
**“EVALUATION OF THE LEVELS OF SERUM ZINC, IRON
AND HEMATOLOGICAL PARAMETERS AND THEIR
CORRELATION WITH RECURRENT TONSILLITIS -
A ONE YEAR OBSERVATIONAL STUDY AT KLES
DR. PRABHAKAR KORE HOSPITAL”**

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ABSTRACT

BACKGROUND

Trace elements such as **zinc and Iron** play essential roles in the metabolic and immune functions of the body. In the practice of medicine zinc supplements are used both prophylactically and therapeutically for infections with a surge during the recent COVID pandemic. Anaemia which also suppresses immunity was assessed. The **neutrophil -lymphocyte ratio (NLR)** is an upcoming predictive factor in inflammation and infections. The present study was done to elucidate the correlation of zinc, anaemia and NLR to chronic and recurrent tonsillitis.

AIM

To determine if Neutrophil -Lymphocyte Ratio and serum iron and zinc levels, Iron deficiency anaemia can be used as indicators for recurrence in chronic tonsillitis

MATERIALS AND METHODOLOGY

A 1 -year observational study was conducted and 100 patients with chronic, recurrent tonsillitis were included. Serum zinc and iron were estimated by atomic absorption spectrometry performed directly by the author in the laboratory and Neutrophil Lymphocyte Ratio was estimated using the CBC. Then using Spearman's and Pearson's correlation Coefficients detailed statistical analysis was done to look for correlation between recurrence and the above parameters and their relevance as predictive indicators was assessed.

RESULTS

88% of the patients had a **low serum zinc**, **56% had low serum iron**, **84%** were **anaemic and NLR was raised in 86% of the study population**. There was a significant correlation between low serum zinc and low serum iron among the study population. There was a **raised NLR** in those with low serum zinc and a significant positive correlation between recurrent tonsillitis and raised NLR, the Spearman's correlation coefficient r value was **0.824** and the p value was less than 0.05.

CONCLUSION

In order to plan the treatment of tonsillitis and predict the prognosis accurately we need to **assess the patient's immune status, for which serum zinc and iron and markers of anaemia** can be used. **The NLR is a novel and economical prognostic biomarker** which can be embraced in the modern, evidence-based practice of otorhinolaryngology.

KEYWORDS:

Recurrent Tonsillitis, Serum Iron, Serum Zinc, Neutrophil Lymphocyte Ratio, Iron deficiency Anemia,

LIST OF ABBREVIATIONS

NLR	-	NEUTROPHIL LYMPHOCYTE RATIO
CBC	-	COMPLETE BLOOD COUNT
WBC	-	WHITE BLOOD CELL
DLC	-	DIFFERENTIAL LEUKOCYTE COUNT
TLC	-	TOTAL LEUKOCYTE COUNT
AAS	-	ATOMIC ABSORPTION SPECTROMETRY
FAAS	-	FLAME ATOMIC ABSORPTION SPECTROMETRY
Zn	-	ZINC
Fe	-	IRON
Cu	-	COPPER
Co	-	COBALT
Mg	-	MAGNESIUM
Mn	-	MANGANESE
TIBC	-	TOTAL IRON BINDING CAPACITY
CuZnSODs	-	SUPEROXIDE DISMUTASE
C₂H₂	-	ACETYLENE
DDW	-	DOUBLE DISTILLED WATER
PPM	-	PARTS PER MILLION

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INTRODUCTION

While practicing Otorhinolaryngology, we most commonly encounter infections and inflammatory pathologies in the tonsils of the palatine region such as tonsillitis which recurs or like hypertrophy.¹

The tonsils of the palatine region constitute the ring of Waldeyer's which is made up of lymphoid tissue with special function and it is located in the upper part of the tract of respiration, at level of the naso-pharynx.²

We can define recurrent tonsillitis as presence of episodes of tonsillitis which are more than seven in number in 1 year or above 5 episodes in a year occurring for 2 years in a row or episodes more than 3 in number of tonsillitis every year for three years; and these episodes are disabling and prevent routine normal functioning.^{2,3} The management of such recurrent or chronic cases is primarily by surgical excision of the palatine tonsils especially in cases not controlled by medical management³. Iron deficiency anaemia is a known predisposing factor for development of infection by affecting immunity⁴, so in our study we have considered low haemoglobin levels as an added variable alongside serum iron and zinc. The number of studies done regarding quantity of serum trace elements such as copper, zinc, cobalt, iron with respect to infection and inflammatory conditions or infections of the head and neck that too of the palatine tonsils are limited^{5,6}.

There have been quite a few studies done regarding trace elements in other acute as well chronic infections and their levels have shown significant correlation to the severity and recurrence of infection.⁷⁻¹⁵ The ratio of neutrophils with lymphocytes is the newly used factor of prediction for chronic infections within upper respiratory

tract, which hasn't been used in such a study in comparison with zinc and trace elements in the past was also included.¹⁶

In the current practice of medicine several trace elements are even given prophylactically to prevent infections, thus we did our study for elucidating levels in the serum of these core elements with respect to a common otorhinolaryngological infection in the form of recurrent tonsillitis.

OBJECTIVES

1. To correlate serum levels of zinc and iron among patients with tonsillitis which is recurrent.
2. To assess the Ratio between Neutrophils and Lymphocytes (NLR) and prevalence of anaemia in recurrent tonsillitis, and its correlation to ZN/Fe levels in recurrent tonsillitis patients

REVIEW OF LITERATURE

The study by as Somuk Battal Tahsin et al, compared amount of trace elements such as zinc as well as iron of tonsillar tissue in cases of tonsillitis which was recurrent and in tonsils which were hypertrophied and they concluded that lower tissue concentrations of these elements led to predisposing the patients to recurrent infections of tonsils which meant that Iron and zinc played a vital part in the function of the immunity as well as in developing infections.¹⁴

In their study Donald.V et al of USA have described that Zinc is a catalyser, and by having structural and regulatory functions, zinc haemostasis affects immunity, apoptosis and oxidative stress, required for the operation of enzymes such matrix metalloproteinases, carbonic anhydrase, and superoxide dismutase. As a component of the enzymes peroxidase and other enzymes that produce nitrous oxide, which is essential for the immune system's enzymatic functioning, iron contributes to the immune system's growth.

The literature is replete with papers describing changes in the plasma concentrations of trace elements in a variety of illnesses. The study by LJ. Srinivas et al. discovered a drop in serum Zn but a rise in serum Cu levels in various acute bacterial infections (septicemia, pneumonia, erysipelas, and meningitis), with the exception of individuals with erysipelas. For viral infections, their outcomes were comparable, although less apparent.

According to K.H. Brown et al the serum Zn levels fell and serum Cu levels rose after acute infection. The CuZnSOD (superoxide dismutase) distribution in paediatric patients tonsils and adenoids varies, and many associated clinical features

regarding its expression were discovered.⁸ Studies also demonstrated that the trace element status played a crucial role in significantly preventing acute infections.¹³

In their research, Tekin Baglam et al. evaluated the accuracy of NLR (neutrophil lymphocyte ratio) in predicting DNSI (deep neck space infections) complications in a paediatric population with acute bacterial tonsillitis. They found that NLR had high specificity and sensitivity for predicting DNSI (deep neck space infections) complications associated to acute tonsillitis of bacterial origin, and their findings further demonstrated the value of NLR as a diagnostic tool.^{13,15}

The research done by Battal Tahsin Somuk et al which was done in Turkey had a sample size of just 20 subjects, results of such a study could have been more accurate and statistically significant if a larger sample size were used.

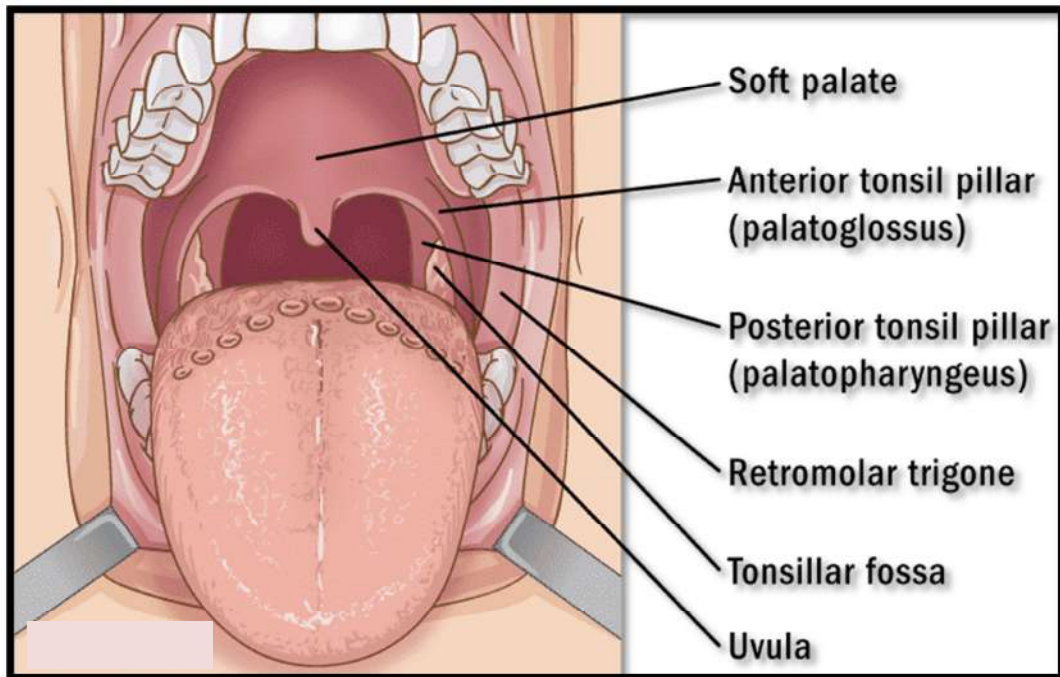
The study done by Onerci et al only included the quantities of zinc and that of iron within the tissues and cells of the tonsils, it did not consider serum levels which would have provided a better understanding of role the played by the trace elements in the general susceptibility to recurrent attacks of tonsillitis in the subjects of study.

Most of these studies have been done in Europe and other western countries due to which their relevance and applicability to the practice of otorhinolaryngology in India cannot be directly ascertained, as the racial factors, prevalence and incidence of tonsillitis is different in our country.¹⁵

ANATOMY OF THE OROPHARYNX

The nasopharynx continues in the caudal direction as the oropharynx, which forms the starting of the aerodigestive tract, and thus is a communication between the nasal cavity, oral cavity and the larynx. It participates in two important functions, being swallowing and breathing. The following anatomical structures contribute the formation of this region – the base of tongue, vallecula, the anterior pillars also known as the faucial pillars, palatine tonsils, the tonsilo-lingual sulci, posterior pillars and soft palate's lower surface, along with musculus uvulae and the walls of the pharynx which were behind and lateral. Each of these structures bear a clinical significance, some of them such as the tonsilo-lingual sulci and the tongue basal region, and the tonsils of the palate are commonly known in malignancies, especially such as squamous cell carcinoma secondary to Human Papilloma virus infection. Whereas the palatine tonsils are commonly known to be the seat for localized infection in the form of tonsillitis and peritonsillar abscess. The regions like the valleculae and the tonsillar sulci due to their alignment to one another also lead to lodgement of foreign bodies such as pieces of food or bone which is another common presentation at an otorhinolaryngology clinic. In this manner it is important to examine each of these structures in detail for complete analysis^{6,7}.

FIG 1- ANATOMY OF OROPHARYNX⁷



THE PALATINE TONSILS

The tonsils of the palate region are tissue of lymphoid follicles whose structures is ovoid in shape and two in number, located in the lateral pharyngeal wall in between the pillar in front which is formed by the palatoglossus as well as pillar behind formed by the muscles of Palatopharyngeous and its folds. Palatine tonsils are unique by virtue of being encapsulated by non-keratinized, epithelium which is squamous and stratified. This surrounding layer is of surgical significance as it provides us a plane of dissection. The extent of mass of the palatine tonsils extends more than what we visualized, the tissue extends superiorly towards the palate and also below into the tongue base and towards the palatoglossal arch region anteriorly, in this manner it has two surfaces, one lateral and another medial surface. It is the medial surface that consists of the epithelium which is thrown into folds, forming crypts that open onto the medial surface about twelve to fifteen in number. There are two poles to the palatine tonsils one superior an another inferior, towards the upper part is the deepest crypt known as the crypta magna it is said to be the representation of the second pharyngeal pouches' ventral part. These crypts get filled up with epithelial cell debris, bacteria or even food, and the caseous deposits get expressed upon exerting pressure over the anterior faucial pillars, a sign named as the "Erwin Moore's sign".⁸

The outer surface of faucial tonsils is formed with a fibrous capsule and the capsule sits on the tonsillar bed, this bed is formed by alveolar tissue is loose, the lower pole of the palatine tonsils is where they attach to the tongue base by a small triangular folded membrane known as the "plica triangularis" this encloses the space known as the anterior tonsillar space and above it is attached by the plica semilunaris

which is a semilunar shaped fold, between the two pillars and this encloses a space that is the supra tonsillar fossa. Between the tongue and the tonsils lies the hidden space which is the tonsilo-lingual sulcus it is clinically significant owing to the chances of development of carcinomas in this region.¹⁰

The next important region is the bed of the tonsils that's contain beneath several important structures. The superior constrictor muscle and the styloglossus form the tonsillar bed, the glossopharyngeal nerve is embedded within this muscle layer, and if the styloid process is elongated it too can come to lie in this bed. Then outside this muscle layer comes the facial artery, submandibular gland, medial pterygoid muscle, the digastric muscle belly which is based posteriorly and, lastly the mandibular angle^{1,2}.

Palatine tonsils are richly supplied with blood vessels and the efferent lymphatics.

There are five major arteries that supply the palatine tonsils, these are all branches of the carotid artery's external branch as follows: the tonsillar branch of the artery of the facial region, the ascending artery of the palate, artery of the palate that is descending which branches from the artery of the maxilla, the ascending pharyngeal artery as well as lastly the branches of lingual artery which arise dorsally.

Para tonsillar vein which is another bed structure is the main vein draining the palatine tonsils which then drains into common vein of facial region and then the plexus of veins of the pharynx.

The way the lymph drains the tonsils only has efferent which enters deeper upper nodes of the cervical region mainly entering the jugulo-digastric nodes.

The palatine tonsils have nerves to supply it coming from the ganglion of the sphenopalatine region through its branches, and the 9th cranial nerve is the sensory nerve supplying the palatine tonsils.^{8,9}

