge is differentiating between primary and chronic infection and result of IgG and IgM testing can often be difficult to interpret, for this reason it is important to consult with an expert area when confirming the diagnosis.

The presence of IgM cannot be considered reliable for making diagnosis for acute toxoplasmosis infection, its titer rise from five day to week following acute infection and reaching maximum after one to two months and decline rapidly than IgG.[11] Also IgM antibodies can decrease to low or undetectable levels in many cases.

IgG antibodies appear later than IgM and are usually detectable within one to two weeks after infection, with peak reached within 12 weeks to 6 months after acute infection, but it will be detectable for years after acquired infection and usually present thorough life.[12]

- If IgG and IgM are both negative this indicate the absence of infection or extremely recent acute infection. [13]
- If testing reveals positive IgG and negative IgMit indicates an old infection (more than one year ago).
- If both IgG and IgM are positive this indicates either a recent infection or false positive test result.[12]

If acute infection is suspected repeat testing is recommended within two or three weeks [11, 12], rise in IgG antibodies titers between tests indicates a recent infection.[14]

There for when positive result is appear, it should confirm by confirming test such as ELISA, Sabin Feldman test and IFAT [11, 12].

Knowing when infection occurred during pregnancy is very important in evaluating the risk of fatal transmission, so initial antibiotic therapy and ensure appropriate prenatal counseling [13].

d) Justification

Seroprevalence of T.gondii infection particularly in pregnantwomen, are still in conclusive. In River Nile State has no published studies on the seroprevalence of T.gondii infection among pregnant women and this were motivated us to carry out this study to determine the seroprevalence of T.gondii infection among pregnant women in Atbara and ALdamer.

e) Objectives

i. General objectives

To determine the prevalence of T.gondii infection among pregnant women in Atbara & ALdamer - River Nile state.

ii. Specific objectives

- To detect the presence of T.gondii antibodies (IgG,IgM) among pregnant women.
- To comparison betweenexposure to the risk factor and acquiring of T.gondii infection.
- To know the age of infected pregnant woman.
- To comparison between Latex agglutination test and ELISA test for diagnosis infection.

III. Material and Methods

a) Study design

This study was Cross sectional study.

b) Study area

The study performed in ALdamer & Atbara Towns, River Nile state.

c) *Study period*

From April to June 2017.

d) *Study population*
Pregnant womenfrom 18to 40 years old.

e) *Inclusion Criteria*
-   Pregnant women were enrolled certified that to be medically fit by the specialist physician and from Atbara and ALdamer Towns.
-   Age between 18-40 years.
-   Didn't Received blood.
-   Didn't Received organ.

f) *Exclusion criteria*
-   Doesn't pregnant women.
-   Pregnant women out of Range (18_40) years old.
-   Received blood.
-   Received organ.

g) *Sample size*
        A total of 50 blood samples were drawn from pregnant women who come to Atbara and ALdamer Hospitals. This figure was arrived at using the relation

$$N = \frac{Z^2 \, XP(1 - P)}{ERROR^2}$$

Where

N= Sample size, Constant set by convention
Z= 1.96,
P= Previous study's prevalence.
P = 92.5% (0.996). Error was calculated at 5% (0.05).
N= [1.962 X 0.996X (1-0.996)]/0.052

And Questionnaires were administered, completed and returned for analysis.

h) *Sample Collection*
        The blood samples were collected by venipuncture using 5 ml syringe into plain containers.

i) *Sample processing*
        Serum obtained by centrifugation of the blood at 5000 rpm for 10 minute. Detection by Latex Agglutination test. Then +ve Result Confirmation by ELISA Test.
        Firstly sample diagnosed with latex agglutination test then confirm with ELISA. Total of 50 pregnant women were enroll in this test from Atbara hospital, data collect by medical field. Take about 5 ml of venous blood by disposable syringes under sterile aseptic technique 2.5 ml in plain tube for latex agglutination test and 2.5 ml also in plain tube for confirming + ve result by detecting IgM and IgG Abs of *T.gondii*.

j) *Principle of latex agglutination test*
        Latex agglutination is observed when sample containing the specific antigen (or antibody) is mixed with an antibody (or antigen). Which is coated in the surface of latex particles.
        The reaction between aparticular antigen and an antibody results in visible clumping called agglutination.

k) *Principle of ELISA Test*
        Enzyme Linked Immunosorbent Assay Combine the specificity of antibodies with the sensitivity of simple enzyme assays, by using antibodies or antigens coupled to an easily assayed enzyme. ELISAs can be provide useful measurement of antigen or antibody concentration.

l) *Study variables*
-   Presence of cats in the house
-   History of abortion in family
-   History of delivery
-   Type of Delivery
-   Educational level
-   Raw meat and vegetable habit
-   gestational period
-   blood and organ transfusion
-   Nature of home ground surface.

m) *Ethical consideration*
-   Approval from EAEUEC (Elsheikh Abdallah Albadri University Ethical Committee)
-   The purpose and procedures involved in this study were explained and written inform consent were obtained from all participants. Blood were collected with the consent of the volunteers

n) *Data analysis*
        Statistical analysis of data was done by using Statistical package for social science (SPSS).

IV. Result

        Out of 50 samples of serum collected from pregnant women in Atbara and ALdamer Hospitals and screened by using latex agglutination test, the number of positive cases was found to be (22.2%) Table (1)

Table 1: The number and percentage of positive cases of toxoplasmosis

Sample size	Positive	Negative
50	12(24%)	38(76%)

Table 2: Prevalence T. gondii antibodies(IgG & IgM) among pregnant women

IgG	frequency	Percentage%
positive	12	24%
Negative	38	76%
IgM		
positive	9	18%
Negative	41	82%

Table 3: Age of participants

Age	frequency	Percentage
18-20	13	26%
21-30	24	48%
331-40	13	26%

Table 4: Comparison between age and infection with toxoplasmosis

Age group/years	Positive	Negative
18-20	5	8
21-30	4	20
31-40	3	10

Table 5: Comparison between latex agglutination test and ELISA test IgG & IgM

Toxoplasmosis latex agglutination	ELISA IgG test	
	Positive	Negative
Positive	12	0
Negative	0	38
Toxoplasmosis latex agglutination	ELISA IgM test	
	Positive	Negative
Positive	9	3
Negative	0	38

