Study Of Matrix Metalloproteinase 9 And Histopathology In Sinonasal Diseases Before And After Endoscopic Surgery

by

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LIST OF ABBREVIATIONS USED

MMP-9	Matrix Metalloproteinase-9
FESS	Functional endoscopic sinus surgery
HPR	Histopathology
NP	Nasal polyposis
AC	Antrochoanal polyp
CRS	Chronic rhinosinusitis
IL-8	Interleukin 8
IL-5	Interleukin 5
TIMPS	Tissue inhibitor of matrix metalloproteinase
QQL	Quality of life
ECM	Extracellular matrix
URT	Upper repiratory tract
LRT	Lower respiratory tract
BM	Basement membrane
LP	Lamina propria
DPN	Deep petrosal nerve
GSPN	Greater superficial petrosal nerve
FN	Facial nerve
ANS	Autonomic nervous supply
SSN	Superior salivatory nucleus
MALT	Mucosa associated lymphoid tissue
ODA	Outward dynein arm
IDA	Inward dynein arm

ABSTRACT

BACKGROUND:.

Headache and facial pain are one of the most common symptoms encountered in day to day ENT practice. One of the reasons for this is disease of sinomucosa which causes distress to the patient in carrying out their daily activities. Incomplete treatment of these diseases leads to chronicity. Understanding the pathogenesis and the changes occurring at molecular and histological level in the sinonasal mucosa can help in better outcome of treatment.

Methods:

A comparative study was carried in 55 patients with sinonasal disease in the ENT dept from November 2017 to June 2019.All 55 patients underwent Endoscopic Surgery.

Nasal mucosa biopsies was taken (preoperatively and postoperatively 1st, 3rd and 6 month) for histopathological study.

Concentration of MMP-9 was measured by using commercially available ELISA.

RESULTS:

The present study was conducted in 55 patients presenting with sinonasal diseases who underwent FESS. It was found that MMP-9 value significantly decreased over time($P<0.05$).The fall of mean MMP-9 was maximum (53%) from pre-op to 1month which subsequently subsidized from previous value. Histopathological findings like presence goblet cell hyperplasia ,inflammatorycells,stromal edema and angiogenesis were found to be positvely correlated with higher level of MMP-9 values. These findings were consistent over the follow up time of 1st,3rd and 6th month.

It was also found that the presence .of fibrosis was negatively correlated with the level of MMP-9

CONCLUSION:

Expression of MMP-9 in epithelium was positively and statistically significantly correlated with a higher number of inflammatory cells in nasal epithelium and mucosa.

A decreased number of inflammatory cells, decline in hypertrophy of the mucous glands indicate a positive effect of treatment on the inflammatory process, which may also account for a more functional nasal epithelium and lower nasal airway resistance. This may also account for the post treatment improvement of symptoms and in quality of life.

Key Words:

MMP-9,histopathology

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INTRODUCTION

Headache and facial pain are one of the most common symptoms encountered in day to day ENT practice. One of the reasons for this is disease of sinomucosa which causes distress to the patient in carrying out their daily activities. Incomplete treatment of these diseases leads to chronicity. Understanding the pathogenesis and the changes occurring at molecular and histological level in the sinonasal mucosa can help in better outcome of treatment. One of the most common sinonasal disease is chronic rhinosinusitis. Approximately 134 million Indians suffer from chronic rhinosinusitis.[1]

Pseudostratified columnar epithelium lines the sinonasal mucosa . This epithelium has several number of goblet cells(~20%), ciliated cells (~75%) and basalcells (~5%) which are present on an acellular basement membrane. Healing of wound is an exceptionally organised process, including inflammation ,coagulation, proliferation of cell, remodelling or matrix deposition or coordinated by a wide assortment of growth factors and cytokines .

The standard defination of Chronic rhinosinusitis , inflammation of the nose and paranasal sinus mucosa persist for > 12 weeks without reaching normal levels even in intervals free of acute episodes.

Neutrophils, macrophages, eosinophils and lymphocytes are the trademark inflammatory cells present in the mucosa of the sinus. Inflammatory process ultimately leads to thickening of the sinonasal mucosa, fibrosis and impediment of the osteomeatal complex. Since the mid 1990s, different chemokines and cytokines in various sorts of rhinosinusitis have been tended to in vitro and in vivo investigations for a superior comprehension of the basic mechanism of CRS.