FIG 2- THE PALATINE TONSILS AND THE OROPHARYNX¹

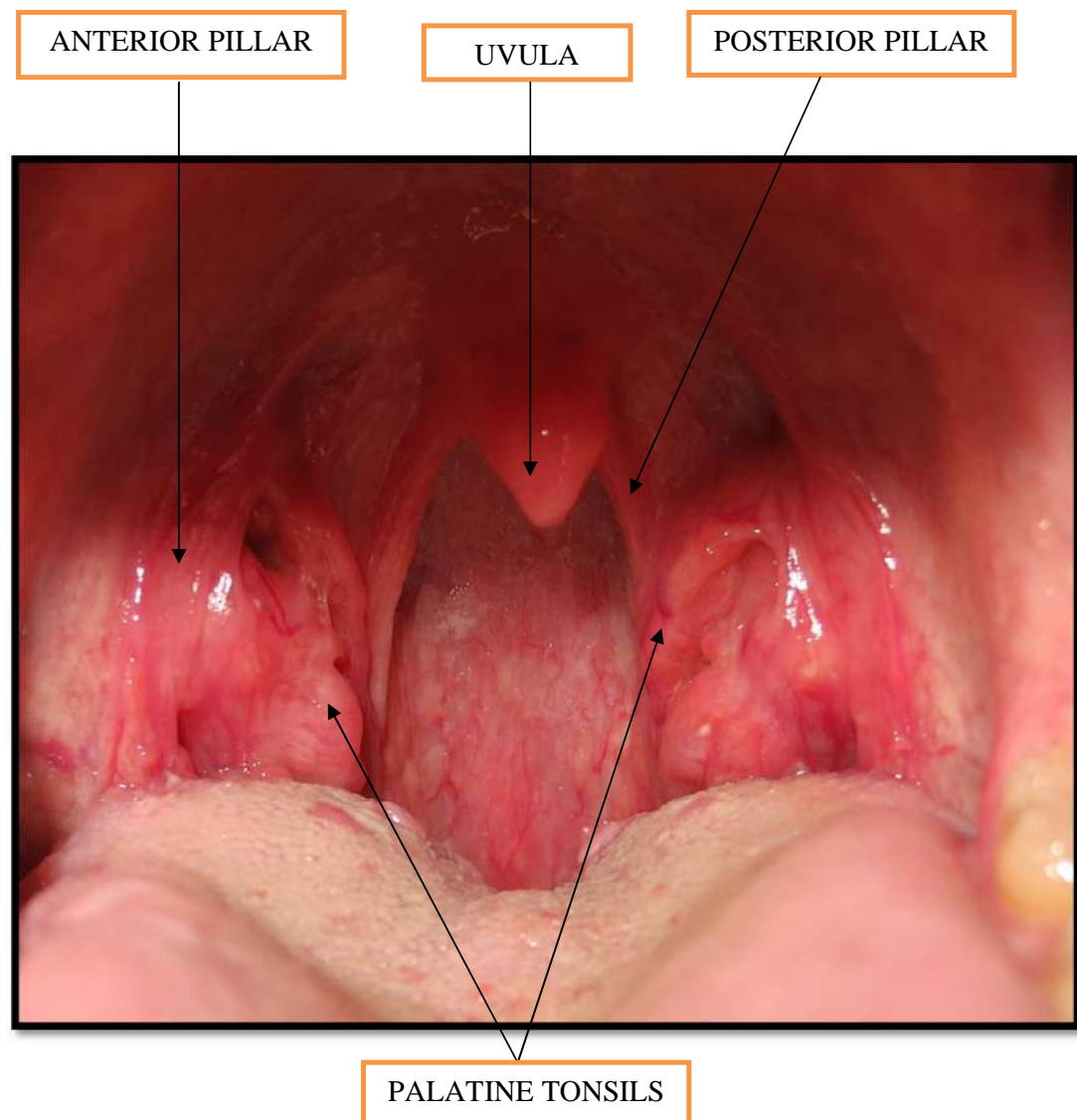


FIG 3- SCHEMATIC REPRESENTATION OF THE TONSILLAR BED

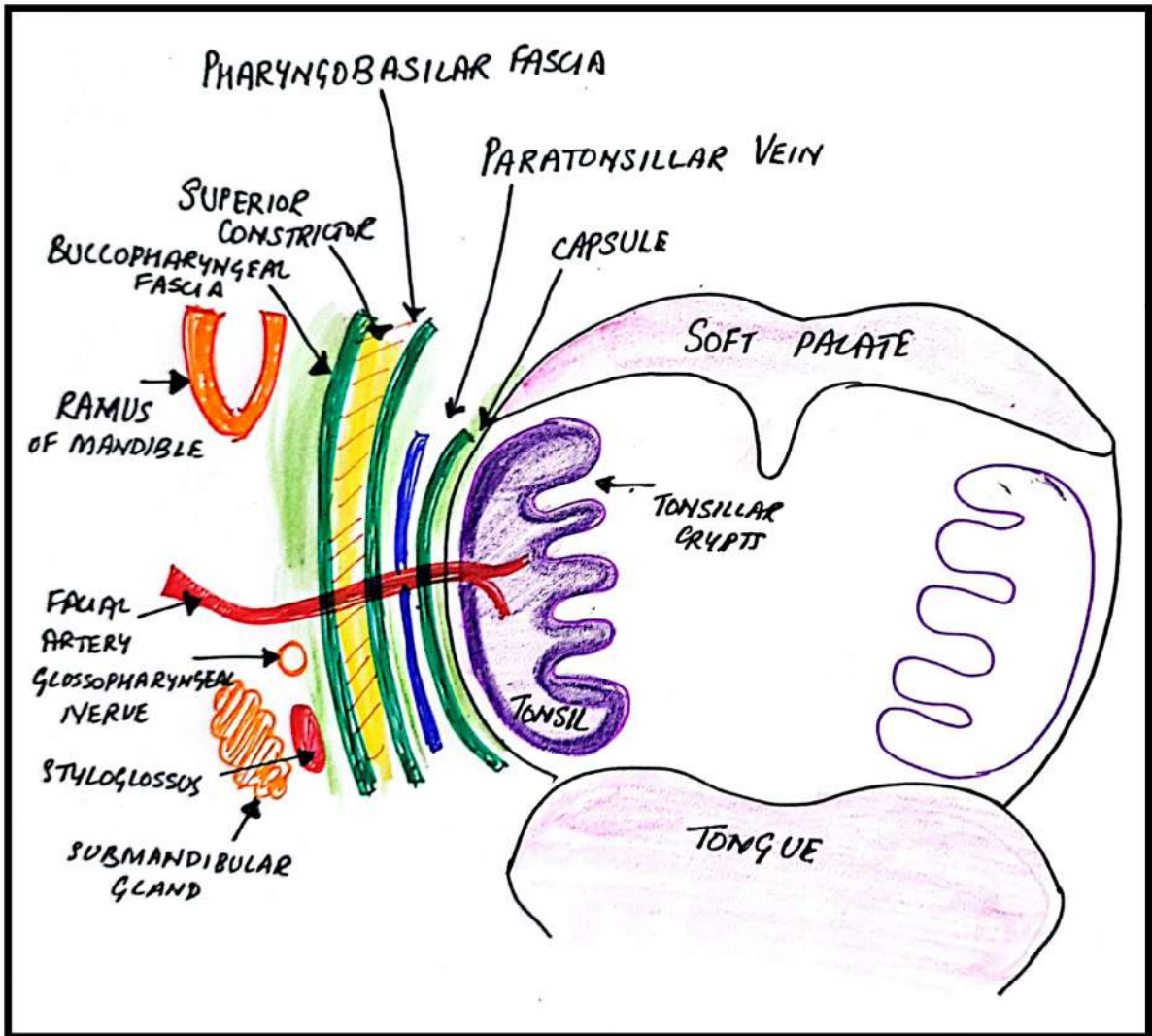
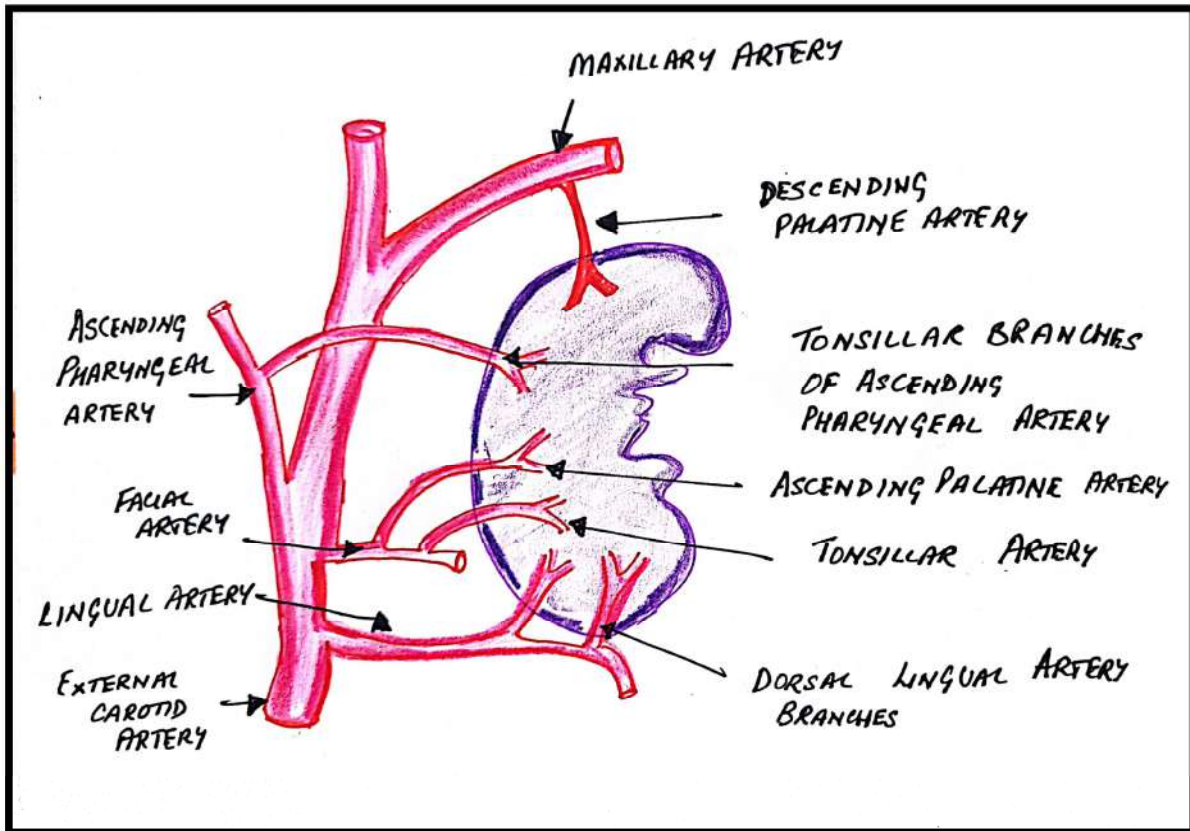


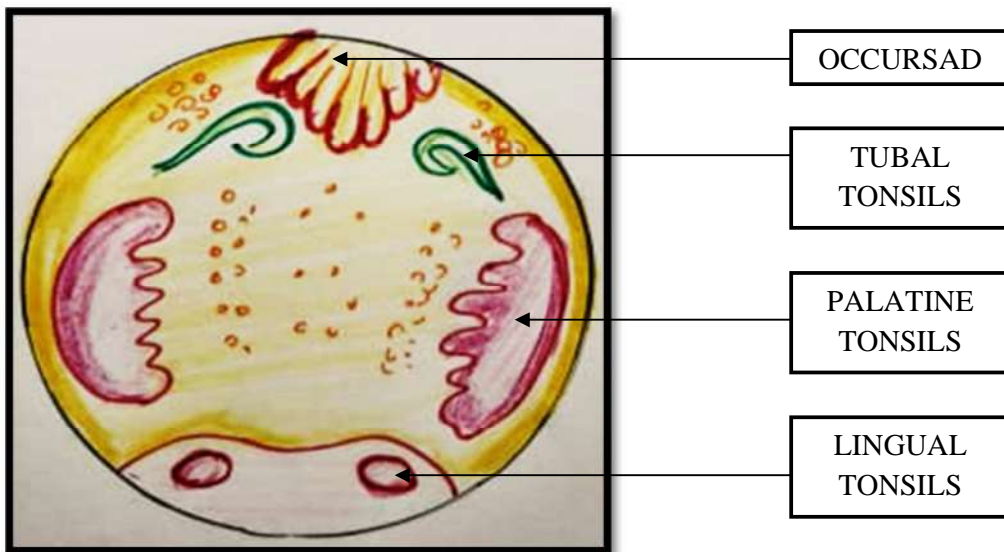
FIG 4- SCHEMATIC REPRESENTATION OF BLOOD SUPPLY OF THE TONSILS



IMMUNITY AND THE TONSILS

The palatine tonsils along with the other lymphoid follicles of the Waldeyer's ring have a pivotal part in the human system of immunity. They provide both local immunity as well as behave as gatekeepers which can detect pathogens and help prepare the system of defence against any further attacks. This local immunity is conferred upon the palatine tonsils due to the presence of m cells and antigen presenting cells which are present in the squamous epithelial layer, that is in direct contact with the pathogens on its external surface. Within the tonsillar tissue lie the lymphoid follicles which have a germinal centre that contain B lymphocytes and the mantel zone around contains several lymphocytes, and upon this exposure the B-cells are stimulated and they in turn form plasma cells which produce antibodies and also participate in phagocytosis in this way the palatine tonsils localize and tackle infection. The other mechanism is by surveillance by activating b cells and in turn memory T-cells and they in turn active cell mediated as well as immune complex mediated immunity. The tonsillar tissue participates in this manner in the immune system maximally between the age group of 4 to 10 yrs and they start to involute by the age of puberty this helps to promote the b cell production ^{8,9,12}.

FIG 5 - WALDEYERS RINGS



TONSILLITIS AND INFECTION OF PHARYNX

It is localized swelling and infection of the tonsils is called as tonsillitis which is a form of localized pharyngitis. It is said that bacterial infection in the form of Group A β Streptococcus pyogenes which are Haemolytic is a common cause of infectious pharyngitis both generalized or localized. This is followed by less common causes such as staphylococcus aureus, H. influenza, Corynebacterium diphtheriae, Moraxella catarrhis, and Bacteroides fragilis, Bacteroides Oris, viral and fungal infections may also occur.^{8,9,11}

Clinical presentation: patients typically present with complaints of throat pain, fever sometimes with or without chills, painful swallowing, difficulty in swallowing, they may even give history similar complaints in the past, recurrence or exposure to those with similar complaints. At the offset based on history it is difficult to differentiate between pharyngitis or tonsillitis of bacterial aetiology from that of viral aetiology, but following detailed clinical examination there are some features that go in favour of each. The presence of high-grade tonsillar hypertrophy with purulent exudates and necrotic debris in crypts, with cervical lymphadenopathy go in favour of bacterial aetiology, whereas if there are associated complaints of diarrhoea, presence of exanths and sneezing with rhinorrhoea we can think in terms of viral pharyngitis.

Depending upon the duration of symptoms tonsillitis maybe acute or chronic. Based on the involvement of each component of the tonsillar structure the tonsillitis can be further classified into:

1. Acute tonsillitis with catarrh
2. Acute tonsillitis with follicles
3. Acute tonsillitis with parenchyma
4. Acute tonsillitis with membranes

FIG 6 -ACUTE CATARRHAL TONSILLITIS¹



FIG 7-. ACUTE FOLLICULAR TONSILLITIS¹

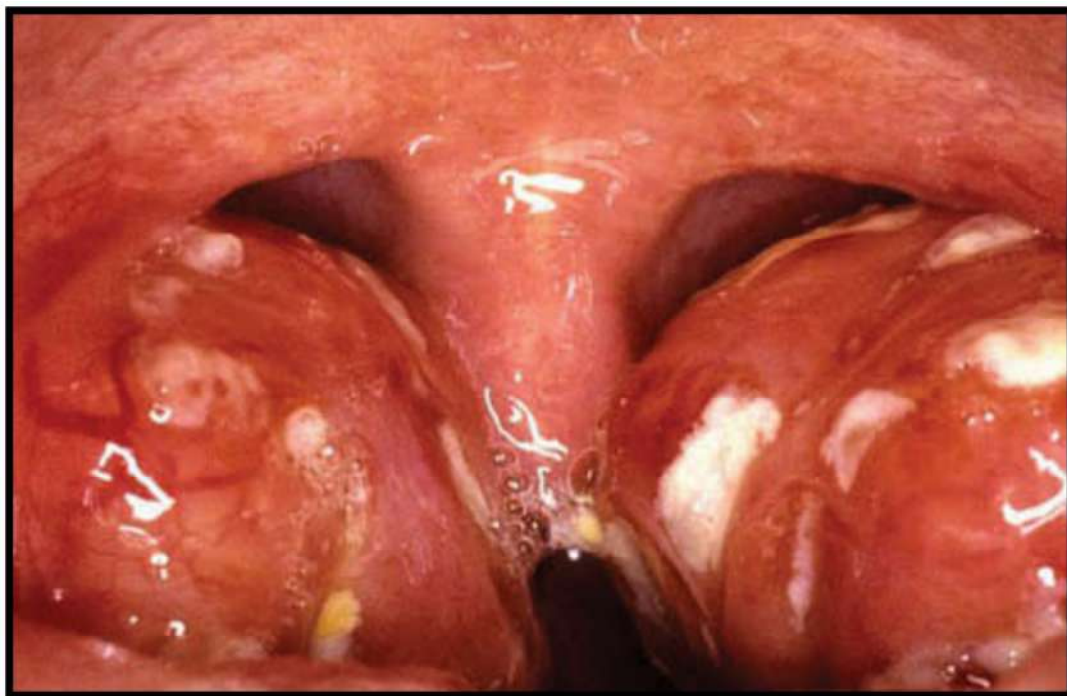


FIG 8-ACUTE PARENCHYMATOUS TONSILLITIS¹



FIG 9 ACUTE MEMBRANOUS TONSILLITIS¹



These conditions need to be treated as soon as can be as they can lead to several complications if left untreated. Chronic or recurrent tonsillitis can occur, peritonsillar abscess or parapharyngeal abscess can form, further suppuration of the lymph nodes such as the jugulodigastric can lead to cervical abscess. Through the eustachian tube the infection can spread and lead to otitis media, in such a case the patient will have symptoms of otitis which coincide with the duration of the tonsillitis. There can be development of dire rare complications such as glomerulo-nephritis and even bacterial endocarditis especially in cases of patients with low immunity and thus utmost care must be given to manage this condition and it must not be dismissed casually.^{2,3,11}

The presence of a membrane must immediately alert the otorhinolaryngologist to keep in mind several differentials such as diphtheria, Vincent's angina, infectious mononucleosis, agranulocytosis must be ruled out, malignancies and leukaemia must be borne in mind.

Now coming to the area of our study in particular – chronic tonsillitis, it can occur as a consequence of acute tonsillitis or due to spread of infection from the sinuses, it is more common in the paediatric age groups and rarely occurs after the age of 50 years.

These include chronic parenchymatous tonsillitis, chronic fibrinoid tonsillitis, and chronic follicular tonsillitis. The presenting complaints will again be like those of an acute infection but with a history of recurrence.

Recurrent chronic tonsillitis can be diagnosed in a patient if his/her symptoms fall into the following criteria, known as the paradise criteria given by JL paradise in the year 1984, where it is specified that a person must have experienced 3 tonsillitis episodes per year for 3 years, 5 episodes in two consecutive years, or 7 episodes in a year. and it is this criterion that we too have used in our study ^{7,10}.

THE TRACE ELEMENTS IN THE BLOOD

IRON- PHYSIOLOGY AND DEFICIENCY

In the body, iron is a crucial trace element that is carried in the plasma while attached to the transport protein transferrin. Duodenum is where the absorption occurs, and the bloodstream carries it from there. Anaemia is the primary result of an iron shortage because haemoglobin synthesis is impaired. The most vulnerable groups to iron deficiency are children, women belonging to reproductive age range, and those from poorer socioeconomic backgrounds. This condition results in anaemia, exhaustion, decreased cognitive development, growth retardation, and lowered immunity. Level of ferritin in serum, total binding capacity to iron, (TIBC) iron levels in serum, faecal occult blood, and complete blood counts are used to measure iron. Heme and non-Heme iron are the two types of dietary iron¹². Iron in Heme form is simpler to use it is available in poultry products and red meat in more amounts but in lesser quantity in the green leafy vegetables and pistachio beans, lentils .¹³ The diagram below shows the physiology of iron absorption and its conversion to active form.

FIG 10- METABOLISM AND TRANSPORT OF IRON

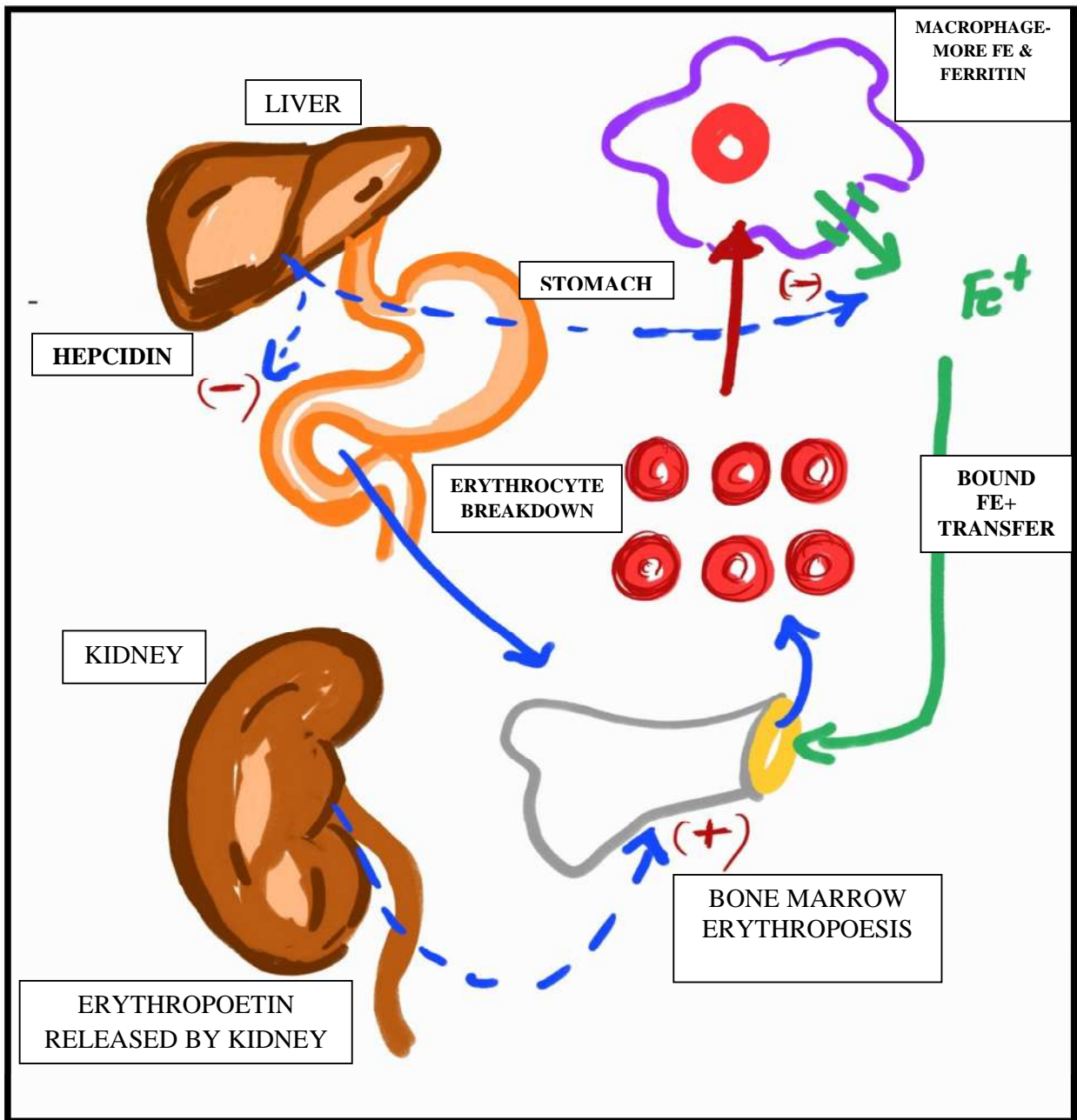
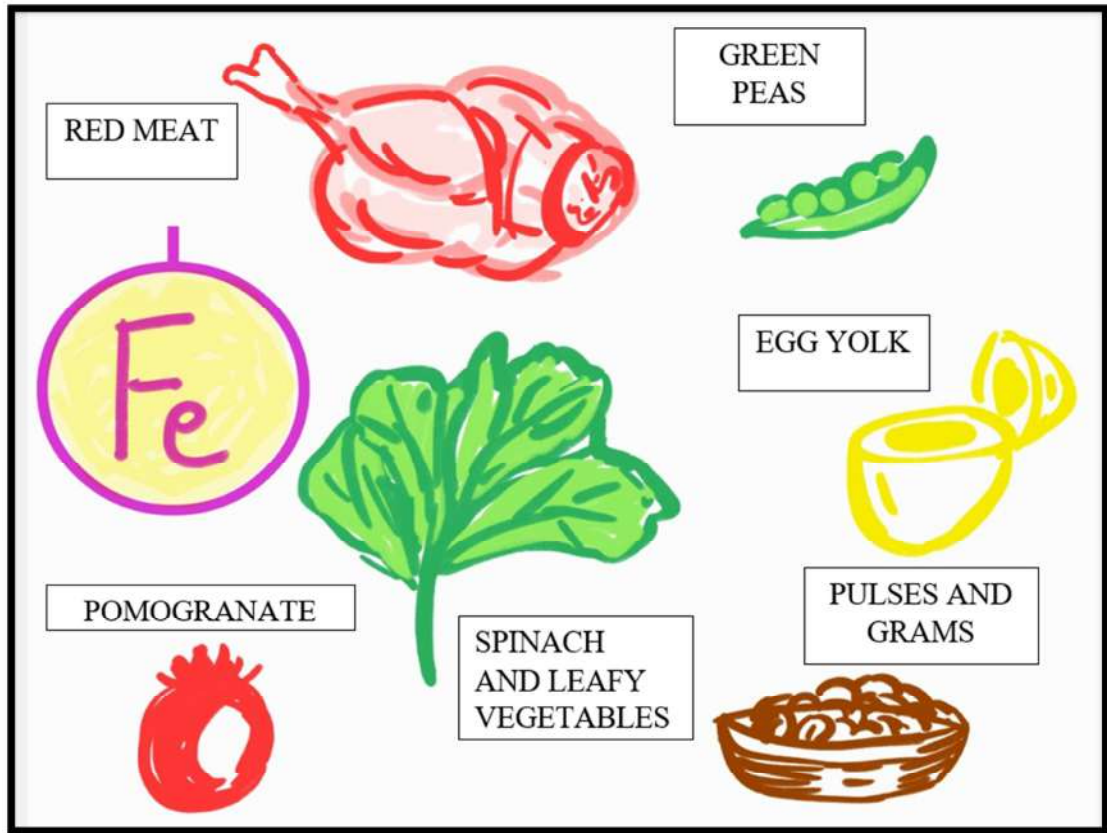