Bar Chart
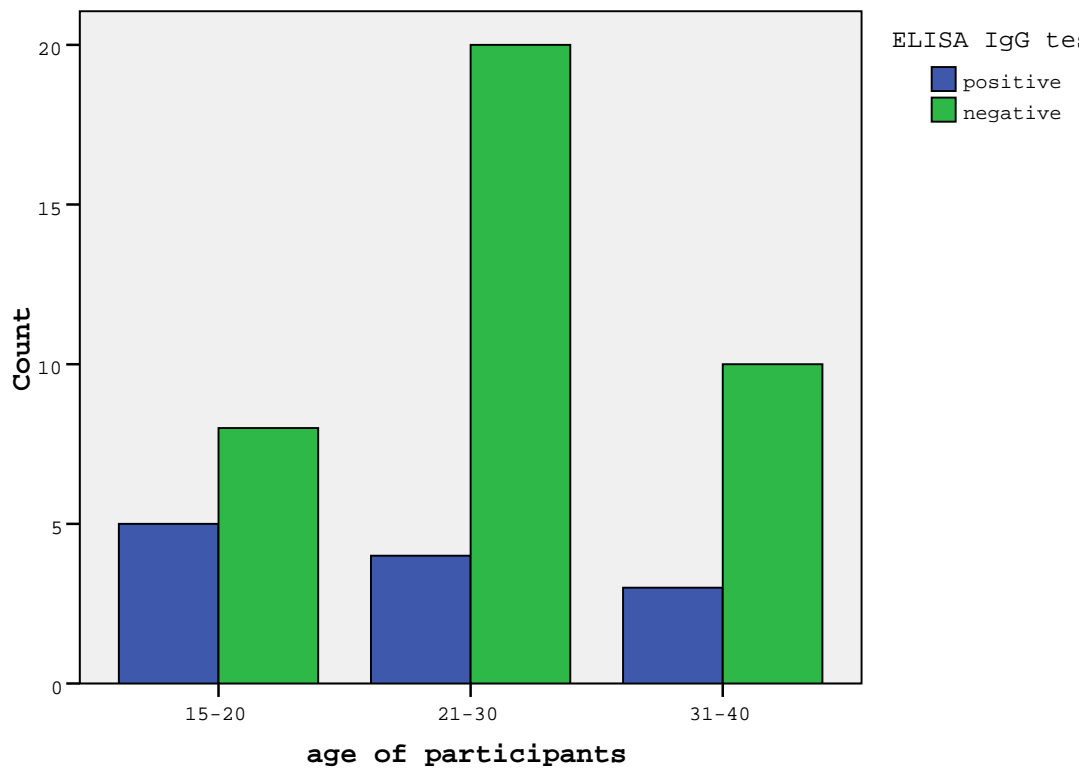
Figure 1: T. gondii IgG seroprevalence by age

Bar Chart
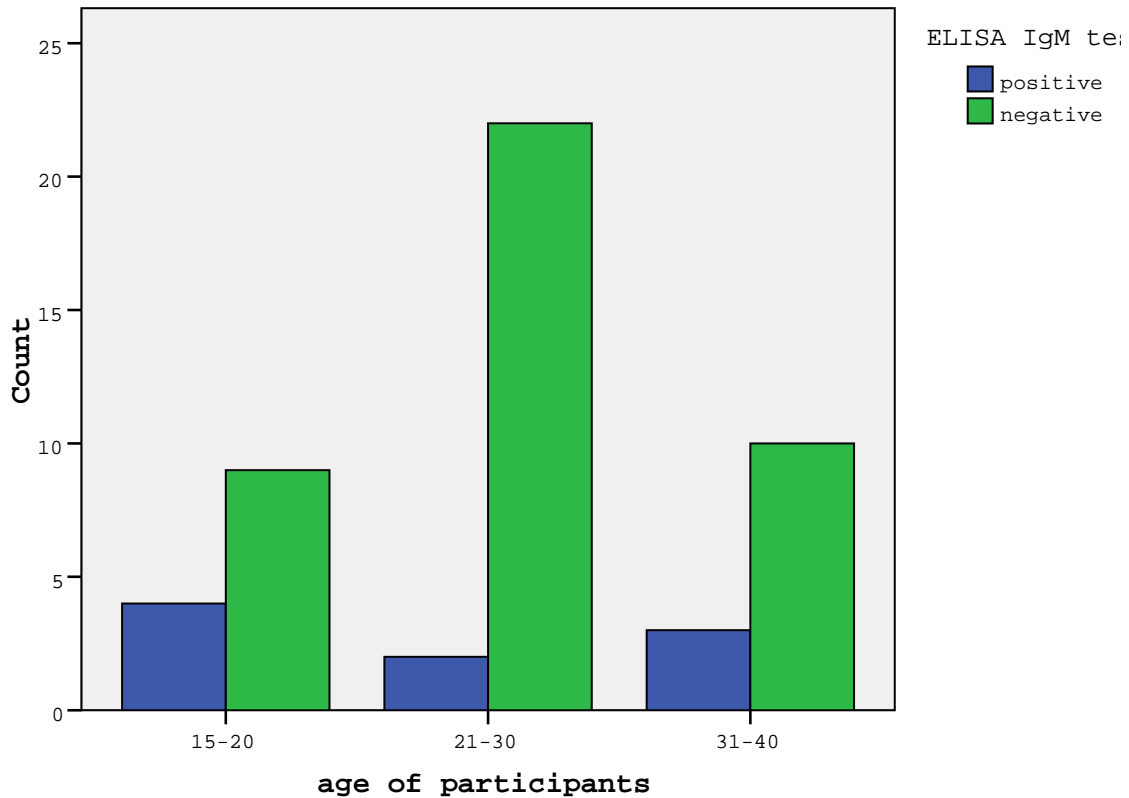
Figure 2: T. gondii IgM seroprevalence by age

V. Discussion, Conclusion and Recommendations

The current study is one of the few studies carried out to explore the seroprevalence of T. gondii infection among pregnant women in ALdamer and Atbara Towns and further studies are required with large sample size and various diagnostic methods.