Recently, MMP-9 were found to play a significant role in inflammation, normal physiologic tissue remodeling and tumour spread. MMP are a group of Zinc dependent endopeptidases, with more than 20 known members, they are able to degrade almost every component of the extracellular matrix in normal physiological process.

Functional endosopic sinus surgery(FESS) insignificantly remains the most generally acknowledged treatment for CRS and Nasal polyp after failure of therapeutic treatment. FESS intends to clear diseased mucosa and to reestablish both drainage and ventilation of the sinus cavities. However the functional outcome is affected by healing quality of the tissue. Defects in the healing quality of the respiratory mucosa results in infection or synechiae, making revision surgery necessary.

AIMS AND OBJECTIVES

1. To study & compare the histopathology of sinonasal mucosa before and after endoscopic sinus surgery of sinonasal diseases.
2. To study the inflammatory changes and Matrix Metalloproteinase-9(MMP-9) protein concentration before and after endoscopic sinus surgery of sinonasal diseases.
3. To study if Neutrophil derived MMP-9 helps in predicting the healing quality after sinus surgery.

REVIEW OF LITERATURE

In 1992 kinnedy prefaced his classification saying that there is only a limited understanding of the etiology , pathology and prognostic factor involved in inflammatory disease.

Messerldinger identified ventilatory defect in the middle meatus, anterior and middle ethmoid in patient with chronic and recovering sinuses infection .

Due to anatomic malformation the area of persistent mucosal contact underwent inflammation and hyperplasia ultimately leading to infection. Mucociliary clearance was disrupted due to this inflammation and resultant in poor drainage of the frontal and maxillary sinus leading to potential site for recurrent infection of the sinuses .The endoscopic examination of nose and sinus help in determining the requirement of surgical intervention or not. Therefore diagnostic nasal endoscopy has become a routine procedure for the evaluation of sinonasal disease .

By the 3rd of this century FESS for the treatment of acute as well as chronic sinusitis was well established .It depended on the admirable anatomical studies of Zuckerkandl, onodi and Grunwald. Transnasal endosopic sinus medical procedure was presented in the mid 1980s.The term FESS was instituted by Kennedy and Michael in the mid 1990s.

"The concept of FESS is the removal of tissue obstructing the osteo meatal complex and the facilitation of drainage while conserving the normal non obstructing anatomy and mucous membrane." The rigidfibre optic nasal telescope gives wonderful intraoperative representation of the Osteomeatal complex, enabling the medical procedure to be focused definitely on the key zones .The picture can be anticipated on to a TV screen through a little camera joined to the eye bit of the endoscope .

Microdebriders evacuate the necrotic tissue while safeguarding ordinary mucosa. In the course of recent years the endoscopic sinus medical procedure has been generally utilized as a protected and compelling treatment of paranasal sinus issue. Powered instrumentation and stereotactic picture guided medical procedure have improved proficiency and wellbeing of this technique. Endoscopic ways to deal with the considerate nose tumours, sinus, front cranial fossa and the circle are presently ending up generally settled. The mix of suction with powered dissection has changed the endoscopic sinus medical procedure. Anyway the potential for inconvenience has shadowed the strategy. Endoscopic sinus medical procedure displayed a progression of inconvenience in the late 1980 and mid 1990s.however new innovation of instrumentation has created undisputable advances.

INDICATIONS OF FESS

1. Recurrent sinusitis
2. Chronic sinusitis refractory to medical treatment
3. NP (Nasal polposis)
4. AC polyp(Antrochoanal polyp)
5. Excision of selected tumours
6. Sinus mucoceles
7. Orbital decompression etc

J.B watelet et al., (2006) did a study on 23 patients, biopsies were collected from patients undergoing FESS for CRS and nasal polyposis.MMP-9 expression was correlated with healing quality. Conclusion was MMP-9 predicts healing quality after sinus surgery.[5]

Peter W Hellings (2009) suggested that endoscopic sinus surgery for CRS is most often successful for sinonasal symptoms, but may also improve bronchial symptoms and reduce medication use for bronchial asthma.[7]

Neil Bhattacharyya (2011) did a study on histopathology of sinus mucosa and concluded that although pathologic inflammation of the paranasal sinuses is inherent to CRS, increasing pathology severity at endoscopic sinus surgery does not predict poorer symptomatic outcome after FESS.[8]