FIG 11- DIETARY SOURCES OF IRON



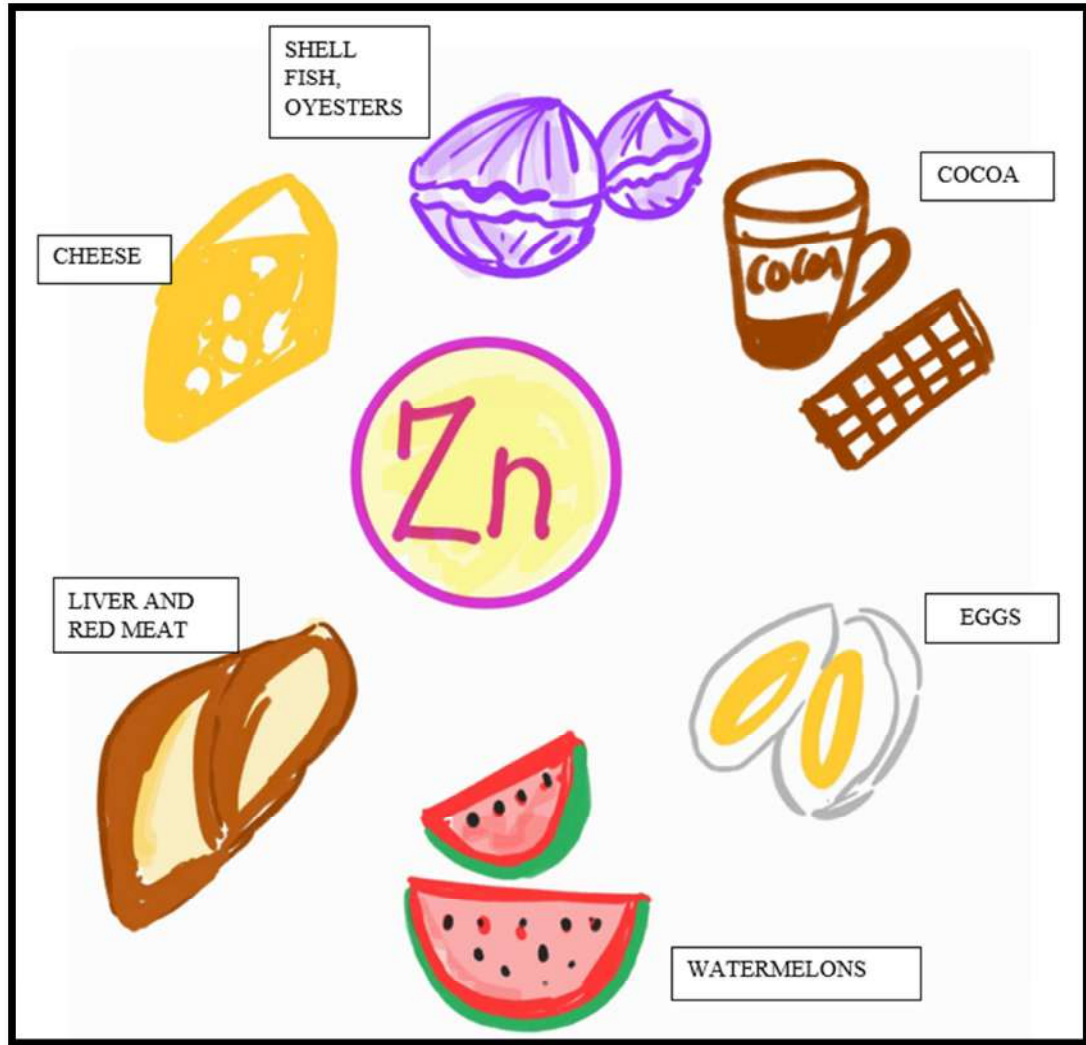
ZINC – PHYSIOLOGY AND DEFICIENCY

Zinc being an important heavy metal element in the body it is 2nd most abundant one in the humans. Zinc is in most tissues, from blood to the connective tissues. Zinc plays many roles in the physiology of the body, it is a catalyst for several metabolic reactions, it is a structural component as well as a regulatory role in genetics. It is necessary in the structure of several enzymes, and in production of rhodopsin needed for vision and also in blood coagulation pathway. It is mainly taken into the blood from food in the lesser intestine i.e. The jejunum and transported via the portal circulation and stored in the liver while the excess is excreted via the bile juice. Its recommended daily allowance is about 8-11mg/d.¹³

The deficiency of zinc mainly affects any of the functions which need zinc containing metalloproteases, thereby causing dysphagia and dysgeusia. Diarrhoea is also caused and that further depletes the zinc. Severe zinc deficiency can lead to night blindness due to reduced rhodopsin production and reduced vitamin A release by the liver. Also, skin gets affected and rashes can be seen. The oxidative damage to cells is increased due with DNA damage as more reactive oxygen species are released. Immunity is greatly affected due to the vital role played by zinc in important enzymes.

The dietary sources rich in zinc are red meat, the meat of oysters and crabs and other crustaceans and in poultry. In the Indian scenario these are not commonly consumed or available and thus a vast majority of the population has a slightly lower serum zinc.¹⁴

FIG 12 -DIETARY SOURCES OF ZINC



ROLE OF TRACE ELEMENTS IN TONSILLITIS

There are several vital functions in the human body which require trace elements some of these are such as nucleic acid synthesis, protein synthesis as well as their degradation. These elements are also necessary in the metabolism of alcohols and connective tissues of the body. In this manner these elements have a vital part in human immune response to infections as well as the entire cascade of reactions which occur during such a response to pathogens. To study the roles played by these components and the how they contribute to one's daily functioning has become more important in today's fast changing world where in the human body has been put to test by exposure to a wide range of pathogens some more deadly than others. For this the levels of the elements in diet and comparison between different age groups, different infections and inflammatory conditions have been done. In the our subject one of the commonest infections which we see is that of tonsillitis and so there have been several studies done to understand the role of certain metallic elements like iron, manganese, magnesium, zinc and copper. Some results have shown that low levels of these elements predispose to infection and also reduce the degree of immune response produced by the individual and that this is attributed to the need of these trace elements for production and activation of T cells.^{14,15}

ROLE OF TRACE ELEMENTS IN ANTIINFLMMATORY ENZYMES

During inflammation a series of complex reactions occur, each of which have several enzymes and cofactors. During inflammation reactive oxygen species are released which increase inflammatory mediators and causes tissue damage. There are certain enzymes such as super oxide dismutase which have an antagonistic effect on these free radicals and they are of two types and amongst them the cupper zinc super oxide dismutase is of major significance.^{21,22} This enzyme requires both zinc and iron to be produced and in this manner these trace elements are of high clinical significance. Several studies have been done in otorhinolaryngology to understand their role, and have assessed the importance of CuZnSODs.²² In the pathogenesis of chronic otitis media, recurrent adenoiditis and tonsillitis, and even in cases of laryngitis. All of these studies have shown a significant association between the function of these enzymes. They also observed lower levels in these trace metals that contribute to the structure of the enzyme, affect ²⁶ the patient's anti-inflammatory response these infections.

ESTIMATION OF LEVELS OF TRACE ELEMENTS

In biological samples we have two types one being the liquids such as serum, whole blood, CSF, urine and the other being tissue¹. Estimation of levels of heavy metals which are in the form of trace elements is most commonly done by “spectrometry” within which the use of a flame of a standardized gas which on exposure to these cations makes ligands leading to colour changes and these are detected and the method is known as AAS- atomic absorption spectrometry and use of flame give it the name flame atomic absorption spectrometry.^{48,49} There is a need of using acids to first allow the adsorbent to get adhered and then use of liquid-based ligands for further processing and before exposing the sample to the flame.⁵¹

These are all done manually and the flame colour change is measured to calibration by the use of the spectrometer.

WHITE BLOOD CELLS AND THEIR COUNTS

❖ NEUTROPHILS:

They make up over 70% of the body's total leukocyte population and are essential to the immune system. Bone marrow produces neutrophils, which are further broken down into subtypes like segmented and banded cells. Along with other WBCs such as basophils and eosinophils they form the group known as polymorphonuclear cells. They have a lobed nucleus with 2-5 lobes. Their name is derived from the neutral pink stain that they get in haematoxylin-eosin staining. They are phagocytes, which are the immune system's front-line fighters, and they are crucial to the acute phase of the immunological response. They travel in the blood stream and go towards the site of acute inflammation or infection by chemotaxis, where in certain chemicals are released which act as signals, such as FLMP, Leukotrienes and interleukins and peroxidase. These neutrophils contribute to formation of pus at the infection site and they account for the whitish colour of pus, these cells thus are described as a 'hallmark' of acute inflammation.²⁰

Clinical significance is as follows, when the neutrophil count is low it is termed as neutropenia. There are two sets of causes for this, either congenital or acquired. Congenital causes are like aplastic anaemia where in the bone marrow is suppressed, or acquired causes as seen in cases of patients post chemotherapy or radiation, side effects of any drugs. Such neutropenia makes patient highly immunocompromised. Conditions such as familial Mediterranean fever where in there is a mutation in the pyrin gene can lead to neutropenia, some studies even show a link between neutropenia and hyperglycaemia following a lack of normal functioning of myeloperoxidase in the biochemical reaction path of neutrophils. Thus, in order to

look for neutropenia and neutropenic fever we must calculate the ANC absolute neutrophil count as a gold standard, if ANC is less than 1500 cells/mm³ then it is considered as neutropenia²¹.

❖ **LYMPHOCYTES**

These are white blood cells engulf pathogen and hence known as the “natural killer cells” as they play a role in phagocytosis and they have both memory cells and the killer cells. they account to about 40% of the leukocyte number. They function by cell mediated immunity and act after the responses of neutrophils. They are of two type the T cells and the B cells. T cells are the ones responsible for immunity mediated by cells and B lymphocytes take part in immunity. Lymphopenia is the term used for a decreased number of lymphocytes and lymphocytosis is the term used for increase in their count. Such lymphocytosis occurs mainly in viral infections and leukaemia. Infections such as pertussis caused by *Bordetella pertussis*, we notice a characteristic lymphocytosis much in contrary to the usual where in bacterial diseases cause neutrophil count to increase. The low levels of this or lymphopenia is seen commonly in HIV infections where in the virus destroys these cells, leading to immune suppression. A specific subtype the CD4 T cells are particularly affected and their count drops in HIV patients, slowly due to this patient develop AID.

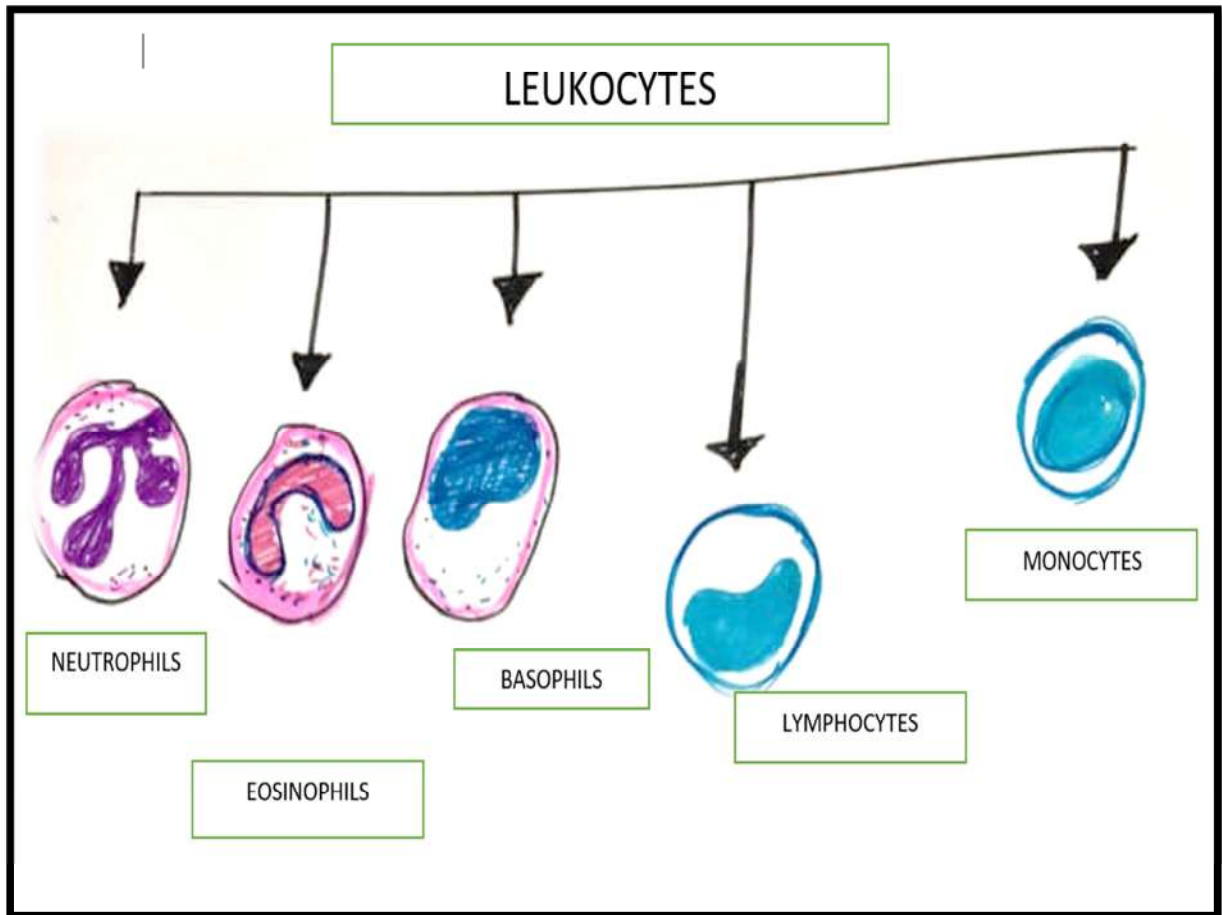
In this manner the rise and fall of these types of white blood cells each have a significance and help asserting and diagnosing several diseases^{43,44}.

❖ **THE RATIO OF NEUTROPHILS AND THE LYMPHOCYTES**

This ratio relatively newer variable is now being assessed in several studies as it is now known to be an indicator of bacteraemia, infection and even sepsis. This neutrophil -lymphocyte ratio is calculated as the term suggests as a ratio between the neutrophils and the lymphocytes, it has been found to be higher in cases of inflammatory conditions, cardiac diseases and especially in bacteriemia.³² The concept is that during infection due to excessive demand and use of lymphocytes both B and T cells which then deplete by apoptosis following release of mediators and phagocytosis there is lymphopenia, likewise during the acute phase of infections and in even in cases of chronic infections, there is a milieu of neutropenia by bone marrow activation of the precursors of neutrophils, in this manner the value of this NLR ratio is higher in these conditions. As this is very easy to arrive at and has no excessive cost it can be used as a diagnostic indicator in infections, alongside or even instead of those such as CRP, ESR, procalcitonin which are more complex tests and more time consuming not to fort get expensive. It's been seen that the normal range of this neutrophil lymphocyte ratio is about 0.8 to 3.5. Increasingly this is getting applied as an indicator of acute infections, especially gaining significance in the light of the recent pandemic of COVID-19. Considering the acute on chronic tonsillitis, is an infection it too is expected to show a raised NLR. With respect to tonsillitis there have been very few studies that have calculated the NLR ratio and assessed it, we in our study have attempted to do calculate this ratio among our patients and look for a reliable corelation between NLR and recurrent Tonsillitis³². NLR has now been extended to understand its prognostic value in cancer as well, studies done have reported higher incidence of carcinoma among patients with higher levels of NLR and also some studies also show that with higher NLR there is poorer prognosis of the

cancer. But the number of studies on NLR are limited and it is still an upcoming clinical tool needing more detailed understanding and elucidation.^{40,44}

FIG 13- TYPES OF LEUKOCYTES



MATERIALS AND METHODOLOGY

A study which was observational was carried out over the course of a year at the department of otorhinolaryngology and head and neck surgery.

Following given written consent and in accordance with the criteria of exclusion and inclusion, 100 patients who presented to the otorhinolaryngology and head and neck surgery OPD with recurrent tonsillitis were included in the study.

The history was procured in a detailed manner to ascertain recurrent case of tonsillitis and clinical examination was done the grade and severity of tonsillitis was assessed and documented.

Following which venous blood samples for CBC, ferritin in the serum, total capacity of binding to iron and levels of Iron in serum were collected and sent to the laboratory.

For evaluation of serum zinc the standard AAS – atomic absorption spectrometry was performed at the Basic research centre of our institution by the investigator using the standardized flame of Acetylene. Neutrophil lymphocyte ratio was calculated based on the values obtained from differential counts of the patients. Following this the results were collected and assessed.

Inclusion criteria-

- Patients from the age of 6yrs to 50 yrs. presenting with recurrent tonsillitis.
- Recurrent tonsillitis was classified as having more than seven episodes in a calendar year, more than five episodes in a calendar year for two years in a row, or more than three episodes in a calendar year for three years in a row.

Exclusion criteria

- HIV/Immune compromised patients
- Patients with diabetes mellitus on steroid medications
- Patients already on iron and zinc supplements
- Patients with bleeding disorders
- Patients who are known cases of malabsorption syndromes.

Sample size formula:

The minimum sample size formula based on prevalence rate is

$$n = \frac{z_{\alpha}^2 P(1-P)}{d^2}$$

where P is the percentage of prevalence and d is the percentage likely difference in the prevalence.

z_{α} is linked with the level of significance. For 5% level of the significance $z_{\alpha} = 1.96$.

With P = 66.67% and d = 15% of P = 10.0%, the sample size is 85.

To make the study more confirmative, the sample size will be raised to 100.

Ref- Önerci M, Kuş S, Öğretmenoğlu O. Trace elements in children with chronic and recurrent tonsillitis. International journal of pediatric otorhinolaryngology. 1997 Jul 18;41(1):47-51.

SAMPLE PROCESSING AND TESTING

Venous blood samples of the patients were centrifuged and the serum obtained was then frozen for preservation. These samples were then thawed and then lysed using a standard for zinc and iron estimation which was then done after preparing extracts and run on FAAS- flame atomic absorption spectrometer using acetylene gas. Following which the data was analyzed. Differential counts were used for neutrophil lymphocyte ratio and automated testing was done for Hb, TIBC and ferritin.