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Bovine Mastitis: Prevalence, Risk Factors and Isolation of Streptoccocus Species from Small Holders Dairy Farms in and Around Haramaya Town, Eastern Ethiopia

By Bayan Amin, Yosef Deneke & Nejash Abdela

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Abstract- Mastitis is the most complex and costly disease of dairy cows occurring throughout the world including Ethiopia. Streptococcal mastitis is the commonest and economically important. However, mastitis caused by this species is not well investigated. A cross-sectional study was conducted from November 2016 to April 2017 to determinethe prevalence of mastitis, associated risk factor and also to isolate pathogenic streptococcus species from lactating dairy cows in and around Haramaya town, Eastern Ethiopia. A total of 384 milking cows and 1536 quarters were examined, out of which 189 and 677 were CMT positive at cow and quarter level respectively. The overall prevalence 49.2% (189/384) at cow level and 45.68% at quarter level were determined, respectively. Out this, 7.5%(29/384) were clinical mastitis and 41.7% (160/384) were subclinical and 6.8%clinical and 38.86% sub-clinical were found to be mastitis positive on CMT at cows and quarter level, respectively. Among total of 1536 quarters examined, 54 (3.5%) had blind teats. The age, lactation stage, parity and hygienic milking practice were found to have significant (p<0.05) influence on the occurrence of mastitis.

Keywords: isolation, mastitis, prevalence, streptococcus species.

GJMR-C Classification: NLMC Code: QW 4

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Bayan Amin [α], Yosef Deneke [σ] & Nejash Abdela [ρ]

Abstracts- Mastitis is the most complex and costly disease of dairy cows occurring throughout the world including Ethiopia. Streptococcal mastitis is the commonest and economically important. However, mastitis caused by this species is not well investigated. A cross-sectional study was conducted from November 2016 to April 2017 to determinethe prevalence of mastitis, associated risk factor and also to isolate pathogenic streptococcus species from lactating dairy cows in and around Haramaya town, Eastern Ethiopia. A total of 384 milking cows and 1536 quarters were examined, out of which 189 and 677 were CMT positive at cow and quarter level respectively. The overall prevalence 49.2% (189/384) at cow level and 45.68% at quarter level were determined, respectively. Out this, 7.5%(29/384) were clinical mastitis and 41.7% (160/384) were subclinical and 6.8%clinical and 38.86% sub-clinical were found to be mastitis positive on CMT at cows and quarter level, respectively. Among total of 1536 quarters examined, 54 (3.5%) had blind teats. The age, lactation stage, parity and hygienic milking practice were found to have significant (p<0.05) influence on the occurrence of mastitis. The prevalence was relatively higher in old than adult and young, in earlier and late lactation stage than mid lactation stage, in cows with many calves than those with moderate and few calves, as well as not wash pre and post milking udder than pre milking and wash pre and post milking udder. However, there was nostatistically significant difference (p>0.05) among the risk factors, breed andaddress of animals. 127CMT positive cows sample were bacteriological examination. Out of 127 samples taken 49 (38.58%) samples were positive for isolation of streptococcus species with 21 (16.5%) *Streptococcus agalactiae,* 15(11.8%) *Streptococcus. uberis* and 13(10.2%) *Streptoccocus dysgalactiae* were identified.The study showed that mastitis is an important problem and a serious threat for dairy industry in the study area. Generally, the study forwarded to improved control of mastitis in the area and hygienic milking practices important tools of mastitis control in this area. Subclinical mastitis of dairy cows in the area and hence warrants serious attention.

Keywords: isolation, mastitis, prevalence, streptococcus species.

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I. Introduction

Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country, and still promising to rally round the economic development of the country. Cow represents the biggest portion of cattle population of the country (CSA 2016). Milk produced from these animals provides an important dietary source for the majority of rural as well as considerable number of the urban and per-urban population. However; milk production often does not satisfy the countries requirement (FAO, 2003).

Mastitis is the common and costly disease causing loss in milk yield, treatmentcost, milk discarded, and reduction in quality and quantity of milk produced by a cow. Bacterial contamination of milk from affected cows may render it unsuitable for human consumption by causing food poisoning or interference with manufacturing process or in rare cases, provides mechanism of spread of disease to humans. Zoonotic diseases potentially transmitted by raw cow milk include brucellosis, leptospirosis, listeriosis, Q-Fever, Staphylococcal food poisoning and tuberculosis (Radostits *et al*., 2007).

By definition mastitis is inflammation of mammary gland parenchyma which is caused by non - infectious agents or microorganisms usually bacteria that invade the udder, multiply and produce toxins which are harmful to the mammary gland (Erskine, 2003, Mekonnen *et al., 2005*), is classified as clinical and sub clinical. Clinical mastitis is characterized mainly by appearances of changes in the milk such as flakes and clots and presence of signs of inflammation on the mammary glands such as swelling, heat, pain, and edema (Christos, 2011). Subclinical mastitis refers to inflammation of the mammary gland in the absence of visible gross lesion in the udder or its secretion with the presence of pathogenic microorganisms and usually high number of somatic cells in the milk (DACA, 2006), milk production decreases, bacteria are present in the secretion, and composition is altered (Blowy, 2010).

Majority of microorganisms that are responsible for mastitis and spoilage of milk are bacterial origin include *Staphylococcus aureus, Streptococcus agalactiae, Escherichia* coli and *Streptococcus uberis*as dominant and pathogenic (Mungube *et al*., 2005). Streptococci are one among the major mastitis pathogens which have a considerable impact on cow health, milk quality and productivity (Mungube *et al*., 2004). *Streptococcus-agalactiae* is causes contagious mastitis, an obligated pathogen of the mammary gland, which is transmitted directly among cows during milking (NMC, 2004). It infects the gland cistern and ducts of the mammary gland causing irritation; swelling and subclinical mastitis (Hillerton and Berry, 2003). As a result, *S. agalactiae* can spread widely within a herd, causing immediate loss due to reduced milk yield (Zoccone, 2006).

Streptoccocu. dysgalactiae is described as alpha hemolytic and associated only with IMI among the environmental streptococci; *S. dysgalactiae* is one of the most prevalent, which may infect mammary glands as favorable conditions arise (Hillarto *et al.,* 2005). *Streptoccocus. uberis* is an important udder pathogen in the modern dairy industry (Pullinger *et al*., 2006). The severe economic impact caused by the high prevalence of environmental streptococci in well managed dairy herds (Leigh, 1999).