Fatih Kemal soy et al., (2013) came to a conclusion after doing a study on 57 pts that for most of the patients quality of life significantly improved after surgery. Mucosal eosinophilia did not correlate with the absolute change of the rhinosinusitis disability index.[9]

Argyro J bizaki et al., (2016) after a RCT study on sinonasal mucosa suggest that chronic rhinosinusitis reveals findings consistent with inflammation, absence of cilia, metaplasia of epithelium, Thickened epithelium, angiogenesis, hyperplasia of mucosal glands and increased inflammatory cells were observed in the majority of samples which were collected preopratively. Hyperplasia of blood vessels hypertrophy of the mucosal glands and mucosal edema decreased after balloon sinuplasty and uncinectomy. In epithelium expression of MMP-9 was found to be strongly and significantly correlated with a increase number of inflammatory cells in nasal epithelium and mucosa.[10]

ANATOMY OF NOSE AND PNS

INTERNAL NOSE

Internal nose is divided by nasal septum into right and left nasal cavities. Each nasal cavity is consists of skin lined portion which is known as vestibule and mucosa lined portion which is nasal cavity proper . Nasal cavity is bounded by medial wall ,lateral wall , roof and a floor . Anterior 3/4 of floor is formed by palatine process of maxilla and posterior 1/4th is formed by horizontal process of palatine bone .Nasal septum form the medial wall of the nasal cavity which consists of columellar septum, membranous septum and septum proper .

Lateral wall is formed by

- Ascending process of maxilla
- Nasal bone
- Ethmoid
- Medial part of maxilla
- Inferior turbinate
- Perpendicular plane of palatine bone
- Medial pterygoid plate

Lateral wall has 3 bony projection called turbinates /concha

1. Superior (part of ethmoid)
2. Middle (part of ethmoid)
3. Inferior (separate bone)

Sometimes a 4th turbinate may also be present which is known as Concha Supreme . Below and lateral to each turbinate is corresponding meatus.

OSTEOMEATAL COMPLEX

The middle meatus lies in the space below and lateral to the middle turbinate which is often functionally referred to as the OMC .The anterior ethmoid , maxillary and frontal sinuses drains through this meatus . This meatus is most commonly involved in the pathophysiology of CRS.

BULLA ETHMOIDALIS

It is the most largest and constant anterior ethmoid air cell . It is located within the middle meatus directly posterior to the uncinate process and anterior to the basal lamella of the middle turbinate .

HIATUS SEMILUNARIS

It is a crescent shaped gap between the uncinate process and the anterior wall of the ethmoidal bulla .

ETHMOIDAL INFUNDIBULUM

It is a passage through which the secretion from various anterior ethmoidal cell , maxillary sinus are channeled in to middle meatus .

UNCINATE PROCESS

It forms the floor and medial wall of infundibulum .

PARANASAL SINUSES

There are filled space present in skull bone and directly communicates with the nasal cavity through their ostia, there are 4 on each side.

Anterior group - Frontal, maxillary and ant ethmoidal.