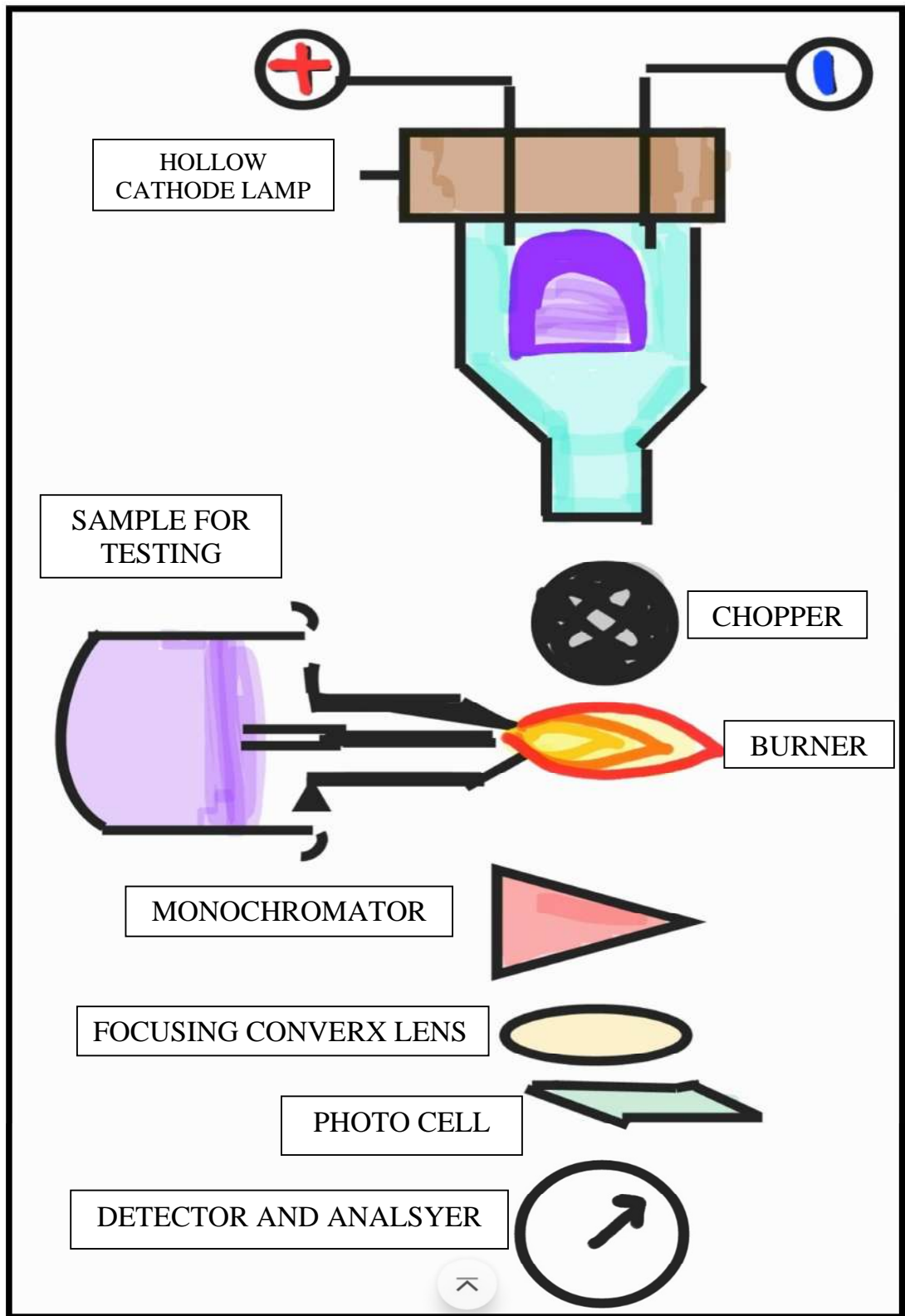
ATOMIC ABSORPTION SPECTROMETRY

This is an ideal and common method which is used to measure the quantity metals like Zn, Mn, Mg, Cu, Fe and Co in fluids in the body of human beings such as complete blood, serum, urinary content, CSF and ever since the 20th century it has been employed as a trusted and standardized method⁷⁰. Now it is available as a automated machine and is employed for biochemical testing.

The concept is simple the system uses a lamp which can be a hollow cathode lamp or electrodeless lamp with a flame which leads to the electrochemical bonds to break from other chemicals, after which the element comes to its state of ground and absorbs wavelength specific light.^{71,72} When this is exposed to a electrode lamp the element emits a light of a typical characteristic wavelength which passes through lens and a detector then measures intensity of its emission and finally this way the quantity of the element is determined. The figure 15 is a representation of the same.

In our study we have used model AA-7000, Atomic Absorption Spectrometer CAT NO. 206-77000-34, and the flame used was Air-Acetylene (Air -C₂H₂) Flame. The software application used for interpretation of the AAS rest was Wizaard, Shimazdu corporation, Version 5.00

FIGURE 14-ATOMIC ABSORPTION SPECTROMETRY



Preparation of the standards for deriving linearity curve.

1. 500µl of stock solution of Zn standard added to 4000µl DDW in 1 test tube – gives the 100ppm solution
2. 9.8ml of DDW with 0.1 ml of this previously made solution gives 2ppm solution
3. This manner serial dilutions were done to prepare 0.125ppm.
4. And then 3ppm was also prepared to check the linearity curve.
5. After which the curve was set and prepared and then the samples were tested.

Statistical Analysis:

Excel and the R programme, of 4.1.2 version used to analyse the data. Frequency tables are used to present categorical variables. To compare the mean of variables over anaemia, a two-sample t test is utilised. Whitney Mann U test is employed to compare the anaemia-related variable distributions. To compare the distribution of variables over serum zinc, utilise the KW test. As a post hoc analysis, Dunn's test is employed. A P-value of 0.05 or less denotes statistical significance.

Connection based on the test of correlation such as Spearman and Pearson were used and the researchers looked for a connection between blood iron, serum calcium, and tonsillitis severity, recurrence rates, and serum levels of zinc, anaemia. and the neutrophil lymphocyte ratio.

RESULTS

Data contains measurements on 100 subjects ranging between 5 to 50 years, $M= 19.47 \pm 11.45$ years. The following table gives the distribution of subjects according to different variables.

Table 1: representation of subjects by Age

Variables	Sub Category	Number of subjects (%)
Age (years)	≤ 10	25 (25%)
	11-20	38 (38%)
	21-30	22 (22%)
	31-40	6 (6%)
	41-50	9 (9%)
	Mean \pm SD	19.47 ± 11.45
	Median (Min, Max)	17 (5, 50)

Table 2: Representation of subjects by gender

Gender	Female	37 (37%)
	Male	63 (63%)

For the analysis of Iron deficiency anaemia, we have evaluated serum FE, TIBC and ferritin the results of which have been represented in the tables as follows.

A majority of the subjects (56%) had low serum Iron with all of them having elevated levels of TIBC which showed Iron deficiency anaemia

Table 3: serum Iron distribution among subjects

Serum Iron	Low	56 (56%)
	Normal	44 (44%)
	Mean \pm SD	67.57 \pm 36.90
	Median (Min, Max)	57.50 (19.50, 152)

Table 4: Distribution of subjects as per TIBC

Total Iron Binding Capacity	Low	2 (2%)
	Normal	42 (42%)
	High	56 (56%)
	Mean \pm SD	489.21 \pm 146.21
	Median (Min, Max)	475.5 (200, 874)

The average value of the NLR among the study population was 4.52 with a standard deviation of about 10.30 again indicating towards a higher NLR among the cases of recurrent tonsillitis. The values of the sma ehave been shown in the table below.

Table 5: Neutrophil and Lymphocyte Ratio Distribution among subjects

Neutrophil	M± SD	66.33 ± 16.63 66 (30, 99)
Lymphocyte	M ± SD	26.68 ± 10.46 30 (1, 50)
N/L Ratio	Mean ± SD Median (Min, Max)	4.52 ± 10.30 2.34 (0.75, 99)

Table 6: Distribution of subjects based on anaemia

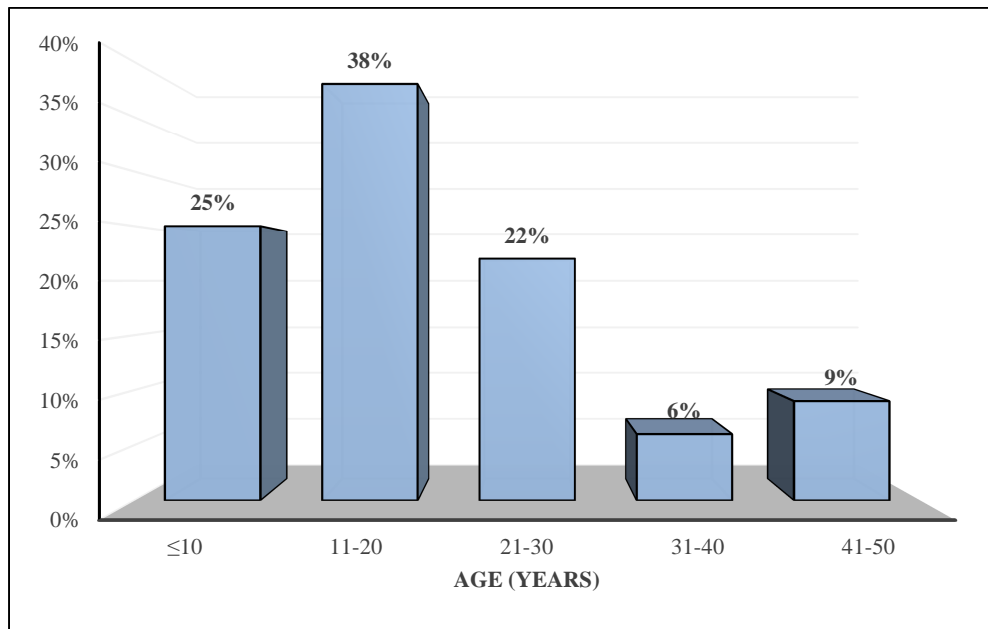
Anaemia	Anaemic	84 (84%)
	Normal	16 (16%)
	Mean ± SD Median (Min, Max)	10.67 ± 1.53 11 (7, 13.6)

Table 7: segregation of the subjects according to serum Zinc

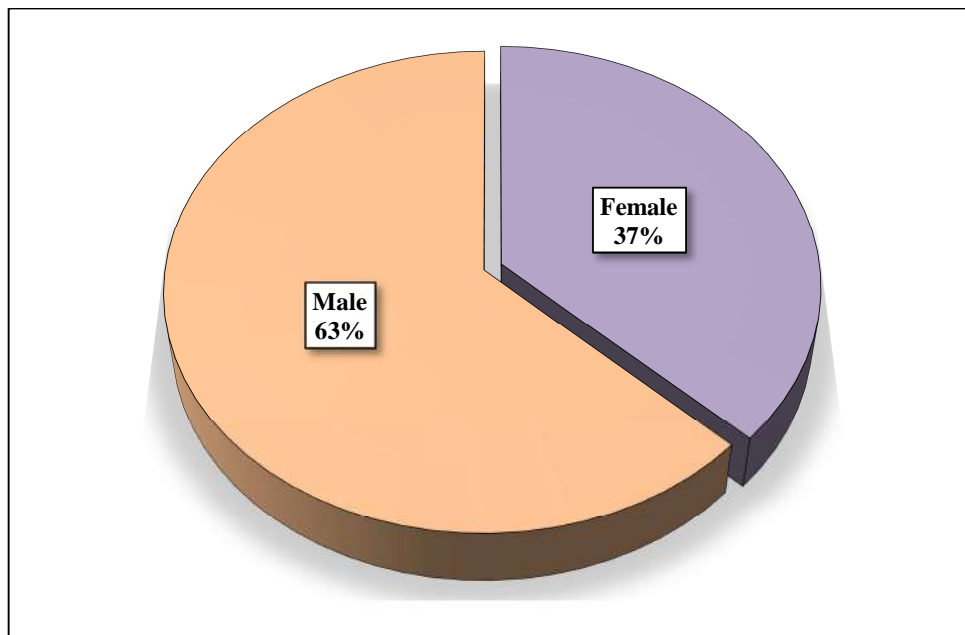
Serum Zinc	Low	88 (88%)
	Normal	5 (5%)
	High	7 (7%)
	Mean ± SD Median (Min, Max)	0.32 ± 0.35 0.21 (0.01, 1.75)

Out of 100 subjects, 63% =male gender, 37% female gender, with ratio of 1.7:1. 56% subjects had low serum iron, 42% had normal total iron binding capacity, 84% subjects were anaemic and 88% had low serum zinc.

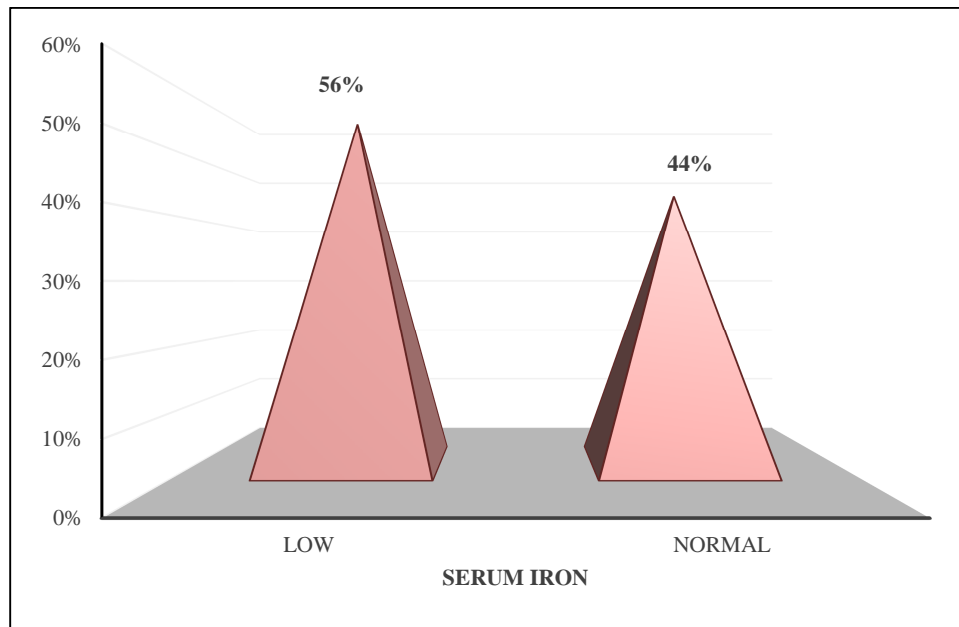
Graph 1: age distribution of subjects



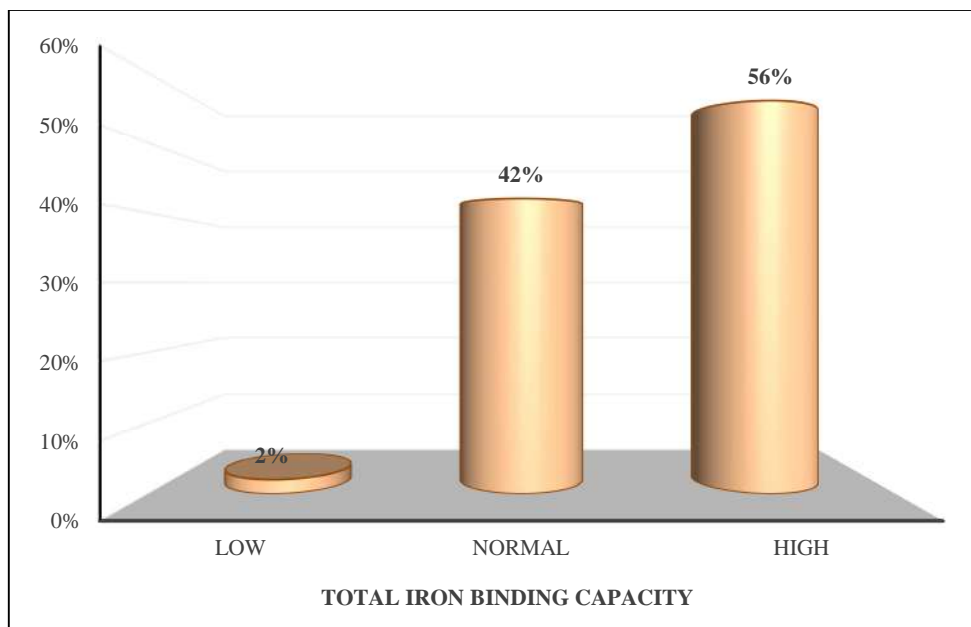
Graph 2: Gender distribution of subjects.



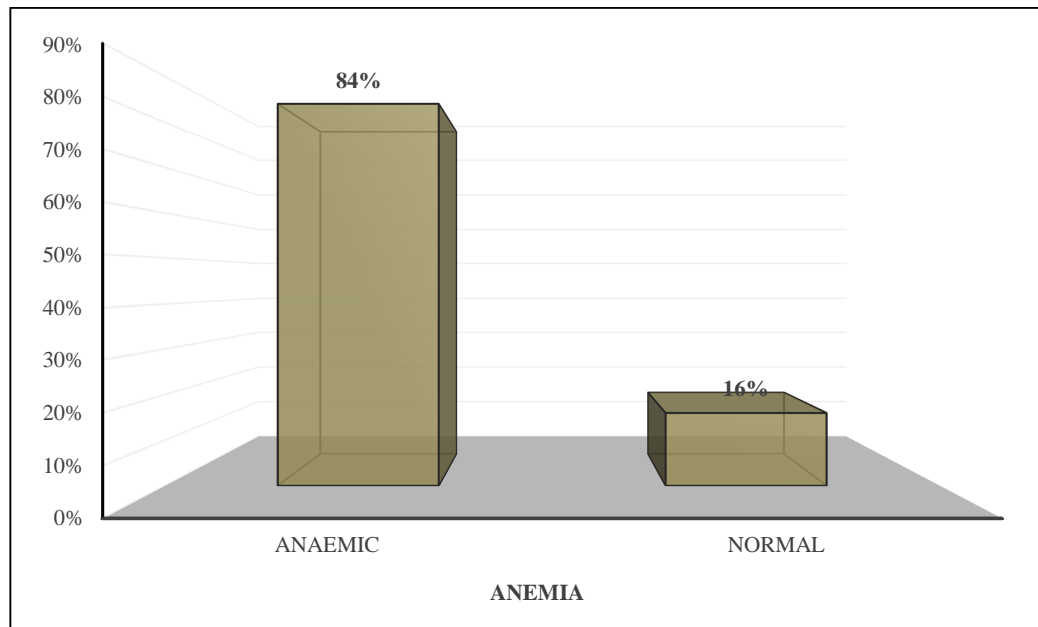
Graph 3: Distribution of subjects according to serum iron.



Graph 4: Distribution of subjects according to total iron binding capacity.



Graph 5: Distribution of subjects according to anaemia.



Graph 6: Distribution of subjects according to serum zinc.