Mastitis is an important factor that limits dairy production due to its heavy financial losses involved and the existence of latent infections characteristics (Lasagno *et al*., 2011). The control and prevention of such important disease in the dairy sector require a rigorous and systematic research on the status of the disease. However, in some parts of Ethiopia, the disease is insufficiently investigated and information relating to its magnitude, distribution and risk factors is scant.Moreover, many investigations on bovine mastitis in Ethiopia focused on *Staphylococcus aureus*, *Escherichia coli*, and rarely streptococcus. Despite therecognition of streptococcal mastitis all over the world (Lasagno *et al*., 2011), the information on bovine streptococcal isolates from Ethiopia is scarce. Therefore, the objectives of this study were to estimate the prevalence of bovine mastitis, assess the risk factors and also to isolate streptococcus species from lactating dairy cows in and around Haramaya town.

II. Material and Method

a) Study Area

The study was conducted in and around Haramaya town, such as Haramaya townAdelleWaltaha, Tuji-gabisa and Ifa- Oromiakebele at Haramaya district of Eastern Hararghe,Oromia region. Haramaya district is located in the Eastern Hararghe Zone of the Oromia Region of Ethiopia, which are about 506 kilometers from Addis Ababa and 12 kilometers far from the city of Harar and 35 kilometers from Dire Dawa and 5 kilometers from Haramaya University at an altitude of 2047 meters above sea level (m a.s.l.) between latitude 9°24″N and longitude 42°01″E. The mean annual rainfall is 870 mm with a range of 560 to 1260 mm and the mean maximum and minimum temperatures are 23.4°C and 8.25°C, respectively relative humidity of 68% (HADB 2016). Small holder mixed farming system is the dominant mode of production of the farmers in the area. The district has about 76,336 cattle, 65,083 sheep, and 84,916 goats, 22,355 donkeys, 356 camels and 89,800 chickens. The area receives an average annual rain fall of approximately 900 mm, with a bimodal distribution pattern (PSE, 2015).

b) Study Population

The study populations were lactating cows of small holder dairy farm which were breeds kept under the semi-intensively husbandry practice and there milking practice was by hand (manual). Lactatingcowsin Haramayatown, Adele Waltaha, Ifa-Oromiya and Tuji Gabisa, were the animals included in the study. These animals werekeptunder the semi-intensive management system whereby cattle are grazed freely on pasture but received supplementary feeds in the morning and evening when they were milked and during last pregnancy. All cows were hand milked twice daily, in the morning and evening. The milk yield of the cows ranged from (4-8 L) per day for cross breeds while (2-4L) for local breeds.

c) Sample Size Determination

Across sectional study was conducted to determine the prevalence of both clinical and subclinical mastitis after a total 384 cow's milk samples were collected by simple random sampling from expected prevalence is 50% CMT with the 95% confidence level and desired precision of 5% using the formula described by Thrusfield (2005).

$$n = \frac{1.96^2 \times P_{exp}(1-P_{exp})}{d^2} = 384$$

Where:

n= required sample size
Pexpe= expected prevalence
d^2 = desired absolute precision
z = 1.96^2

d) Sampling Strategy

A cross- sectional study was carried out to determine bovine mastitis from November, 2016-April, 2017 conducted on simple random sampleselected local and cross breed lactating dairy cows from selected area in and around Haramaya town at cows level based on udder inspection for clinical mastitis manifestations and indirect test (California mastitis test) for sub clinical

mastitis, questioner survey for risk factor and milk sample collection for microbial isolation.

e) Sampling Method

Sample collection was made to examine all functional teats of each study animals and CMT positive cases with relevant information about lactating cows in the small dairy farm was gathered and the sample was employed from CMT for the bacterial isolation.

f) Questionnaire Survey

A semi-structured questionnaire was developed and pretested, and all information relating to the study objectives was recorded. Data collected include address and Pertinent to cow-level factors, including lactation dairy cows age, parity, lactation stage, breed and milking practice where the owner of cows were wash hand and udder before and after milking, wash hand and udder before milking and wash hand only before milking. Age of the animals was determined from birth records and dentition characteristics and categorized as young (>3 to 6 years), adults (>6 to 10 years), and old (>10) according to Jonsan(1999) who classification of age depending dentition. Stage of lactation was categorized as early (1st to 3th month), mid (4th to 6th month), and late (7th month to the beginning of dry period). Parity was categorized as few with (1-3 calves), moderate (4–6 calves) and many (7 and above calves).

g) Clinical Inspection of the Udder

Each cow was clinically observed for the manifestation of general clinical signs related to udder and teats and presence of any gross abnormalities. The udder was first examined visually and then through palpation to detect possible fibrosis, inflammatory swellings, visible injury, tick infestation, atrophy of the tissue, and swelling of supra-mammary lymph nodes. The size and consistency of mammary quarters were inspected for the presence of any abnormalities, such as disproportional symmetry, swelling, firmness, and blindness. Viscosity and appearance of milk secretion from each mammary quarter were examined for the presence of clots, flakes, blood, and watery secretions. The udder was also inspected for the presence of any grossly visible injury on location, size, and nature injuries the teats were part of the indicators for clinical mastitis (Quinn *et al*., 2002).

h) Milk Sample Collection, Methods of Transportation and Storage of Samples

The Californian mastitis reagent was used to screen cows with sub clinical mastitis milk sample collection was according to the procedures recommended by national mastitis council (NMC, 1999). The result of the test was indicated on the basis of gel formation. The interpretation (grades) of the CMT was evocated and the results graded as 0 for negative and trace 1, 2 and 3, for positive (Quinn *et al*., 2002).

The milk sample was taken from cows, washing by clean water and dry the teat by cotton and the teat were wiped thoroughly with 75% ethyl alcohol and the first stream (2-3) of milk from each quarter was discarded and collected milk in the sterile milk collection bottle for good collection of sample. After collection of the milk sample, all samples were clearly labeled with the appropriate identification of the cows, quarter using permanent marker on the test tube and all samples were transported with ice box to the laboratory without delay and it were processed (Quinn *et al*., 2002). In the laboratory, samples were cultured immediately or stored at +4^{0}c in any case of delay (NMC, 2004). Analysis o f specified samples was performed on isolation and identification of pathogenic bacteria at Haramaya University collage veterinary medicine laboratory in microbiology laboratory.