Posterior group - sphenoid and posterior ethmoid

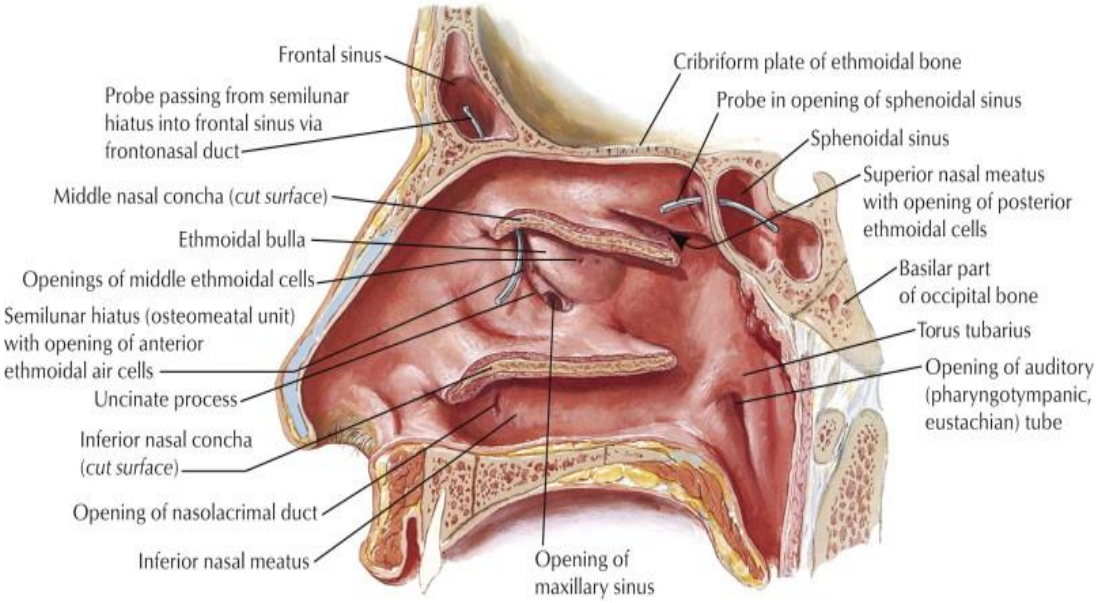


Fig 1:Lateral wall of nose

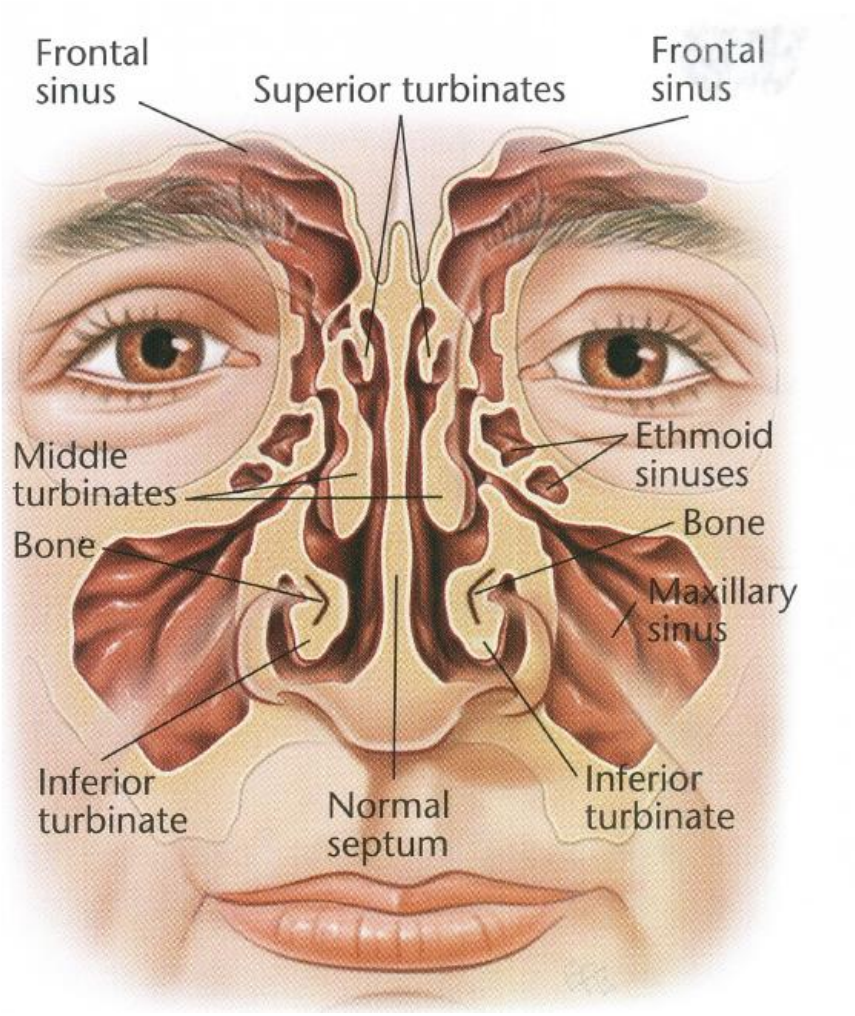


Fig 2 :Frontal sinus, Ethmoid sinus and maxillary sinus

MAXILLARY SINUS

1. pyramidal shape sinus
2. It is the largest paranasal sinus
3. capacity is 10-20ml.

It is bounded medially by nasal cavity posteriorly by pterygopalatine and infratemporal fossa .Anteriorly by soft tissue of cheek.

FRONTAL SINUS

It is present between outer and inner table of frontal bone. Capacity is 5-10ml. Anteriorly bounded by outer table of frontal bone and posteriorly by inner table of frontal bone . Medially septum present between two frontal sinuses. Floor is by thin bone separating sinus from orbit.