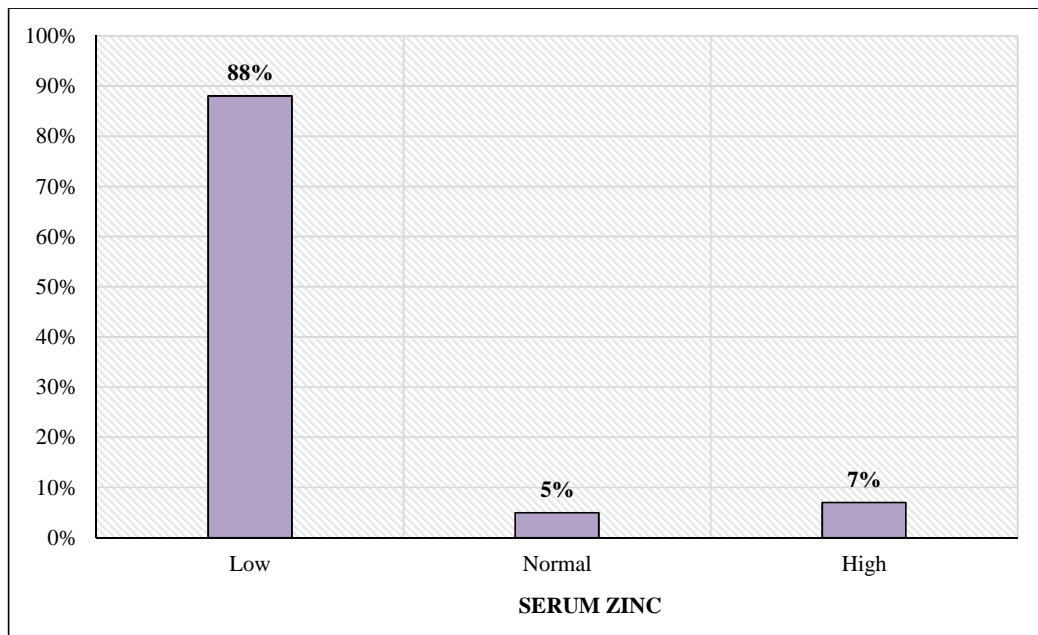
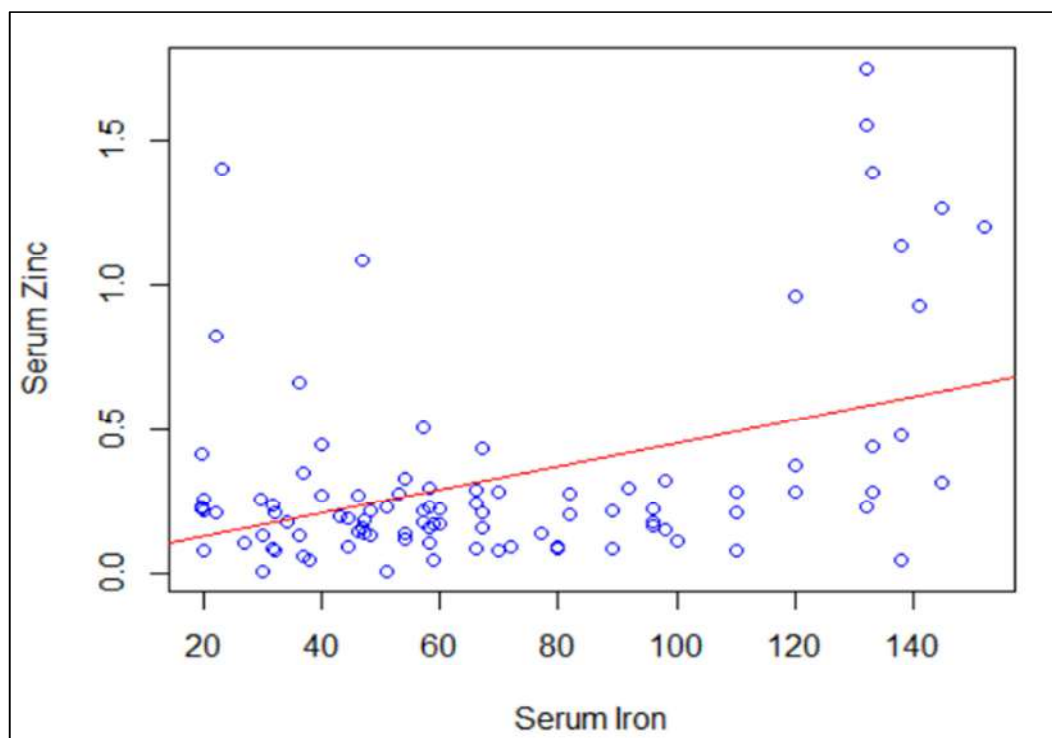


Table 8: Correlation of serum Fe and Zn in study population

Variables	Correlation coefficient	p-value
Serum iron and Serum zinc	0.2437	0.0146^{SP*}

Abbreviation: SP – Spearman’s rank correlation test,

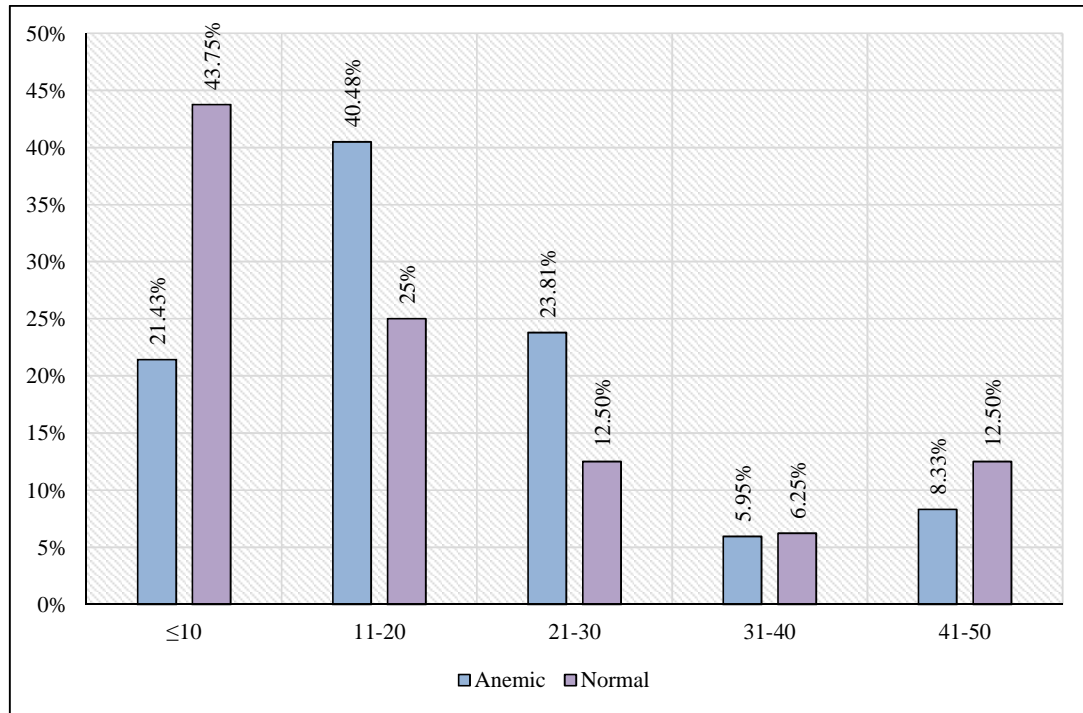
Graph 7: Scatterplot of serum Zn and Fe



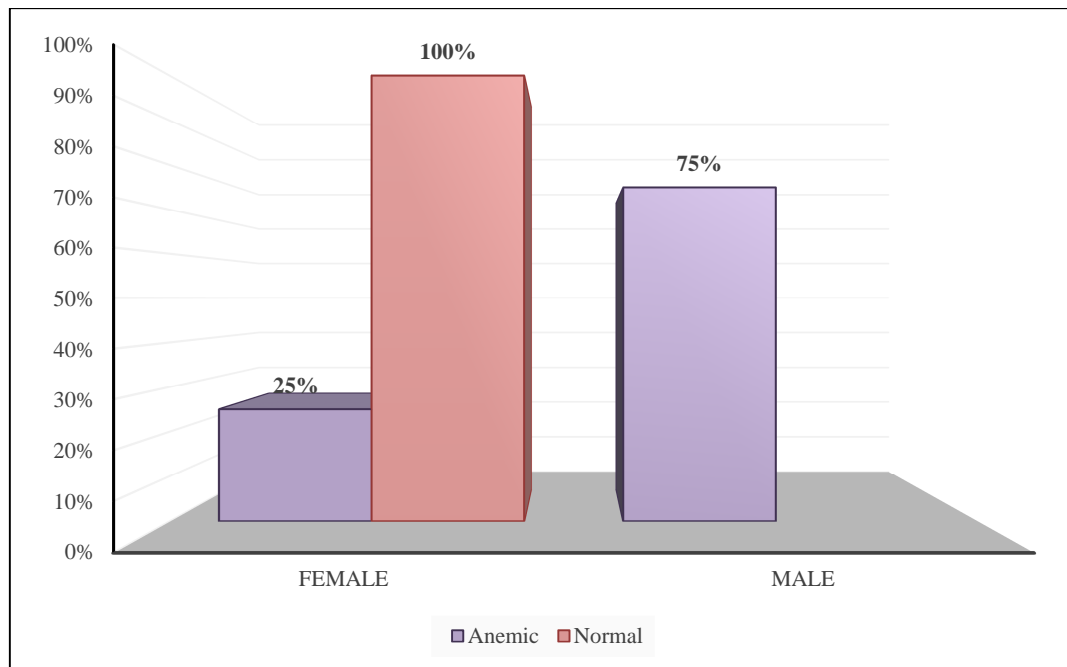
Chi square test shows association of gender with anaemia. There is no significant association of Age (years), Zn, Fe, TIBC over anaemia.

From two sample t test, we observe that, there is significant difference in mean Neutrophil over anaemia. There is no significant difference in mean Lymphocyte over anaemia. From Mann Whitney U test, we observe that, there is no significant difference in the distribution of age, with TIBC and Fe, N/L ratio and serum zinc over anaemia.

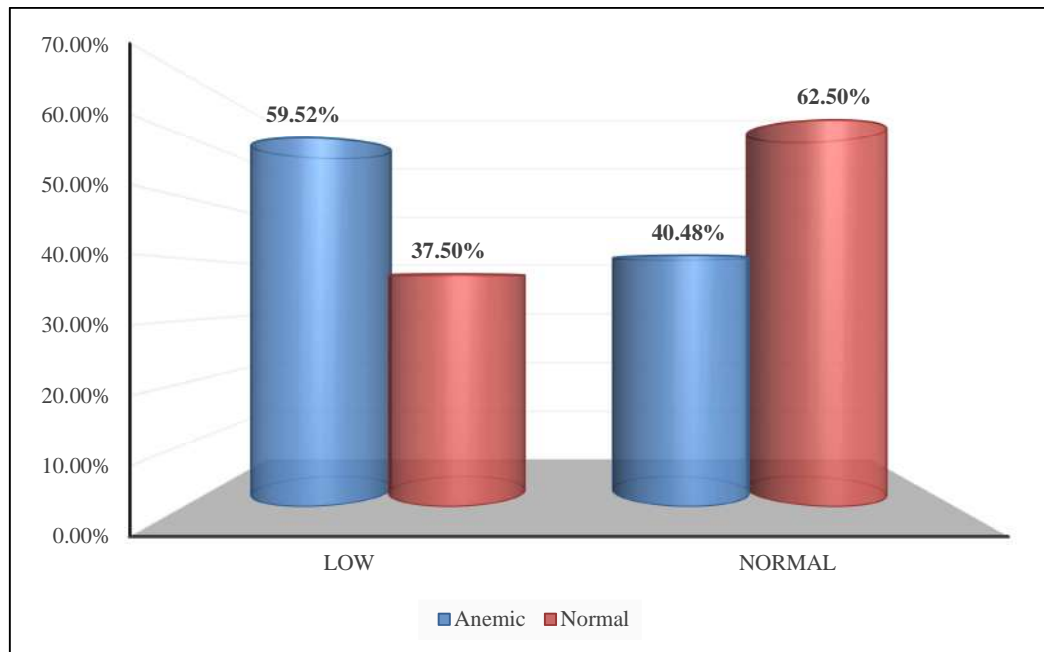
Graph 8: subjects distributed according to age over anaemia.



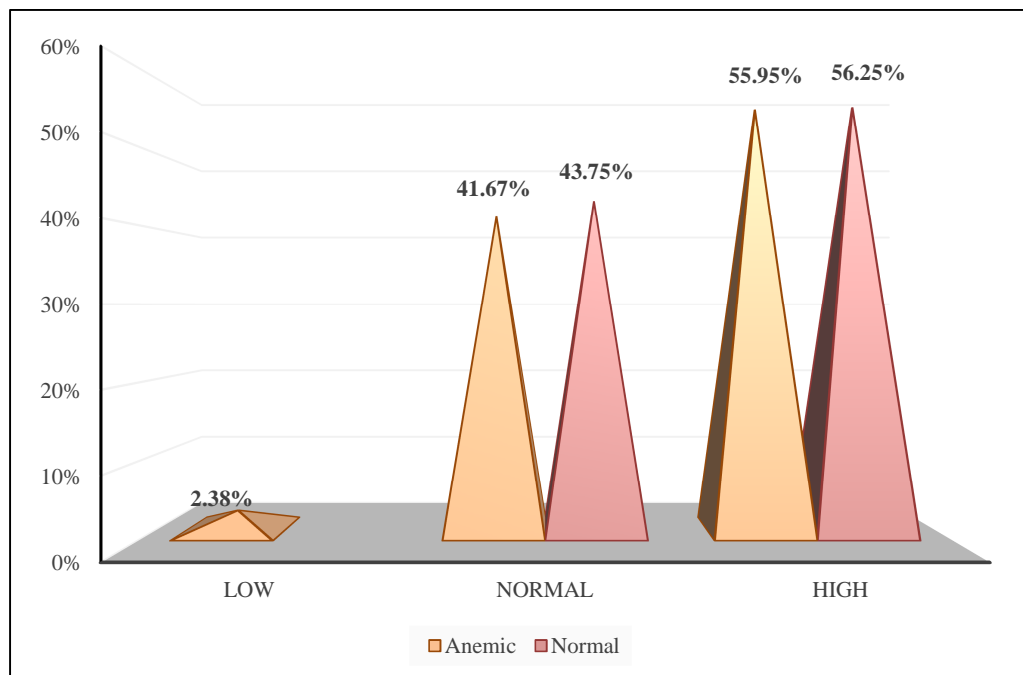
Graph 9: subjects distributed according to gender over anaemia.



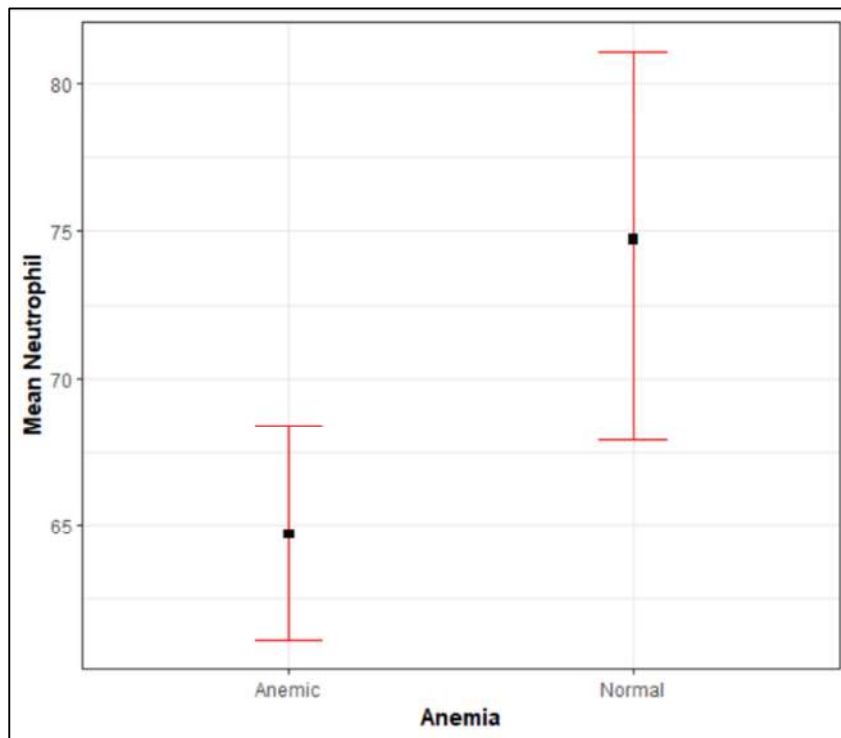
Graph 10: subjects distributed according to serum iron over anaemia.



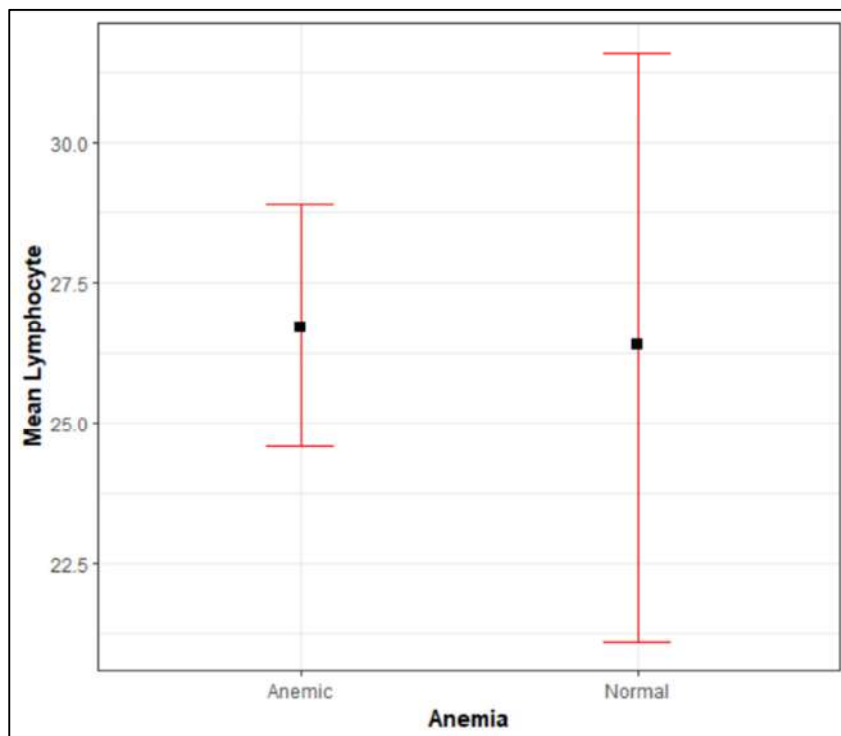
Graph 11: subjects distributed according to Total Iron Binding Capacity over anaemia.



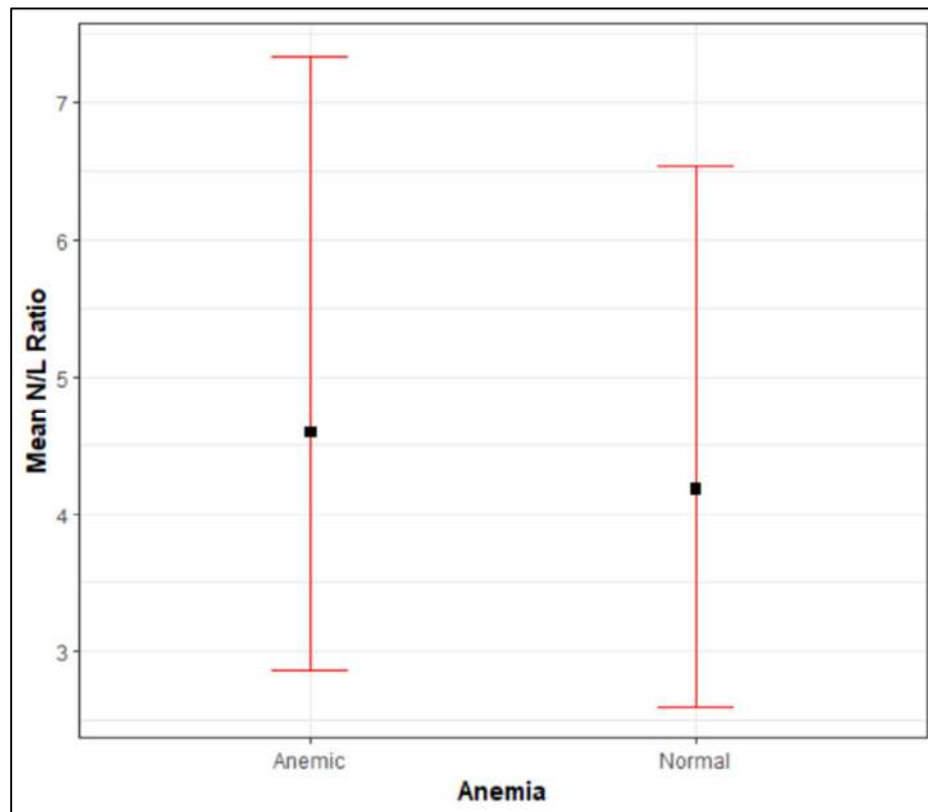
Graph 12: Mean plot of Neutrophil over anaemia.