i) Detection of sub-clinical Mastitis

Mastitis was detected using the California Mastitis Test (CMT) and results of clinical inspection of udder (Quinn *et al.,* 1999). Grades of the CMT were evaluated and the results graded as 0 for negative and1, 2 and 3 for positive (NMC 2004). Subclinical mastitis was diagnosed based on CMT results and the nature of coagulation and viscosity of the mixture, which show the presence and severity of the infection, respectively (Harmon 1994)

j) Preparation of Culture Media, Culture and Bacterial Isolation

i. Preparation of Culture Media

To prepare media for bacterial culture, the manufacturer's instructions was be followed, besides few additional general points were included, all glass wares used for the preparation of media were first sterilized using appropriate equipment like autoclave, hot air oven, the appropriate amount of dehydrated media were weighed out of using sensitive balance and the required amount of distilled water were added to the powder media. Dehydrated media containing agar were dissolved in heating mantle until it boil and frothy appearance was settled (removed), then the media were sterilized by autoclave at 121^{0}C for 15 min holding time, and cooled in water bath at 50^{0}C before poured in to the Petri dishes. Some media like blood agar and modified Edward medium requires addition of blood after it is cooled to 50^{0}C since RBC are not tolerate higher temperature, adapted from (Quinn et al., 2002). The common media used during the study were blood agar, MacConkey agar, modified Edward medium (Oxiod England), Aesclinehydirolaysis media and Manitol salt agar.

ii. Culture and Bacterial Isolation

After Milk samples were collected from all quarter with clean and aseptically procedure for microbiological culture and species identification,

according to the procedures of the (NMC, 1999). Culturing of milk sample collected from individual cows, in search for mastitis producing organisms in standard of examination for mastitis (Radostits *et al*., 2007). One standard loop (0.01 ml) of milk sample was streaked using the quadrant streaking method for each cows on streptococcus selective agar of modified Edward medium (Oxiod England) at around Bunsen burner to reduce contamination. In case of refrigerated milk samples, as bacteria might be concentrated in the cream layer and held with in clumps of fat globules, dispersion of fat and bacteria was accomplished by warming the samples at 25 °C for 15 min before plating on modified Edward medium agar the inoculated plates were then incubated aerobically at 37 °C for 24 to 48 hrs.

Then the inoculated plates were examined from 24hr incubation to 48hrs for growth, morphological features, such as colony size, shape, and color, and hemolytic characteristic, the growth colonies on selective media were sub-cultured on 7% sheep blood agar (Oxoid, UK) for further investigation hemolytic types and growth character. After pure colonies were obtained, Gram stained smears were done for primary identification of bacteria to genus level, such as Gram reaction (Gram positive and Gram negative), and cellular morphology (coccus or rods). Other primary tests had done include catalase, oxidase and growth or absence of growth on MacConkey agar (Oxoid, UK) and the secondary biochemical tests such as, CAMP test Aesculin hidrolaysis test, etc were done for bacterial species identification. annex 3

k) Data Management and Analysis

The collected data were entered to Microsoft office excel 2010 program and analyzed using SPSS version 20. Descriptive statistics were used to summarize the generated data on the rate which was collected through, clinical inspection, CMT, isolation and identification Streptococcus species. Prevalence of mastitis related to specific risk factors was determined as the proportion of affected cows out of the total examined. Effects of specific variables (breed, hygienic practice, age, parity, lactation stage, site, on prevalence of mastitis were investigated using chi-square (X^2) test. Similarly, the variation in prevalence of mastitis-induced blind quarters was assessed using the same statistical method. A statistically significant association between variables is considered to exist if the p value is < 0.05.

III. Results

A total 384 lactating cows were included in thisstudy and 189 (49.2%) cows were found be positive for mastitis on CMT. Out of 189 CMT positive cows, 29/384 (7.5%) clinical and160/384 (41.7%) sub- clinical mastitis were found with statically significance difference (p=0.000) table 1.

Table 1: Prevalence of clinical and sub-clinical Mastitis at cow's level

Status	No. examined cow	CMT positive	Prevalence	X^2	P-value
Sub clinical	384	160	41.66 %	384	0.000
Clinical	384	29	7.5%		
Total	384	189	49.2%		

a) Prevalence of Mastitis at Cows and Quarter Level

A total number of 384 lactating cows and 1536 quarter were included in this study. Out of which 189(49.2%) cows and 677(45.86%) quarter were be found positive for mastitis on CMT. Out of this 29 (7.5%), 160 (41.7%) were clinical and sub-clinical mastitis at cows level respectively and 6.8 % clinical and 38.86% sub-clinical mastitis at quarter levels(table 2).

Table 2: Prevalence of mastitis at cows and quarter level using CMT

Observation	No. Examined	No. Positive	Prevalence	Clinical mastitis. No.%	Sub-clinical mastitis. No. (%)	X^2	p-value
Cows	384	189	49.2	29 (7.5)	160 (41.7)	384	0.000
Quarter	1482	677	45.68	101(6.8)	576 (38.86)		

b) Quarter Prevalence of Mastitis using CMT

A total number of quarters (1536) of cow were checked for the presence of gross abnormalities, 54 quarters were found to be blind teats and 1482 quarter were using CMT screening test out of these 677 (45.68%) quarters were found to be positive mastitis on CMT positive at quarter levels (table 3).

Table 3: Quarter prevalence of mastitis using California mastitis test

Quarter	No. examined teat	CMT positive quarter	Frequency %
Rear right	372	174	46.77%
Rear left	368	167	45.38%
Front left	372	170	45.69%
Front right	370	166	44.86%
Total	1482	677	45.68%

c) Proportion of Blind Teat

All functional quarter (1536) were examined. Out of which 54 (3.5%) quarters which belongs to 42 lactating cows were found to be blind quarters. From cows having blind quarters, 30/42 (71.4%) cows have single blind quarter and 12/42 (28.57%) cows have double blind quarters. With regard to the location of the blind teats, 22.22%, 29.62%, 22.22%, and 25.92% were found to be of the Rear Right (RR), Rear Left (RL), Front Left (FL), and Front Right (FR) position respectively, (table 4).

Table 4: Proportion of blind teat

Blind teat	No. examined teat	No. blind teat	No. Blind teat Clinical	No. blind teat Sub-clinical%	Proportion% of blind teat
Rear Right	1536	12	4	8	22.22
Rear Left	1536	16	4	12	29.62
Front Left	1536	12	3	9	22.22
Front Right	1536	14	4	10	25.92

d) Risk factors associated with bovine mastitis

During the course of study on varies risk factors associatedmastitis among those age, parity, lactation stage, breed, milking hygienic practice and address of animal for examine presence of mastitis at cow's level. The age, parity, lactation stage and milking hygienic practice were found to be significantly (p<0.05) associated with presence of mastitis. On another hand breed and address did not significant effect (p>0.05) on presence of mastitis (table 5).