ETHMOIDAL SINUSES

It is a thin walled air filled cavities present in the lateral masses of ethmoid bone. It is divided in to anterior and posterior group . Lateral wall is formed by lamina papyracea. Medially by superior and middle turbinate, roof is formed by anterior cranial fossa. Inferiorly lies the maxillary sinus. Medially bounded by superior and middle turbinate. Posteriorly bounded by sphenoid sinus.

SPHENOID SINUS

There are 2 sphenoid sinus which are divided unequally by a thin bony septum

RELATION

1. Superior pituitary gland is present.
2. Inferiorly by nasopharynx andvidian nerve.
3. Laterally by cavernous sinus.
4. Posteriorly by brainstem.

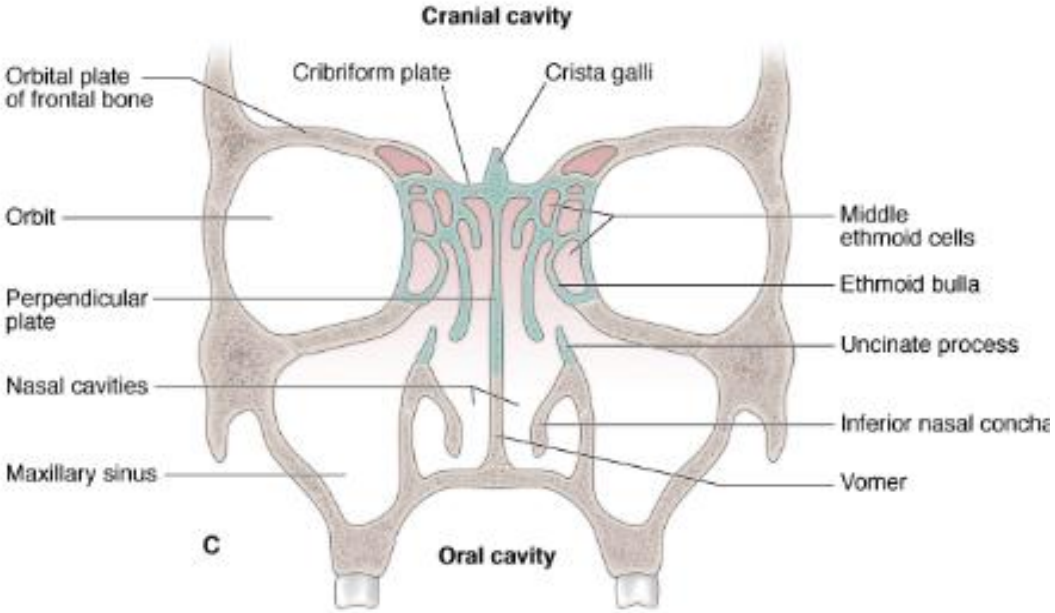


Fig 3:Relation of the maxillary sinus

FUNCTIONS OF NOSE

1. Respiration.
2. Protection of lower airway.
3. Air conditioning.
4. Vocal resonance.
5. Olfaction.
6. Nasal reflex function.

The epithelium of upper airway has a significant role in regulating the inhaled air. The paranasal sinuses is lined by pseudostratified ciliated columnar epithelium which has goblet cells, and various mucus and serous glands. Immunoglobulins, interferons and lysozymes are secreted by serous mucous glands.

DEVELOPMENT

At eight weeks- the nasal cavity begins to form, a hypercellular mesenchymal case conforms to creating nasal structures. In spite of the fact that most of the nasal cavity is comprised of stratified cuboidalcells or undifferentiated cells , the nasal septum is partially differentiated into cartilage as of now, in the roof of the nasal cavity and olfactory epithelium is present .

By nine to ten weeks-the cartilaginous nasal part has differentiated completely, cuboidal epithelium or ciliated pseudostratified columnar epithelium is seen on inferior turbinate and the septum with primitive blood vessels.

Eleven to tweleve weeks-the epithelium of septum starts differentiating into ciliated respiratory epithelium and secretory goblet cells are found, yet the mucosa of lateral wall of nose continues to be less differentiated. The lining of the mucosa of the developing paranasal sinuses remains cuboidal or circular with less cilia and glands.

By seventeen-eighteen weeks-the lateral wall of nose and ethmoid sinus has matured to respiratory epithelium, anterior part has higher concentrations of goblet cells and posterior part has ciliated cells.