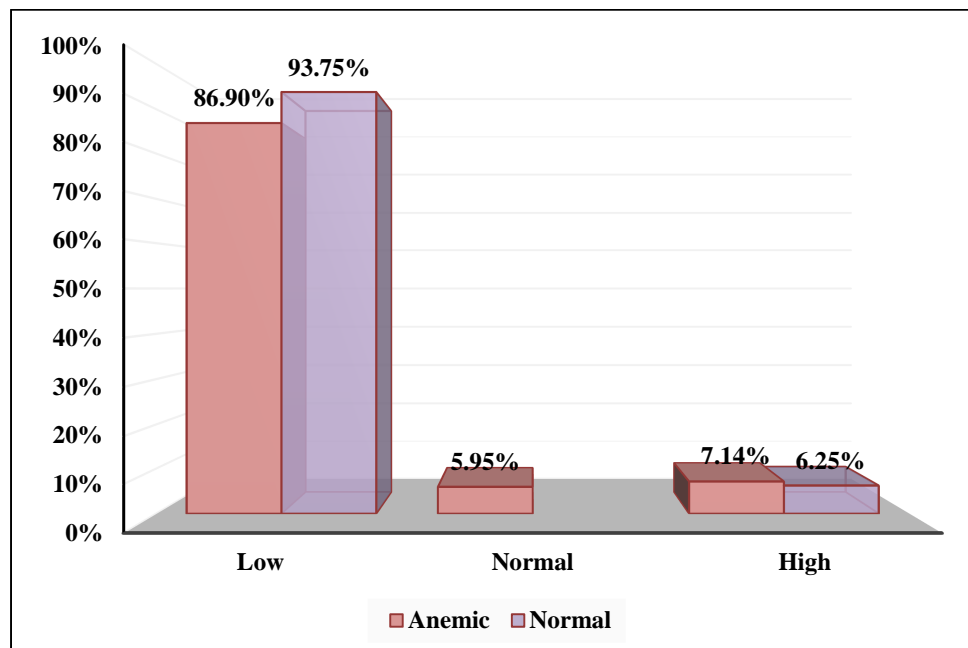
Graph 13: Mean plot of Lymphocyte over anaemia.



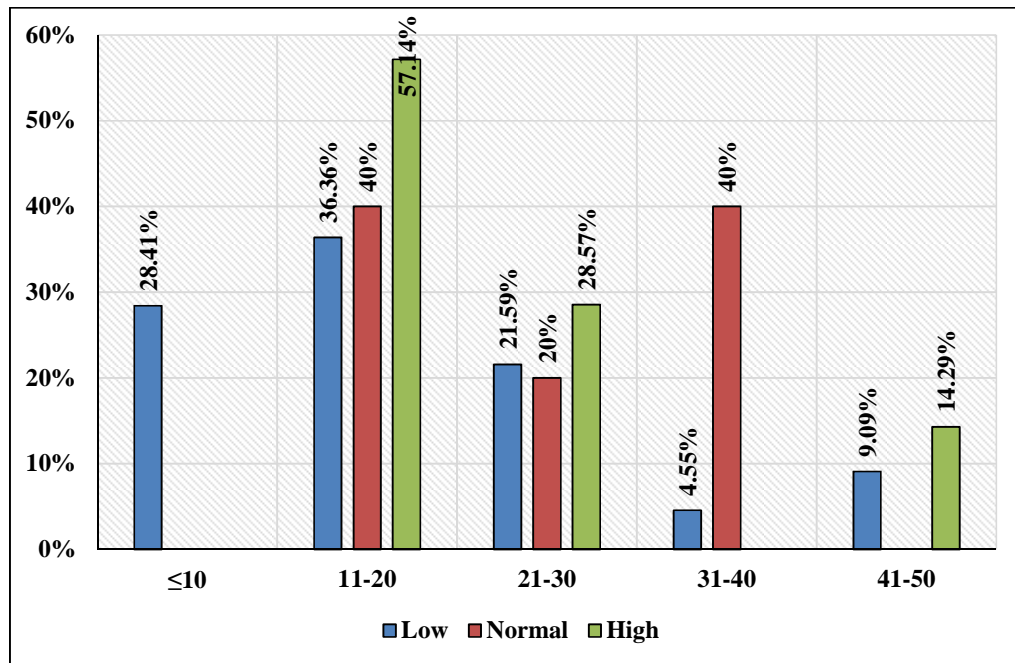
Graph 14: Mean plot of N/L ratio over anaemia.



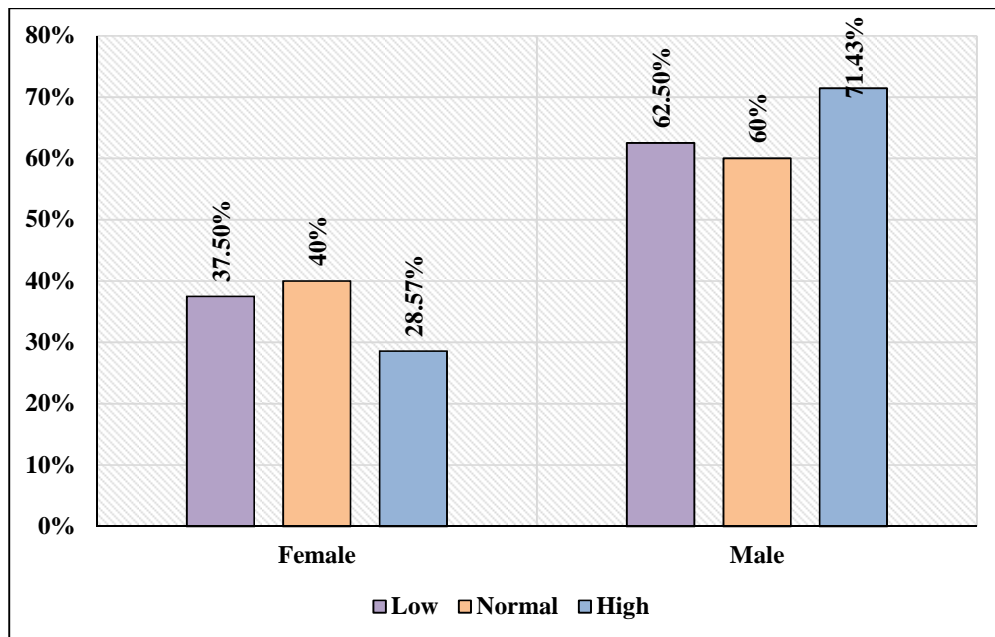
Graph 15: segregation of subjects with respect to serum zinc over anaemia.



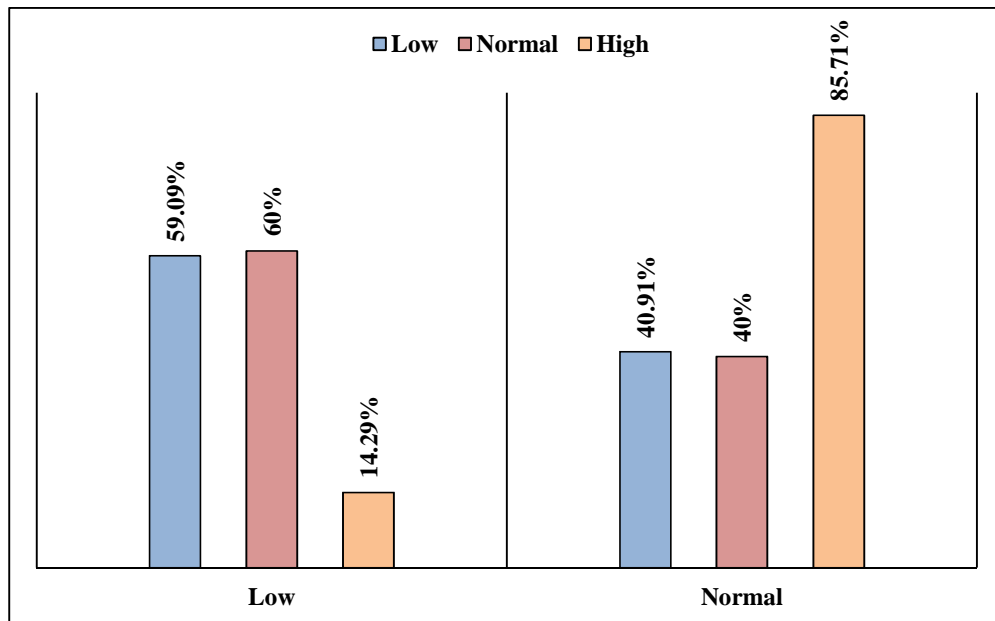
Graph 16: subjects distributed according to age over serum zinc.



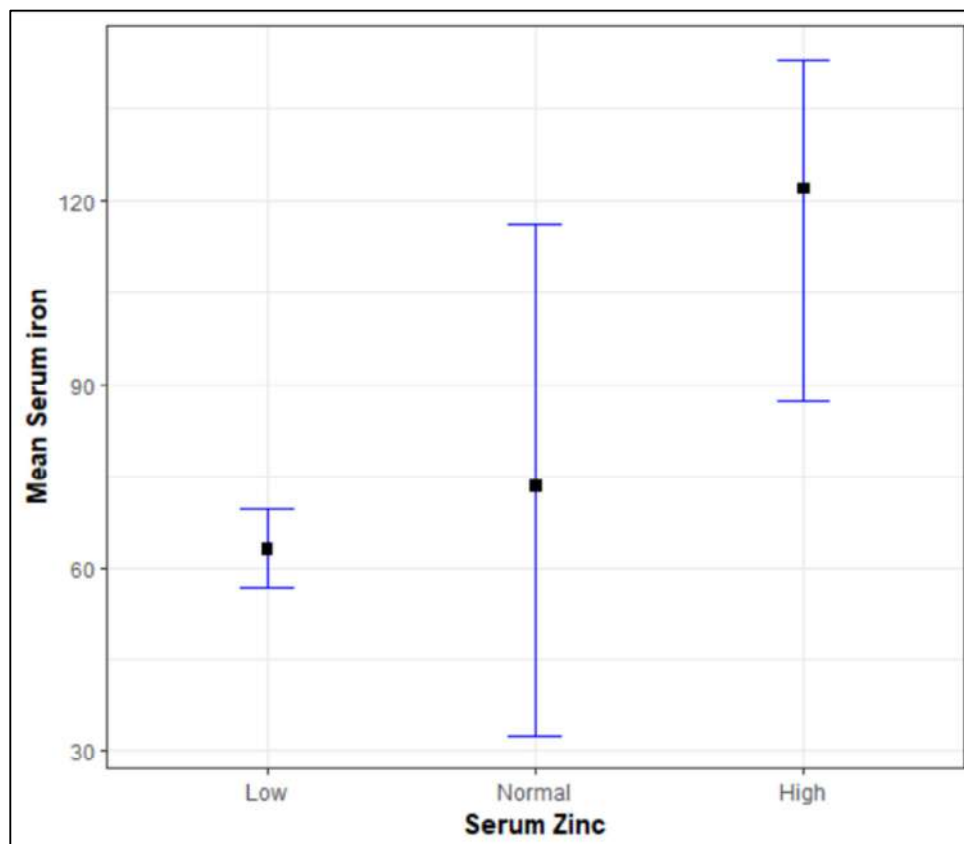
Graph 17: subjects distributed based on gender over serum zinc.



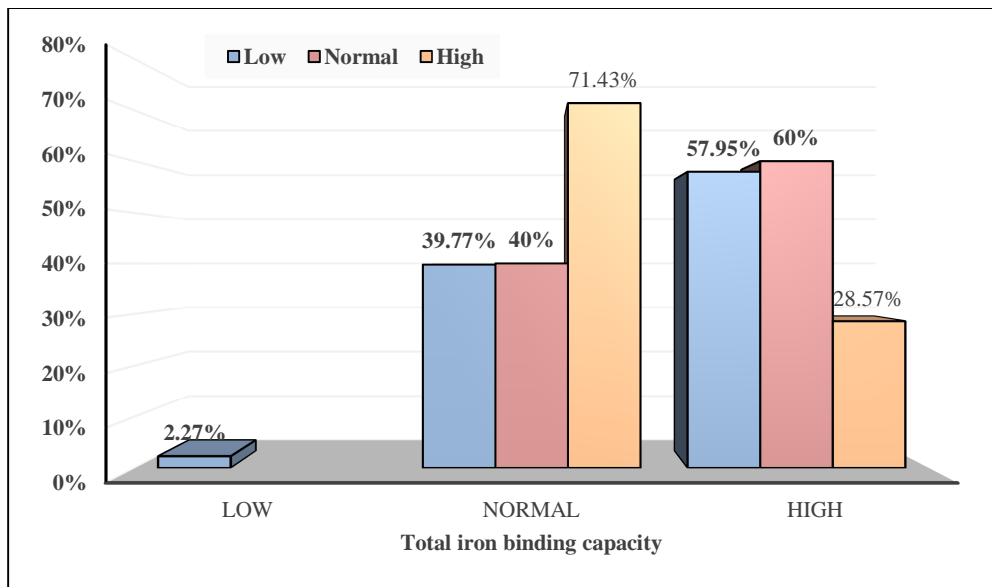
Graph 18: subjects distributed as per serum iron over serum zinc.



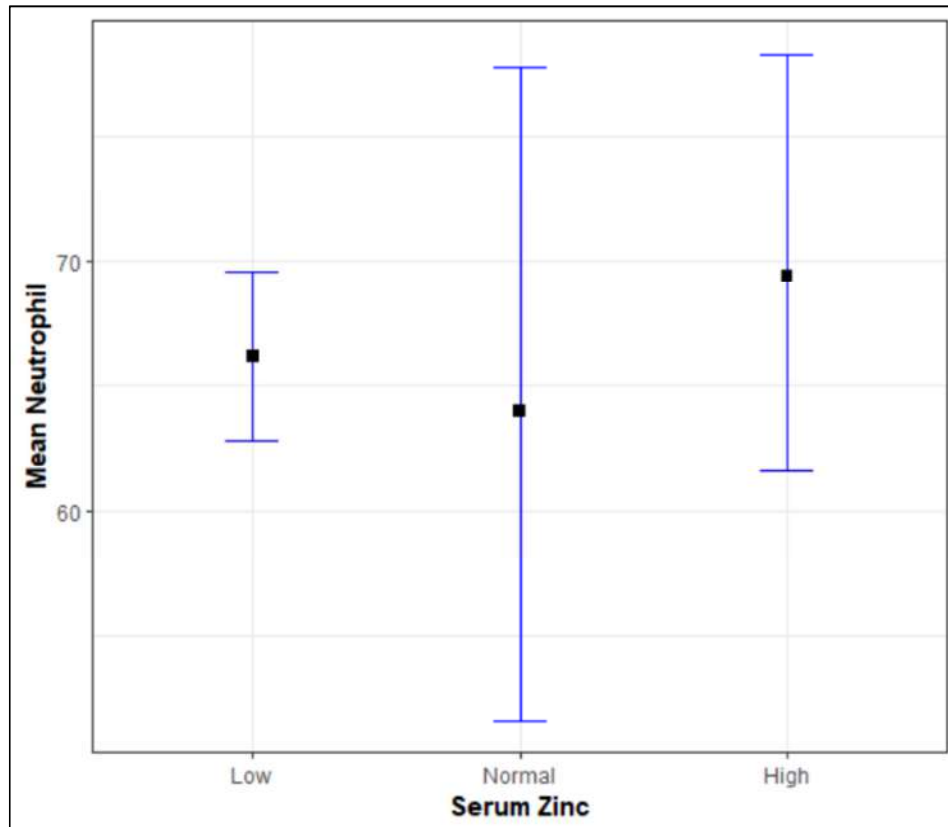
Graph 19: Mean plot of serum iron over serum zinc.



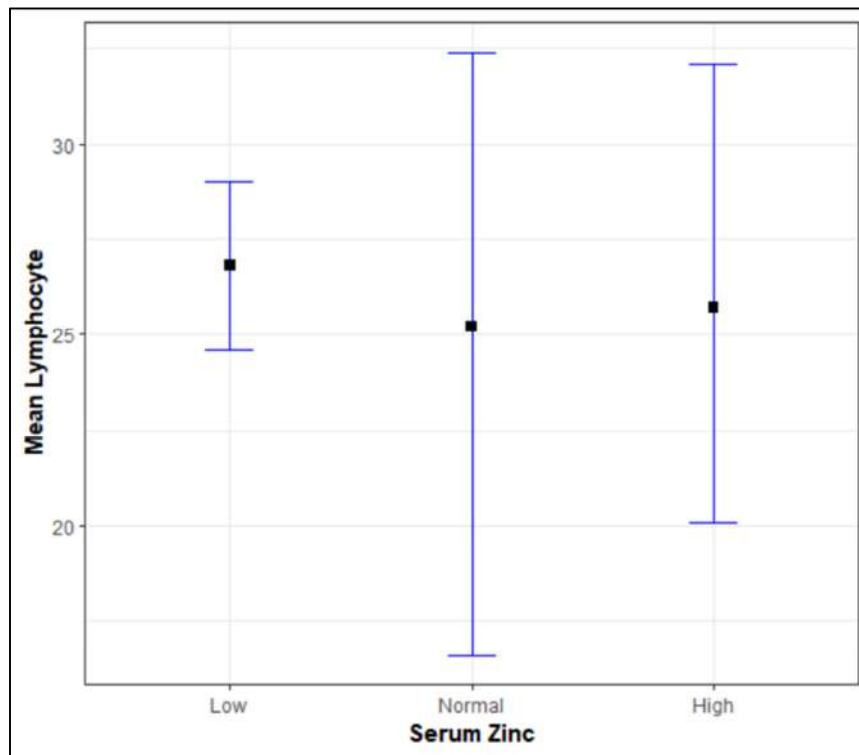
Graph 20: subjects distributed according to total iron binding capacity over serum zinc.



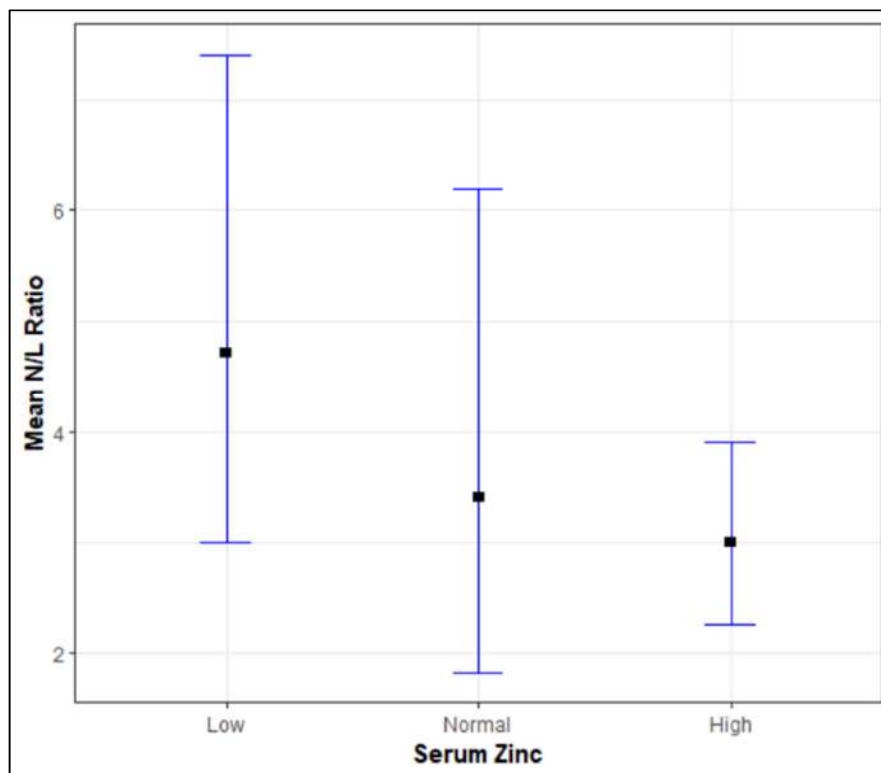
Graph 21: Mean plot of Neutrophil over serum zinc.