There were significant differences in prevalence between cows of different age categories. The highest prevalence (66.6%) was found to be lactating cows at old age (>10 years old) and followed adult cows with age category between (6-10) years (51.6%), and the lowest prevalence (42.5%) was recorded in young cows at age category between (3-6) years old with significant at (p=0.004).

Risk factors with lactation stage between successive lactation stage were significant effect (P=0.000) on the prevalence of mastitis. Higher prevalence (64.3%) of mastitis was observed and recorded in cows of earlier lactation stage between first three months of lactation (1-3 month), followed by cows in late lactation stage (7th month to the beginning of dry period) (52.7%) and lowest prevalence (30.5%) was recorded cows at middle lactation stage between (3 month to 6 month) (table 5).

There was also statically significant difference in prevalence between lactating cows at different parity (P=0.003). The highest prevalence (72.9 %) was recorded in cows which gave birth up to 7 and above calves, followed by cows which gave birth or parity number between 4-6 calves (51.6%) and the lower prevalence (42.9%) was recorded in cows that gave birth to 1-3 calves (table 5).

The effect of breed on the presence of bovine mastitis at study area were revealed that breed with in prevalence of subclinical and clinical mastitis did not vary along with the breed of animal, but relatively higher prevalence was seen in animals at local breed (56.6%) and low in cross breed with prevalence of 43.9%. The result of statistical analysis revealed no significant difference (P > 0.05) among the breed animals (table 5).

The milking hygienic practices of udder during milking were significant effect with Presence of mastitis (p=0.000). The highest were found the cows managed under poor milking hygienic practice (no udder and hand washing) (86.3%), followed the cows which wash udder and hand before milking (33.9%) and lowest prevalence (22.6%) were recorded cows at good milking hygiene practice (wash before and after milking) (table 5). The presence of mastitis with cows address was also studied;but the result on statistical analysis indicated were not significant difference (P > 0.05) among different kebele in the study area (table 5).

Table 5: Prevalence of Bovine Mastitis with Different Risk Factor of lactating dairy cow

Risk factors	Category.	No. examined	No. Positive	Prevalence %	X^2	P-value
Age	Young	202	86	42.5		
	adult	122	63	51.6	11.162	0.004
	Old	60	40	66.6		
Breed	Local	293	149	56.6	1.322	0.250
	Cross	91	40	43.9		
lactation stage	Early	115	74	64.3		
	Middle	108	33	30.5	27.464	0.000

Parity	Late	161	85	52.7		
	1-3(few)	198	85	42.9		
	4-6(moderat)	149	77	51.6	11.847	0.003
	≥7(many	37	27	72.9		
Milking hygienic practice	Wash pre & post milking	141	32	22.6		
	Wash before milking	109	37	33.9	137.079	0.000
	Not wash at all milking	134	120	86.3		
Address	Haramaya town	108	49	45.3		
	IfaOromiya	101	50	49.5		
	Adele Waltaha	86	42	48.8	1.440	0.696
	TujiGebisa	89	48	53.9		
Total		384	189	49.2		

e) Bacterial Isolation and Identification

During the course of the study, a total of 127 milk samples were taken from 29 clinical cows and 98 sub-clinical mastitis cows were cultured on modified Edward media. The growths of different streptococcus species bacteria were observed. Prevalence isolation and identification of major bacterial streptococcus species were carried out on milk from all 29 clinical cows and 98 random sample from sub-clinical mastitis cows by using primary and secondary biochemical tests. Result obtained from this study showed that out of 127 samples taken 49 (38.58%) sample were found to be positive up on growth. Out of which 21 (16.5%), *Streptococcus agalactiae,* 15 (11.8%) *Streptococcus uberis and* 13 (10.2%) S*treptoccocus dysgalactiae* were found to be identified species *(*table 6).

Table 6: Bacterial isolation and identification

Species identified	Clinical	Subclinical	Proportion
Streptococcu, agalactiae	5	16	21(16.5%)
Streptoccocus, dysgalactiae	5	8	13(10.2%)
Streptococcus, uberis	2	13	15(11.8%)
Total	12	37	49(38.5%)

IV. Discussion

In the current study,a total of 1536 quarters and 384 lactating cows in and around Haramaya town east Hararghe were investigated and overall prevalence of mastitis 49.2% at cows levels were recorded. This result was in agreement with (Sori *et al*., 2005), who reported 52.78% in and around Sebeta, 53.25% in Dire Dawa town by Biniam *et al*., 2015 and 46.7% in Adama town by Abera *et al.* (2013). Moreover, the present study was agreed relative to the available reports from other African countries such as 51.6% in Tanzania by Karimuribo *et al*., 2009 and 51.8% in Rewanda by Iraguha *et al*., 2015, but higher than previous study in different parts our country such as findings of Jefar *et al*., 2016 who reported 39.2% in dire dawa town eastern, Ethiopia, Biffa *et al.,* 2005) in Southern Ethiopia (40.40%). Nevertheless, the current finding was lower than findings of Mekibib *et al*., 2010 in Holeta town in Central Ethiopia 71.05%, Birhanu *et al*., 2013 in Asella Oromia regional state, South Eastern Ethiopia. The variability in the prevalence could be suggested the complexity of the disease which involves interaction of several factors, mainly the difference in management of the farms, husbandry system, environment, and factors related to causative agent and host.

In this study 7.5% were found to be clinically positive formastitis up on udder inspection. It was similar with the previous study in different areas in Ethiopia such as 7.8% by Duguma *et al*., 2014 and 7.14% by Tsegai (1997), both at Holleta area, Central Ethiopia and in central high lands of Ethiopia 6.6% by Mungube *et al*., 2004, butlittle bet higher than the result of Kasech *et al*., 2016 in Tullo District West Hararghe 5.2%, in central Ethiopia 5%, by Nibret *et al*., 2012, and Benta and Habtamu (2011) in Batu and its environments, Ethiopia 5.3% on prevalence of clinical mastitis. These variations could be due to improper hygiene during udder preparation and milking, lack post milking dipping of teats and appropriate treatment. Risk factors which influence the occurrence of clinical mastitis were outlined as animal, pathogen, and environmental risk factors, which could contribute in the discrepancies of mastitis prevalence (Radostits *et al*., 2007).