Twenty-twenty four weeks-secretory cells are all equitably disseminated and vascular structures are available all through the lamina propria, resembling devlopment of postnasal part.

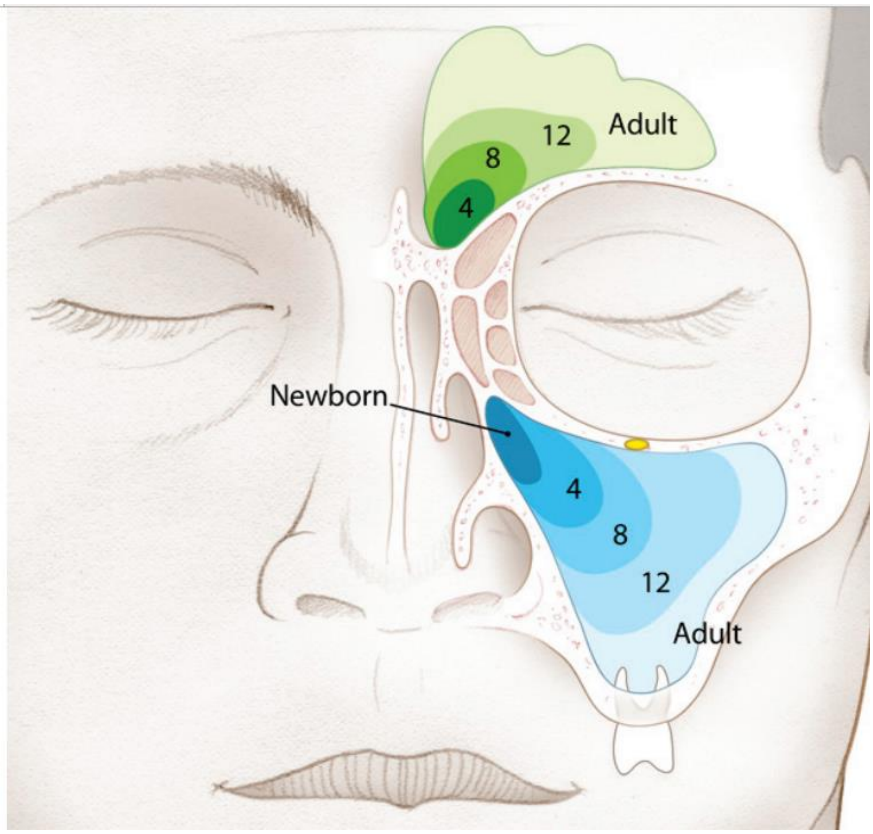


Fig 4:Maxillary and frontal sinus postnasal development

In remodeling, inflammation and tumor spread of normal physiologic tissue MMP were found to play a significant role. MMP-9 are Zn2+-dependant endopeptidases, with in excess of twenty known members, that helps in degrading almost every part of the extracellular matrix. Type IV collagen can be cleaved by active form of MMP-9 and MMP-2. The TIMPs helps in regualating MMPs extracellular activity.

In lung ailments The MMPs and TIMPs plays a significant role . In transgenic creature concentrates raised degrees of MMP-9 and MMP-2 are related with bronchial architecture defect .Eosinophils produces MMP-9 which results in inflammation of the airway in bronchial asthma. In spite of the fact that Interleukin 5 is a important cytokine in Nasal polyposis and eosinophils acts as a source for MMP-9 directly stimulates eosinophils by IL-5,it did not result in increase in MMP-9 production .

Raised levels of MMP-9, MMP-2 and MMP-7 were found in Nasal polyp. Moreover, MMP-9 was found to predict quality of healing of nasal mucosa after sinussurgery . A few cytokines are known to affect the generation of MMP-2 ,MMP-9, and TIMP-1. In epithelium of respiratory tract, IL-8 appears to have the capability of activating of MMP-9 during regeneration of airway epithelium . IL-8 moreover has the capacity to initiate MMP-9 and MMP-2 creation in endothelial cells .