Graph 22: Mean plot of Lymphocyte over serum zinc.



Graph 23: Mean plot of N/L ratio over serum zinc.



The neutrophil lymphocyte ratio was calculated for all 100 cases which were included in the study and correlation factor “r” was calculated. By using Spearman’s correlation coefficient, the r value was of **0.824** and the $p < 0.05$ and as well as Pearson’s coefficient r was **0.4253** and p value less than **0.05**.

Thus, we found high significant correlation among a high neutrophil - lymphocyte ratio and recurrent tonsillitis and also with a positive corelation with respect to degree of tonsillar hypertrophy.

Table 9: Spearman's correlation coefficients with Neutrophil count

PARAMETER	r	p VALUE	INFERENCE
LYMPHOCYTE	-0.5040	0.0000	HIGHLY SIGNIFICANT
N/R RATIO	0.8242	0.0000	HIGHLY SIGNIFICANT
HAEMOGLOBIN	0.1878	0.0614	NOT SIGNIFICANT
SERUM ZINC	-0.2210	0.0272	SIGNIFICANT
SERUM ZINC	-0.2210	0.0272	SIGNIFICANT

Table 10: Pearson's correlation coefficients with Neutrophil count

PARAMETER	r	p VALUE	INFERENCE
LYMPHOCYTE	-0.5896	0.0000	HIGHLY SIGNIFICANT
N/R RATIO	0.4253	0.0000	HIGHLY SIGNIFICANT
HAEMOGLOBIN	0.1895	0.0590	NOT SIGNIFICANT
SERUM ZINC	-0.0690	0.4950	NOT SIGNIFICANT

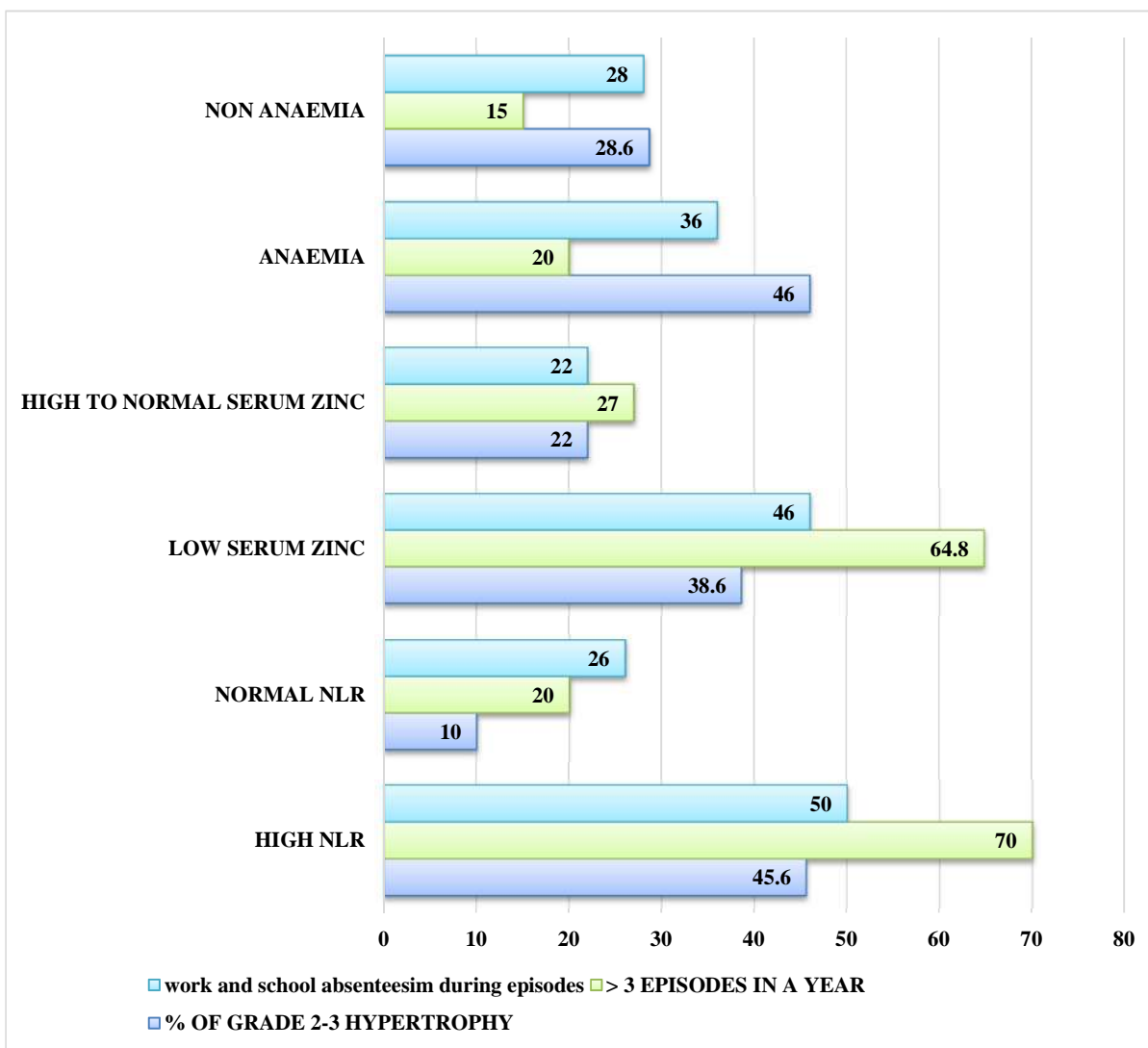
Table 11: Spearman's correlation coefficients with lymphocyte count

PARAMETER	r	p VALUE	INFERENCE
N/R RATIO	-0.8722	0.0000	HIGHLY SIGNIFICANT
HAEMOGLOBIN	-0.0146	0.8853	NOT SIGNIFICANT
SERUM ZINC	0.0773	0.4447	NOT SIGNIFICANT

Table 12: Pearson's correlation coefficients with lymphocyte

PARAMETER	r	p VALUE	INFERENCE
N/R RATIO	-0.5095	0.0000	HS
HAEMOGLOBIN	-0.0287	0.7771	NS
SERUM ZINC	0.0137	0.8922	NS

Graph 24: overall assessment of the variables with respect to absenteeism form work and grade of hypertrophy and number of recurrent episodes in a year



We assessed the variables overall based on the absenteeism form school and work as we know that recurrent tonsillitis leads to DALY and we found results as shown in the figure, almost more than 40% of patients with anaemia, reduced serum zinc and iron had work and school absenteeism. Among them in the raised NLR group about 50% patients had absenteeism and loss of working hours which had affected their work quality or education and led to loss of resources.

We also found that the recurrence of 3 or more than 3 episodes a year was maximum of 70% in patients with a raised NLR and accounted to 64.8% among those with reduced Serum Zinc and anaemia patients about 20%.

With respect to the hypertrophy of the tonsillar tissues we found that repeated episodes and lower levels of Serum Zinc and presence of anaemia was among those with more hypertrophied tonsillar tissue another indicator of recurrent infection.

DISCUSSION

Studies have shown that about more than 11,000 people among every 1 lakh suffer from chronic or recurrent tonsillitis across the globe, amount 20% of those with sore throat are said to have tonsillitis. With such a huge global burden this disease has been researched a lot in the past with several modalities of treatment ranging from medical to surgical. The commonest causes of the inflammation are due to bacterial infection thus making children, anaemic adults and immune compromised individuals more susceptible. It is said that the recurrent tonsillitis among the school and college going age groups accounts to about 0.1 million DALY, owing to absenteeism form work or school and affects their growth and overall performance. In our study as well, we found that about 60% of the patients had a history of absenteeism form work or school due to the recurrent episodes.⁴

Though the diagnosis and treatment of chronic tonsillitis have standard guidelines with several detailed studies being done on their pathophysiology, clinical features and management, not enough data is available with respect to its prognosis or the risk of its recurrence which ultimately decides the line of treatment, this was attempted in our study. So the immunologically active trace elements such as zinc and iron and other blood parameters have been considered by us.

Table 13: Sex distribution: comparison with present study

STUDIES	MALE %	FEMALE%
Yakup Yengin et al	45.8%	54.2%
Tekin Baglam et al	51.3%	54%
Present study	37%	63%

While we have seen the role of trace elements in immune reactions the primary defence is formed by white blood cells. Low levels of serum Iron and presence of anaemia makes the patient predisposed to development of infection and also its recurrence.

There have been studies done to decipher how commonly anaemia occurs by iron deficiency among those with chronic tonsillitis, one such study done by Weijie Weng et al⁴⁷ that shows that children undergoing adenotonsillectomy for chronic and recurrent adenotonsillitis have a higher incidence of iron deficiency anaemia when compared to the general population.

They also propose that it could be attributed to the accepted theory that recurrence occurs due to immune deficiency, and anaemia in turn compromises the immunity making them more prone to recurrent bacterial infections. Considering that our setting is in suburban India, where the general prevalence of iron deficiency anaemia is higher due to nutritional deficiency as well and worm infestations, the population of the study group was more prone to infection.

Table 14: Prevalence of Anaemia among tonsillitis patients

STUDIES	PREVALANEC OF ANEMIA IN TONSILLITIS PATIENTS
A. Busuttil, a. I. G. Kerr	15%
Weiji wang	46.9%
Hans Elverland et al	56.3%
E Mira et al	57.5%
Present study	84.4%

According to Kerr A.I.G. et al in their study certain venous blood samples from children who were admitted for an adenotonsillectomy and had a history of recurrent throat infections revealed low haematocrit., indicating a degree of anaemia, and thus in our study we are evaluating haemoglobin, to look for the prevalence of recurrent tonsillitis in anemia.²⁰

The prevalence of anaemia is higher in our study population perhaps owing primarily to the socioeconomic strata, race of the population hailing from rural India as against other studies done in developed populations of the west.

Somuk Battal Tahsin et al have compared the quantities of metals like such as zinc as well as iron in the tonsillar tissue in cases with tonsillitis which was recurring and in hypertrophic tonsils which demonstrated that lower tissue levels of these elements predisposed to recurrent infections.¹⁴ The same study, also stated that Iron and zinc are trace elements which are important and have important part in

immunity development, functioning , they also said that they partake in the development of infections⁵.

Donald.V et al of USA in their research have described that Zinc plays three pivotal roles by being a catalyser, and by having structural and regulatory functions, zinc haemostasis affects immunity, apoptosis and oxidative stress, also in enzyme functioning such as superoxide dismutase, matrix metalloproteinases and carbonic anhydrase¹⁹.

As a component of the enzymes peroxidase and other enzymes that produce nitrous oxide, which is essential for the immune system's enzymatic functioning, iron contributes to the immune system's growth.

The literature is replete with papers describing changes in the plasma concentrations of trace elements in a variety of illnesses. In some severe bacterial infections (such as meningitis, erysipelas, pneumonia, and septicaemia).

Table 15: Prevalence of low serum zinc in tonsillitis

RESEARCHES	LOW SERUM ZINC PREVALANCE IN THOSE WITH TONSILLITIS
KH Brown et al	90%
Srinivas et al	59.6%
Onerci et al	52%
Present study	88%

The study by Srinivas et al found a decrease in serum Zn but an increase in serum Cu levels, except in patients with erysipelas.

According to K.H. Brown et Al in their study serum Zn levels fell and serum Cu levels rose after acute infection. Numerous clinical characteristics connected to the production of CuZnSOD (superoxide dismutase) were discovered in pediatric palatine tonsils and adenoids, and studies also revealed that the trace element status greatly contributed to the prevention of acute infections.²⁷

In the study by Shi-Nae Park et al, they have suggested that the mode of action to counter infection as well as repeated inflammations may be related closely to CuZnSOD expression in the pediatric patient's tonsils as well as in the adenoids and this enzyme is mainly made up of zinc and Cu trace elements in this manner trace elements play a vital role in development of recurrent infections.⁵²

We in our study have studied the correlation of recurrence and severity of tonsillitis with respect to the episodes and the grade of tonsillar hypertrophy and compared it with the levels of serum iron and zinc to look for increased inflammation and infection in those with reduced trace element concentration in the blood and but we didn't find a significant correlation between them, probably due to limited sample size and also that we correlated blood levels unlike the previous studies that calculated the quantity of these trace metals in the tonsillar tissue itself, which yielded more significant results.

The study done by Onerci et al only included the quantity of zinc and iron in the tonsillar cells and did not consider serum levels which would have provided a

better understanding of role the played by the trace elements in the general susceptibility to recurrent attacks of tonsillitis in the subjects of study¹⁵.

Most of these studies regarding the role played by trace elements have been done in Europe and other western countries due to which their relevance and applicability to the practice of otorhinolaryngology in India cannot be directly ascertained, as the racial factors, prevalence and incidence of tonsillitis is different in our country^{5,6,7}.

Rosilene et al in their study have elaborated the means of performing and analysis levels of the heavy metals in the human blood stream, using standard method which is FAAS, flame atomic absorption spectrometry and how it is not only a cost effective but also very precise tool to arrive at the same.⁶² Several researchers such as Luiz et al, Roseline et al have all performed their researches with the use of the cathode lamp of zinc and n air acetylene flame, and obtained highly precise results of analysis. In our study we have also used the standard flame cytometry with acetylene to obtain our values keeping in mind the international standards.⁶⁵

Today there's another biomarker of inflammation which owing to its ease of calculation and affordability is being researched and applied across inflammation to neoplasia, this is the NLR (neutrophil-lymphocyte ratio). In their research project, Tekin Baglam et al. evaluated the diagnostic accuracy of NLR in identifying DNSI in a pediatric age group with acute tonsillitis of bacterial origin. They found that NLR seemed to have significant sensitivity and specificity for predicting DNSI (deep neck space infections) health problems linked to severe tonsillitis. Additionally, their findings demonstrated the value of NLR as a marker of inflammation and infectious complications associated with bacterial infections of tonsils.⁵³

The research work done by Battal Tahsin Somuk et al which was done in Turkey had a sample size of just 20 subjects, results of such a study could have been more accurate and statistically significant if a larger sample size were used.¹⁴

The ratio between neutrophils and the lymphocytes is a relatively newer addition to the spectrum of markers of infection.

Chronic infections initially deplete neutrophils leading to a milieu of neutropenia, which activates bone marrow precursors of neutrophils thus raising the neutrophil count as against lymphocytes thus the ratio or NLR value as a whole increases². We have applied this concept of NLR to one of the oldest maladies in ENT to aid us in preempting recurrence and accordingly manage the patients.

Table 16: Raised levels of NLR in Infection as a marker

STUDY	NLR LEVELS AMONG THOSE WITH INFECTION
Fatma dogruel et al	Raised in 85%
Somuk et al	RAISED in 76% showed high specificity and sensitivity
Tekin Baglam et al	RAISED in case more than control
Present study	Raised in all cases with $r = 0.824$

At the same time, it is important to understand that there still isn't a universal normal range for NLR, given by any standard guidelines, it is being followed based on previous studies which have been done on large sample sizes of healthy individuals and analyzed in detail. If this were to come into practice as a routine testing modality then it will require larger cohorts and standardization.

Some of the initial studies done with regards to the neutrophil lymphocyte ratio were those among patients of infective endocarditis and how the bacterial endocarditis showed a higher NLR as against those without infection, it also showed that patients with blocks in the coronary circulation and of higher risk has correspondingly higher levels of the NLR. Raymond farah et al_were one of the first to study the relation and the utility of NLR in cases of gastritis with H pylori infection, and they found that it was significantly raised in cases with the acute or active infection and recurrence as against those with gastritis without H pylori. There have been other studies to compare association of the NLR with the severity of infection in cases of appendicitis where in patients with gangrenous appendicitis had a very high and significant NLR as well as those who underwent emergency appendectomy for the same.⁴¹

The microenvironment of cancerous tissue has been seen to have increased lymphocytes and their higher numbers have shown that prognosis is better, whereas the neutrophils and lymphocytes are said to be associated with aggressive tumours, in this manner lymphocytes are proposed to have antitumour activity. The neutrophil lymphocyte ratio thus has been included into the armamentarium of doctors to predict prognosis and recurrence not only in inflammatory conditions but also in tumours.

The study by Fatma et al has shown that neutrophil lymphocyte ratio is positively correlated to the prognosis of patients with odontogenic infections, where in there was an increase in the dose and duration of antibiotics as well as prolongation of the hospital stay, and they concluded that in this manner NLR is a prognostic indicator for such infections of the oral cavity.⁶⁷

Yakup yegin et al⁶⁸ in their recent study have assessed the correlation of the size of tonsils and the ration of neutrophils to the lymphocytes among post tonsillectomy patients and have concluded that the higher the grade of hypertrophy of tonsils being an indicator of chronic infection, there seems to be a higher level of NLR.⁴⁷

Studies have tried to analyze the ability of NLR to predict the survival among patients of metastatic nasopharyngeal carcinomas and found it raised in these patients. Surajeet et al 2018 have concluded that NLR value can be as useful in assessing the prognosis of surgery for chronic tonsillitis those who underwent tonsillectomy.⁶⁹

This is one of the few studies to our knowledge to assess the correlation of NLR and recurrent tonsillitis and use this biomarker for indication of prognosis of tonsillitis alongside serum trace elements. Thus, further studies with larger sample sizes are required to support and ascertain our results.

CONCLUSION

Tonsillitis is a probably one of the most commonly encountered condition in the practice of not only otorhinolaryngology but also general medicine and paediatrics be at the grassroot level, or at highly specialized tertiary care centres. These cases amount to a huge global burden of DALY and lead to work and school absenteeism reducing performance ability of the patients. That is why we have tried to highlight the magnitude of its impact on public health, though at the offset it may seem like a simple ailment.

Over the past decades treatment for tonsillitis in the form of antibiotics and antiseptic gargles and surgery have been vastly explored and several types of surgeries have been developed, not much attention has been given to assess the prognosis or recurrence of infection in such cases. With the modern concepts of evidence-based management in mind we must ensure that prior to suggesting surgery which bears its own complications we must 1st assess the underlying cause and then the chances of recurrence, which can be done by simple investigations as seen in this study.

We suggest that ruling out anaemia especially nutritional iron deficiency anaemia is one of the primary approaches, not only will correcting the anaemia reduce the rate of recurrence, it will also improve general condition of the patient, thereby ensuring better surgical outcome and withstanding intraoperative stress if the patient were to undergo surgery.

We have also studied the correlation of this recurrence with serum zinc and we found a low positive significance between reduced serum zinc and recurrence of tonsillitis. Zinc has always been associated with immunity being an essential trace element for immunologically active enzymes and following the COVID -19 pandemic was further brought into light with use of zinc supplementation. In this manner further studies comparing recurrence rates after supplementing trace elements in those who are predisposed to recurrence due to deficiency must be done to ascertain our findings. The ratio of NLR was found as a marker of increased recurrence with positive correlation to cases of tonsillitis. The NLR is already being used as a biomarker of prognosis in cancers and several systemic infections, but with respect to pharyngitis and tonsillitis it hasn't been explored much, thus larger studies must be done to understand the applicability of NLR and consequently recommend its use in day-to-day practice, due to its versatility and ease of calculation also the cost effectiveness of this investigation.

SUMMARY

- This study was performed at the Otorhinolaryngology department for 1 year and during this time the patients who came with chronic and recurrent tonsil infections fitting into inclusion criteria after consent were included.
- Detailed history taking and examination was done, after which blood samples were collected and analysed.
- Tonsillitis had been a timeworn malady which has kept otorhinolaryngologists busy to combat its acute attacks, chronicity and its complications across all age groups.
- This research results found a positive correlation among anemia as well as low serum zinc among patients of recurrent tonsillitis with a raised NLR among recurrent tonsillitis.
- Spearman's correlation coefficient, the r value was of **0.824 p <0.05**.
- Thus, these trace elements and the NLR play a vital role in the immunity which in turn reflects on the recurrence of infection so the serum zinc, iron, NLR can be used as markers of recurrence.
- This corroborates with other studies in literature, being one of the first studies to consider multiple variables we need larger studies to ascertain our findings and inculcate them into practice.