Out of examined cows, 160/384 (41.7%) were found to be positive forsub-clinical mastitis. This result was in agreement with previous findings such as 40.6% in Batuand its surroundings (Benta and Habtamu 2011) and 43% at Areka town by Gebremichael *et al*., 2013), and higher than 33.8% around Holeta area by Girma. (2010), 10.6% in Tullo District West Hararghe by Kasech *et al*. (2016), but lower than findings such as 51.8% in

eastern Hararghe area by Tesfaheywet and Abera (2017) and 55.1% in Addis Ababa Zeryehun *et al*. (2013). The present study revealed higher prevalence of subclinical mastitis compared to clinical mastitis. Other studies shared similar observations Sori *et al*., 2011, Zeryehun *et al*., 2013. High prevalence of sub-clinical mastitis were observed in our case could be due to the infected animal shows no obvious clinical sign and secrets apparently "normal" milk, lack of regular mastitis screening test such as CMT, lack of dry cow therapy and lack of awareness.

In the current study 45.68% of quarter was found to be positive for mastitis at quarter levels with 6.8% clinical and 38.86% sub-clinical. This result cross agreed with 47.52% at Sebeta Town by Belay (2011) and Mekibib *et al*., 2010, who reported an overall prevalence of 44.9% around Holeta town. This result little bet closed with 5.2% and 42.7% Around Addis Ababa by Zeryehun *et al*., 2013, 10.7% and 46.4%, in Eastern Hararghe Zone by Tesfaheywet and Abera (2017). This result not in agreement with 18.91% and 81.08% in Dire Dawa City, Eastern Ethiopia by Jafer *et al*., 2016 and higher than reports made over as such as prevalence of 35.25% in Pakistan by Bachaya *et al.,* 2011 and 27.57% in Germany by Fadlelmoula *et al*. (2007). The difference may be due to greater experience in drying off, the potential effect of level of milking hygiene and cleanness, and the application of sanitary measures.

The study result revealed statistical significant association of prevalence of mastitis with the age, lactation stage, parity and milking hygiene practice of lactating cows. The present result was coincides with previous study that state increasing age, lactation stage, parity and poor management as the risk of mastitis (Dego and Tareke, 2003) and Nibret *et al*., 2011). The association of age with positivity for mastitis was found to be statistically significant (P<0.05) and high prevalence of mastitis was recorded in old cows. This finding was found to be similarl with previous finding of Girma (2010) in Holeta area and Bitew *et al*., 2010 around Bahir Dar area. The higher prevalence in older cows in the present study might be that older cows have largest teats and more relaxed sphincter muscles that render ease of accessibility and establishment of infectious agent in the cows' udder (Radostitis *et al*., 2007). The association of parity with positivity for mastitis was found to be statistically significant (P<0.05). This finding was comparable with the previous reports (Tamirat, 2007; Mekibib *et al*., 2010; Haftu *et al*., 2012). This might be due to the increased opportunity of infection with time and the prolonged duration of infection, especially in a herd without mastitis control program (Radostits *et al*., 2007) and cows having greater than 5calves were more affected than those with fewer and moderate calves (Zeryehun *et al.,* 2013).

The relationship between the prevalence of mastitis on different lactation stage was studied, the result showed significantly higher infection (p<0.05) in cow with early (63.3%) and late lactation (52.7%) than cow with mid (30.5%) lactation stage. This result was agreed with G/mechael *et al*., (2013) and Biffa *et al*., (2005) who reported lactation stage had significant effect on the prevalence of mastitis in Ethiopia. Early stage and the late stage of lactation were the most susceptible stages. The mid lactation was lower. This could be due to the delayed diapedesis of neutrophils to mammary gland in recently calved cow and at late lactation there is decrement of neutrophil concentration when the cows reach to dry off (Workineh *et al*., 2002) and increased oxidative stress and reduced antioxidant defense mechanisms during early lactation (Sharmal *et al*., 2011). Moreover, absence of dry cow therapy regime could possibly be among the major factors contributing to higher prevalence at early lactation (Green *et al*., 2008), the high rates of new infection following drying off may be associated with the lack of flushing action of milking (Biffa *et al*., 2005).

The current study showed that the effect of milking hygienic practice was statistically significant difference (p<0.05) on the prevalence of bovine mastitis and infection rate was high in cows which not washed udder pre and post milking was (86.3%), followed by wash pre milking only 33.9% and lowest which wash pre and post milking 22.6%. The current study cross checked with previous findings (Lakew *et al*., 2009, Junaidu *et al*., 2011) both were reported that Cows at farms with poor milking hygiene standard are severely affected than those with good milking hygiene practices. The absence of udder washing, increased exposure and transmission of pathogens during milking (Kivaria *et al*., 2004), Whereas under Ethiopian conditions most of households use hand milking and washing hands, udder and teats before milking are not practiced, this could predispose dairy cows for pathogens (Bedane *et al*., 2012).

This current study showed that out of the 127 samples taken and growth 49/127 (38.5%) were found be positive for cultural isolation of streptococcus species. This result agreed with that of Bryson and Thomson 1990 at Bulawayo found to be 37% and 38% respectively and comparable with that of the report of Atyabi *et al*., 2006 at farms around Tehran (33.54%), but higher than previous study such as 29.03% by Ayano *et al*., 2013 in holeta town, 27.7% by Yohanis (2013), in walaytasodo southern Ethiopia and Hawari and Al-dabbas (2008), who reported 26.2% of Streptococcus species in Jordan.

However this study was much higher than the reports of Bitew *et al*., 2010 at Bahir Dar 13.9% and Sori *et al*., 2005 in and around Sebeta (3.73%) and the present findings was lower than that of reported by Tolassa (1987) and Okeke *et al*. (2005), who found

Streptococcus species to be 53.55%, and 80.95% in dairy cows respectively. The relatively high isolation of this organism in this study may due to poor milking time hygiene, absence of post milking teat dipping, lack of proper treatment for clinically infected animals and lack of use of dry period therapy.