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ANNEXURE – I - INFORMED CONSENT

**STUDY TITLE: EVALUATION OF THE LEVELS OF SERUM ZINC , IRON
AND HEMATOLOGICAL PARAMETERS AND THEIR CORRELATION
WITH RECURRENT TONSILLITIS - A ONE YEAR OBSERVATIONAL
STUDY AT KLES DR. PRABHAKAR KORE HOSPITAL**

PRINCIPAL INVESTIGATOR: Dr.

Post Graduate student

Department of Otorhinolaryngology and head and neck surgery.

CO-INVESTIGATOR: Dr.

Associate Professor,

Department of Otorhinolaryngology and head and neck surgery

J.N. Medical College

INTRODUCTION AND PURPOSE: The present study is conducted among subjects with recurrent tonsillitis, between the age group of 1yr to 50 yrs. attending the out-patient department of ENT & HNS in KLE's Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belgaum. You are requested to participate in the study and your participation is completely voluntary.

PROCEDURE: If you agree to participate in this study, the relevant data will be collected as per the proforma and the final diagnosis will be confirmed. After getting inducted in the study, you will undergo detailed ENT examination and venous blood samples will be collected for CBC, serum Iron and serum Zinc.

BENEFITS: Patient will not be eligible for any kind of monetary benefits or free services by virtue of your participation in the study.

RISKS: Methods applied to do the study are safe.

COST OF PARTICIPATION: The cost of the Investigation will be borne by the Study Subject. The other indirect expenses will be borne by the Investigator.

PRIVACY AND CONFIDENTIALITY: The results of the study may be published in journals for scientific purposes. However, your identity will not be revealed. All information collected will be coded so that no one other than the investigator will know your identity.

WITHDRAWAL FROM THE STUDY: You can withdraw from the study at any time if you wish to do so.

AUTHORIZATION TO PUBLISH THE RESULTS: The researcher may use the information gathered from this study for presentation in scientific meetings. However, your identity will not be revealed.

QUERIES AND CONTACT: If you have any queries regarding the study, you can contact Dr. _____ without any hesitation on Mobile no: _____ and the guide Dr. _____, Mobile No: _____.

If you have any questions about rights as a research participant you can contact Dr. Harsha Hegde, Professor, department of Pediatrics, Jawaharlal Nehru Medical College Institutional Ethics Committee on human subjects' research.

CONSENT SUMMARY: I have been explained all the contents of this consent form in my local language and having understood and clarified all my queries about the study to the best of my knowledge, I hereby give my voluntary consent for participation in the study. I do sign the informed consent form in front of an eyewitness whom I recognize.

Name and Signature/ left thumb impression of the participant:

Name and Signature of the interviewer:

Name and Signature/ left thumb impression of the eyewitness (Relative):

Signature of the guide:

Consent statement to participate in a research study

I, Mr. /Mrs./Miss. _____ voluntarily agree to take part in this study, by signing this consent form I am not giving up my legal rights. I may withdraw at any time. I am signing after having read, or been read to me in the vernacular language including risks and the benefits and having all queries cleared.

Signature of the study patient _____

Name of Study patient _____

Name and Signature of Witness _____

Name and signature of investigator _____

Date:

Place:

FOR MINORS INFORMED ASSENT FROM PARENTS/GUARDIAN

ASSENT STATEMENT TO PARTICIPATE IN A RESEARCH STUDY

I, Mr./Mrs.._____ Parent/Guardian of _____ voluntarily agree to let my child/ ward participate in this study, by signing this consent form I am not giving up my legal rights. I may withdraw my child at any time from the study. I am signing after having read, or been read to me in the vernacular language including risks and the benefits and having all queries cleared.

Signature of the parent/ guardian/legally authorized representative_____

Name of Study patient_____

Sign of the Patient: (if 13yrs and above or verbal consent of below 13yrs)

Relationship with the patient_____

Name and Signature of Witness_____

Name and signature of investigator_____

Date:

Place:

ANNEXURE – II - PROFORMA

**EVALUATION OF THE LEVELS OF SERUM ZINC , IRON AND
HEMATOLOGICAL PARAMETERS AND THEIR CORRELATION WITH
RECURRENT TONSILLITIS - A ONE YEAR OBSERVATIONAL STUDY
AT KLES DR. PRABHAKAR KORE HOSPITAL**

Date:

O.P. No:

Name:

Age:

Sex:

Occupation:

Address:

Phone No:

CLINICAL PROFILE:

Chief Complaint:

History of Present Illness:

Past History:

Personal History:

Family History:

I) General Physical Examination -

Blood Pressure:

Pulse:

Respiratory Rate:

- Pallor
- Icterus
- Clubbing
- Cyanosis
- Lymphadenopathy
- Oedema

II) ENT Examination

1. NOSE EXAMINATION

External appearance root

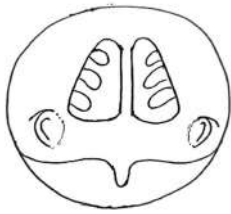
- Bridge
- Dorsum
- Alae
- Tip
- Columella

Cold spatula test

Cottle's test

Anterior Rhinoscopy

Posterior Rhinoscopy



Paranasal Sinus Examination

2. EAR EXAMINATION:

Right

Left

Pinna

Pre auricular area

Right

Left

Post auricular area

External auditory canal

Tympanic membrane

ANNEXURE – III – PHOTOGRAPHS

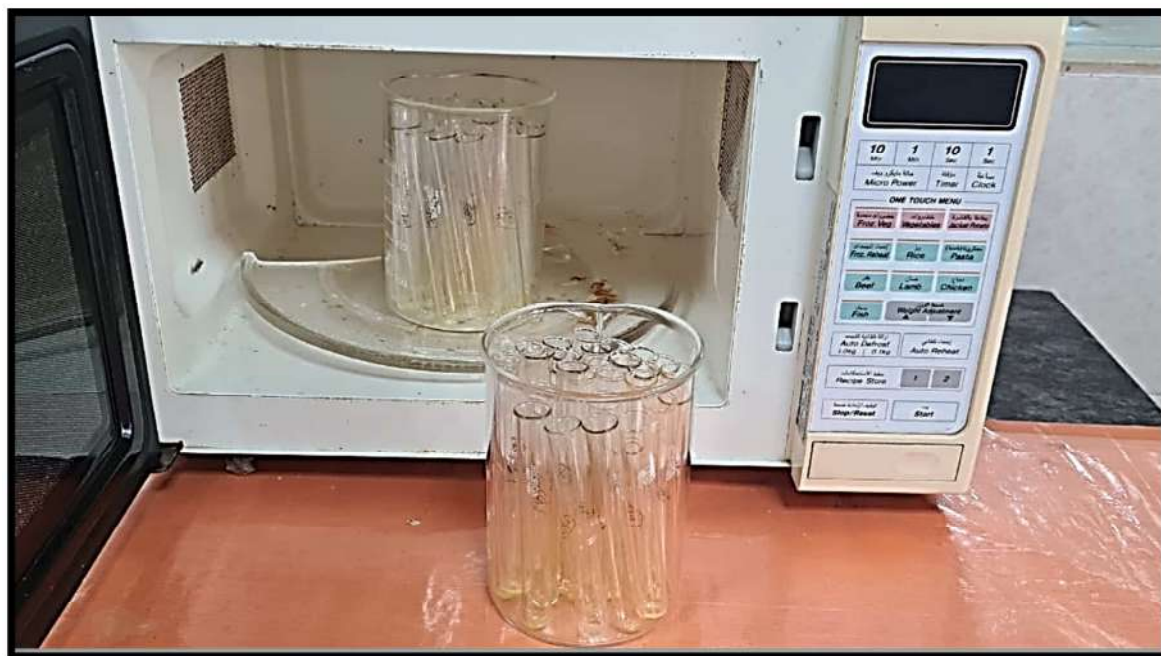
Photograph 1: GASES USED FOR THE FAAS



Photograph 2: THE SERUM SAMPLES AFTER PREPARATION OF SOLUTIONS USING THE STANDARDS FOR ZINC AND IRON



Photograph 3: THE SERUM SAMPLES BEING MICROWAVED TO GIVE THE PRECIPITATE WHICH ARE THEN DILUTED



**Photograph 4: THE DILUTED AND PREPARED SAMPLES BEING TESTED
BY FLAME ATOMIC ABSORPTION SPECTROMETRY**



**Photograph 5: THE SET UP OF FAAS – AA-7000 SPECTROPHOTOMETER
WITH THE ASC-AUTOSAMPLER AND THE WIZAARD SOFTWARE
BEING USED TO TEST ALL THE SAMPLES**



Photograph 6: DETAILED OROPHARYNX EXAMINATION SHOWING RIGHT GRADE 1 AND LEFT GRADE 2 TONSILLAR HYPERTROPHY



Photograph 7- OROPHARYNX EXAMINATION SHOWING BILATERAL GRADE 3 TONSILLAR HYPERTROPHY



Photograph 8- OROPHARYNX EXAMINATION SHOWING BILATERAL GRADE 4 TONSILLAR HYPERTROPHY



ANNEXURE – IV – KEY TO MASTERCHART

M - MALE

F - FEMALE

I - GRADE 1 TONSILLAR HYPERTROPHY

II - GRADE 2 TONSILLAR HYPERTROPHY

III - GRADE 3 TONSILLAR HYPERTROPHY

IV - GRADE 4 TONSILLAR HYPERTROPHY

NLR - NEUTROPHIL LYMPHOCYTE RATIO

TIBC - TOTAL IRON BINDING CAPACITY

Hb - HEAMOGLOBIN

Fe - IRON

Zn - ZINC

ANNEXURE - V - MASTERCHART

SR NO.	NAME	GRADE OF TONSILLIAR HYPERTROPHY	AGE	SEX	SERUM IRON (60-170mcg/dL)	TIBC (Total Iron Binding Capacity) (240-450 mcg/)	NEUTROPHIL	LYMPHOCYTE	NLR (NEUTROPHIL/ LYMPHOCYTE RATIO)	Haemoglobin (13.2-16.6g/dL)	SERUM ZINC (0.6-1.10 mcg/dl)	work/school absenteeism history
1	YASH	III	7	M	80	362	68	30	2.2	13	0.09092	yes
2	GANESH	III	9	M	38	378	47	38	1.23	12.4	0.0468	yes
3	SARIKA	II	30	F	30	560	68	20	3.4	10	0.1308	yes
4	HIRAKANT	III	11	M	40	427	38	50	0.76	11.6	0.269	no
5	ALFA	II	7	F	40	350	51	45	1.13	13.6	0.4462	no
6	TEJAS	III	11	M	23	400	69	25	2.76	11.8	1.406	yes
7	SHRIKANT	III	30	M	141	253	50	21	2.12	10	0.93	yes
8	SHIVANANAD	II	20	M	30	312	65	33	1.99	8	0.0084	no
9	RAJSHREE	I	27	F	19.5	478	70	37	1.89	11	0.4154	yes
10	RAIZA	II	5	F	20	540	78	10	7.8	12	0.0796	no
11	LAXMI	III	6	F	133	276	80	20	4	13	0.4388	yes
12	DRAKSHAYINI	II	24	F	98	312	99	30	3.3	11	0.1504	no
13	SADHIYA	II	8	F	80	427	90	10	9	10	0.0886	yes
14	HAFASAKHANAM	II	17	F	120	657	89	10	8.9	9.6	0.9592	yes
15	LAXMAN	I	17	F	152	328	67	20	3.35	11	1.2022	yes
16	RAKESH	III	11	M	67	700	40	30	1.333	10	0.4302	no
17	VISHWAS	II	11	M	54	314	68	30	2.233	8	0.3264	yes
18	SARASWATI	II	16	F	138	600	50	40	1.24	9	0.4758	no
19	MAHADEV	III	16	F	43	290	98	44	2.22	10	0.1958	yes
20	KUSHI	III	9	F	57	368	50	30	1.66	11	0.1792	no
21	MALLESH	II	9	F	110	390	60	31	1.9	12	0.283	no
22	SHIPA	II	7	F	96	400	66	20	3.3	12	0.2252	no
23	BIBJAN	III	7	F	70	414	54	20	2.7	7	0.284	yes
24	MALLIKARJUN	II	9	M	48.2	436	46	22	2.11	8	0.215	yes
25	RAJEEV	II	9	M	66	379	46	16	2.85	9	0.289	yes
26	NAGRAJ	II	11	M	132	322	54	20	2.7	10	1.75	no
27	SHIVANAND	III	11	M	77	314	56	24	2.33	11	0.1398	no
28	MAHES	II	21	M	51	289	57	30	1.9	12	0.0102	yes
29	RAVI	I	21	M	46	390	58	36	1.61	13	0.1466	yes
30	TEJAS	III	7	M	44.4	417	80	28	2.85	11	0.1924	yes
31	TEJAS	III	8	M	32	540	96	17	5.64	12	0.082	yes
32	MUMTAZ	III	7	F	89	620	78	18	4.33	12	0.2178	no
33	RAKHI	IV	8	F	36	360	96	16	6	13	0.1324	yes
34	RAMESH	II	8	M	47	511	75	20	3.75	10	0.181	yes
35	MAHANTESH	II	11	M	59	436	59	22	2.86	12	0.1678	yes
36	BHAVANI	III	12	F	46.8	360	49	31	1.24	11	1.0868	yes
37	GANESH	III	13	M	34	717	50	36	1.76	10	0.1786	yes
38	RAKHI	II	13	F	31.6	387	80	33	2.42	9	0.086	yes
39	MOHAN	II	10	M	37	436	78	28	2.78	9	0.3442	yes
40	KRISHNA	II	11	M	26.8	525	77	23	3.34	11	0.1028	no
41	RAMAPPA	III	35	M	22	698	60	28	2.12	12	0.826	yes
42	SOMAPPA	I	35	M	58	266	65	30	2.166	11	0.2322	yes
43	MALAPPA	I	50	M	60	487	63	41	1.54	10	0.168	no
44	RAJESH	I	50	M	19.5	646	46	27	1.88	10	0.2282	yes

SR NO.	NAME	GRADE OF TONSILLIAR HYPERTROPHY	AGE	SEX	SERUM IRON (60-170mcg/dL)	TIBC (Total Iron Binding Capacity) (240-450 mcg/)	NEUTROPHIL	LYMPHOCYTE	NLR (NEUTROPHIL/ LYMPHOCYTE RATIO)	Haemoglobin (13.2-16.6g/dL)	SERUM ZINC (0.6-1.10 mcg/dl)	work/school absenteeism history
45	RAKESH	II	25	M	20	456	60	20	3	10.6	0.216	yes
46	SHIVANANAD	III	25	M	133	380	64	26	3.2	10.8	1.3926	yes
47	SHIVANGAIDA	II	40	M	98	560	66	20	3.22	11	0.3218	yes
48	MULAPPA	I	40	M	82	735	75	30	2.45	11	0.203	yes
49	SANGEETA	I	43	F	120	637	73	34	2.13	12	0.3724	yes
50	MALLIKARJUN	II	42	M	145	325	82	15	5.46	11	1.2686	no
51	PREMA	I	44	F	67	534	93	5	14	12	0.2104	no
52	HAVLEPPA	II	45	M	54	524	95	5	18	11	0.1392	no
53	GANGA	II	46	F	138	253	70	18	3.88	10	0.0442	yes
54	BASAPPA	I	42	M	43	330	90	10	9	8	0.1962	yes
55	HAVLEPPA	III	22	M	57	436	55	30	1.446	8	0.2156	yes
56	MOHAMMED	III	24	M	110	564	77	25	3.4	8	0.2124	no
57	ABBAS	II	25	M	96	534	64	30	2.21	8.7	0.1746	yes
58	RAMCHANDRA	III	26	M	72	320	86	32	2.65	10	0.0954	yes
59	GANGAMMA	II	20	F	48.2	424	60	36	1.66	11.6	0.131	yes
60	AMIT	III	18	M	66	434	55	28	1.96	10	0.24	yes
61	GURRAK	II	16	m	132	534	50	32	1.56	13	0.2324	no
62	SUNITA	II	26	f	58	525	51	40	1.25	12.4	0.1034	no
63	ANJALI	III	22	f	51	523	41	30	1.22	10	0.2272	no
64	PRAMOD	III	21	m	46	543	80	20	4	11.6	0.2674	yes
65	RESHMA	I	34	F	44.4	654	82	18	4.5	13.6	0.1936	yes
66	ANIL	I	42	M	32	231	50	20	2.5	11.8	0.2096	yes
67	SOMASHEKAR	II	19	M	89	342	69	24	2.87	10	0.0828	no
68	DUNDAPPA	II	39	M	36	654	72	36	2	8	0.658	yes
69	DAYANAND	II	12	M	47	636	80	28	2.87	11	0.1412	no
70	GANEDH	I	9	M	59	728	90	10	9	12	0.048	yes
71	PRAMOD	II	8	M	46.8	670	91	8	10.11	13	0.1554	no
72	ANAND	II	10	M	34	759	81	10	8.1	11	0.1742	no
73	BASAVRAJ	II	11	M	31.6	567	88	10	8.8	10	0.2346	no
74	CHIRAG	III	15	M	37	534	86	11	7.8	9.6	0.0622	no
75	HANUMATH	II	17	M	29.7	487	30	40	0.75	11	0.2562	yes
76	KAMALESH	II	19	M	22	491	46	40	1.15	10	0.2082	yes
77	KAJAL	II	22	F	58	480	42	30	1.67	8	0.2974	yes
78	LAKAPPA	II	21	M	60	463	72	33	2.36	9	0.2212	yes
79	MANOJ	II	19	M	19.5	473	75	32	2.56	10	0.226	yes
80	MAHAVDEV	II	18	M	20	397	76	32	2.37	11	0.2542	yes
81	SHAINAZ	I	19	F	133	398	85	34	2.5	12	0.2818	yes
82	SADASHIV	I	9	M	92	525	55	35	1.57	12	0.2946	yes
83	PARVATI	III	10	F	82	6354	53	36	1.47	7	0.2744	yes
84	SHIVANI	II	30	F	120	463	36	37	0.13	8	0.2796	yes
85	MANIKANTH	II	20	M	145	384	45	38	1.22	9	0.3154	yes
86	SHALINI	I	25	F	67	487	40	39	1.16	10	0.1582	yes
87	SAHANA	I	26	F	54	597	80	34	2.35	11	0.1196	no
88	PRATIMA	III	28	F	138	698	90	32	2.8	12	1.1344	no
89	RAJESH	I	16	M	53	720	78	36	2.166	13	0.276	no
90	PRAMOD	II	18	M	57	746	60	28	2.3	11	0.5016	yes

SR NO.	NAME	GRADE OF TONSILLIAR HYPERTROPHY	AGE	SEX	SERUM IRON (60-170mcg/dL)	TIBC (Total Iron Binding Capacity) (240-450 mcg/)	NEUTROPHIL	LYMPHOCYTE	NLR (NEUTROPHIL/ LYMPHOCYTE RATIO)	Haemoglobin (13.2-16.6g/dL)	SERUM ZINC (0.6-1.10 mcg/dl)	work/school absenteeism history
91	RAVESAB	II	10	M	110	456	56	30	1.86	12	0.0786	no
92	GUNASHEELA	II	11	F	96	463	76	30	2.533	12	0.1616	yes
93	PANDURANG	II	18	M	70	445	47	39	1.2	13	0.0778	yes
94	BHOOMIKA	II	14	F	48.2	200	85	42	2.02	10	0.1326	yes
95	BHUVANA	III	12	F	66	780	76	33	2.03	12	0.0892	yes
96	PRITHVI	II	11	M	132	635	60	42	1.42	11	1.554	yes
97	GAGANDHAR	II	10	M	58	524	58	31	1.87	10	0.1556	yes
98	KRITIKA	III	20	F	100	874	60	42	1.42	9	0.112	yes
99	SADIQ	III	17	M	46	728	40	20	2	9	0.268	yes
100	ALFAS	I	21	M	44.4	770	52	31	1.66	11	0.09	no