Streptococci species isolated as mastitis pathogens in this study showed the species *S. agalactiae* (16.5%) and *S. dysgalactiae* (10.2%) and *S. uberis* (11.8%). The present result on bacteria isolated *S.agalactiae* was most commonly isolated in clinical and sub clinical case of mastitis in this study case with (16.5%) of all isolate. The high level isolation in this study is related with the findings at different part of Ethiopia Such as 17.8% by Yohanis and Molla (2013) in and around walaita sodo, 15% by Tadesse *et al.* (2014), Holeta area and 18.31% by Fufa *et al.* (2013) and higher than 12.2% by Duguma *et al.*, 2013, but much higher than reported by Lake *et al.* (2009) and Bitew *et al.* (2010) who reported 4 and 8.8%, respectively, but also current findings was lower than that of Bishi (1998) who reported higher isolation rate (27%) for *S. agalactiae.* The reason for the higher isolation rate of this organism is the wide ecological distribution inside the mammary gland. In area where hand milking and improper use of drug is practiced to treat the mastitis cases, lack pre and post milking wash and teat dip, lack of dry cow's therapyand an adequate treatment clinical case. Its domination has been reported by many research scholars. *S. agalactiae* is adapted to survive in the udder an obligate agent of the mammary gland, S. agalactiae is a contagious cause of mastitis within a herd, sources of contagious mastitis are infected cows and transmission is from cow to cow, mainly at milking time through milking equipment, the milker's hands and contaminated wash cloths (Zoccone, 2006).

The present result indicated *S. dysgalactiae* isolated from milk sample (10.2) was similar with the previous findings of Ayano *et al.*, 2013 who reported 10.6% atholota district. However, this finding was found to be higher when compared with Yohannis and Molla (2013), who reported 8.9% in and around walaitasodo, 7.2% by Duguma *et al.*, (2013), 5.6% by Kerro and Tareke (2003) and 0.5% by Bishi (1998), but lower than that of G/Michael *et al.*, 2013 who reported 24% *S.dysgactiae* in and around ereka town. *S. dysgalactiae* are contagious pathogens were higher isolates in current study area might be due to lack of inter-cow hand washing and disinfection in the milking area andcontaminations of milkers' hands were spread of mastitis the present study agreed with previous study that spread of *S. dysgalactiae* between cows within dairy herds may occur directly or by way of the milking machine or environment (Younis *et al.*, 2005).

Present study showed that *Streptoccocus uberis* (11.8%) was isolated which was in agreement with Ayano *et al.*, 2013 who reported 12.1% at holeta district, but much higher than 4.23% by Kerro and Tareke (2003), 1.48% by Almaw *et al.*, 2009 in and around bahirdar and (6.53%), by Mekebib *et al.* (2009) but lower than that of Zerihun (1996) and Iqbal *et al.* (2004) who reported in in Addis Ababa and Pakistan, 27% and 49.98%, respectively. Environmental streptococci may be due to poor housing facilities which predispose to the accumulation of feces on cows which could increase the rate of exposure of the teats and udder to the pathogens, not use dry cloth during milking, wash hand and material by common water, lack of dry therapy and improper of milking. This finding in line with many researches who reported *s. uberis* environmental factor during milking process, between milking, during the dry period and prior to parturition in first-lactation heifers and other environmental risk factor is housing and management practices such as contamination of bedding materials and exposure of teats to environmental streptococci (Hillarto *et al.*, 2005).

V. Conclusion

The present study indicated overall prevalence of 49.2% which was a major health problem of dairy cows in the study area and undoubtedly would have an adverse effect on productivity of dairy industry. Relatively high prevalence of subclinical mastitis in dairy cattle of the study area due to lack of strategic control measures against the disease, lack of proper attention to health of the mammary glands, Lack of maintenance of strict hygiene and good sanitary environment contributory factors in the cause of clinical and subclinical mastitis. The major Streptoccocus species isolated was mainly *Strepotoccocu agalactiae*. Since the bacteria isolated from cows' milk samples was cause of both contagious and environmental mastitis the farmers should ensure strict personal hygiene and that of animals and sanitary condition of the farms should be improved and regular screening for the detection of subclinical mastitis should also be practiced.

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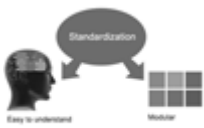
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It is mandatory to read all terms and conditions carefully.

AUXILIARY MEMBERSHIPS

Institutional Fellow of Open Association of Research Society (USA) - OARS (USA)

Global Journals Incorporation (USA) is accredited by Open Association of Research Society, U.S.A (OARS) and in turn, affiliates research institutions as "Institutional Fellow of Open Association of Research Society" (IFOARS).

The "FARSC" is a dignified title which is accorded to a person's name viz. Dr. John E. Hall, Ph.D., FARSC or William Walldroff, M.S., FARSC.

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The IBOARS can initially review research papers of their institute and recommend them to publish with respective journal of Global Journals. It can also review the papers of other institutions after obtaining our consent. The second review will be done by peer reviewer of Global Journals Incorporation (USA) The Board is at liberty to appoint a peer reviewer with the approval of chairperson after consulting us.

The author fees of such paper may be waived off up to 40%.

The Global Journals Incorporation (USA) at its discretion can also refer double blind peer reviewed paper at their end to the board for the verification and to get recommendation for final stage of acceptance of publication.

The IBOARS can organize symposium/seminar/conference in their country on behalf of Global Journals Incorporation (USA)-OARS (USA). The terms and conditions can be discussed separately.

The Board can also play vital role by exploring and giving valuable suggestions regarding the Standards of "Open Association of Research Society, U.S.A (OARS)" so that proper amendment can take place for the benefit of entire research community. We shall provide details of particular standard only on receipt of request from the Board.

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After nomination of your institution as "Institutional Fellow" and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf.

The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.

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- The Fellow can organize symposium/seminar/conference on behalf of Global Journals Incorporation (USA) and he/she can also attend the same organized by other institutes on behalf of Global Journals.
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Note :

"
- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.

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1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5.STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

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Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

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Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

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A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

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Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

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- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
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Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

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Acknowledgements: Please make these as concise as possible.

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References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

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1. Choosing the topic: In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

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13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

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21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

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28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

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Key points to remember:

- Submit all work in its final form.
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- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

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· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

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· Use past tense to describe specific results

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· Shun use of extra pictures - include only those figures essential to presenting results

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- Fundamental goal
- To the point depiction of the research
- Consequences, including <u>definite statistics</u> - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

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- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

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- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

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- Report the method (not particulars of each process that engaged the same methodology)
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- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
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Approach:

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What to keep away from

- Resources and methods are not a set of information.
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The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

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- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
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What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.

- Do not present the similar data more than once.
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Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
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Figures and tables

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- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
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Approach:

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Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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