CRS is a common disease affecting the quality of life of the patient. Because of its expanding commonness, CRS majorly contributes in socioeconomic burden. The pathology depends on the chronic mucosal inflammation of the PNS, which results in secretion, stasis and bacterial infection. Chronic rhinosinusitis can be isolated into those in which NP's are absent and those in which polyps are present. Nasal polyposis occurs due to chronic inflammation of the nasal mucosa wherein the invasion of goblet cells ,epithelial changes and edema are often observed. Nowadays, ESS is most frequently used to treat CRS and NP. Nasal polyposis is viewed as a subgroup of CRS . Mucosa of the sinus in nasal polyps (NP) has massive stromal edema, which comprises of eosinophils, plasma cells and lymphocytes, modifications of the overlying epithelium and at times, submucosal and seromucous gland hyperplasia .Several chemokines and cytokines have been identified in higher levels in chronic sinusitis and NP.

THE MUCOSA

When pathogens and debris measuring >0.5-1mm passes through the first tiers of nasal defense gets trapped in the mucus blanket of the sinus and nose. The mucosa of the sinus and nose acts as a defense upper airway and sinus from pathogens , debris, and toxins .Mucociliary clearance is one of a kind defensive epithelium of the airway. Mucociliary clearance which includes mucus production

and transport, expels both kind of secretions healthy as well as pathological debris from the sinonasal airway. Despite the fact that the cough and wheeze may help in mucus clearance from the upper and lower respiratory tract respectively, mucociliary clearance remains the main defense of the paranasal sinuses and respiratory tract.

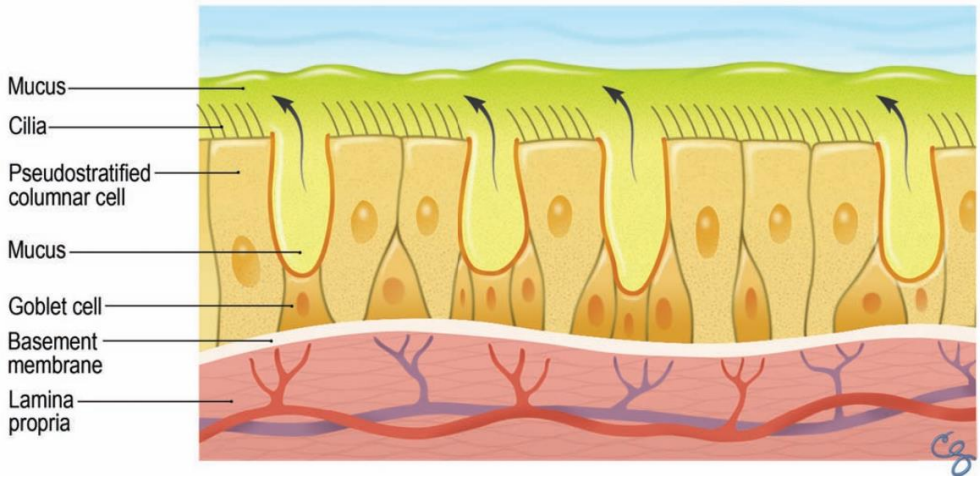


Fig 5: The sinonasal mucosa with all the layers

"The superficial layer epithelium of the nasal mucosa consists of an acellular basement membrane, goblet cells, a thick lamina propria containing vascular and glandular layers and the periosteum ". The nasal epithelium is specific to suit its specific region. The nasal vestibule's anterior border is made up of stratified squamous epithelium, which consists of sweat glands, sebaceous glands, and finer hair(vibrissae). Like the skin of the rest of the face ,the stratified squamous epithelium acts as a protective layer. The stratified squamous epithelium converts to the pseudostratified columnar ciliated epithelium near the nasal valve region which is found all through the nasal cavity (except for the olfactory epithelium). This area where epithelium undergoes transition is one to two mm in thickness, overlies anastomosis of several blood vessel. This anastomoses lead to Kiesselbach's plexus or Little's area, which is a very common area for nasal bleed.[13]The area of transition increases as the age of the individual increases.

The columnar epithelium in the anteroinferior may change to squamous metaplasia due to years of exposure to the inspired airflow. Therefore squamous epithelium area increases as the individual ages. The sinonasal mucosa comprises of 80% of columnar epithelium,20% of goblet cells and less than 5% of basal cells. The epithelium of nasal cavity is most commonly made up of psuedostratified epithelium which means it has both non ciliated and ciliated cells.

The epithelium of paranasal sinus mostly is simple cilliated columnar epithelium.This epithelium is made up of several immotile microvilli, which are tiny actin filaments and measures about one to two milimeter in length.There is an increase in total surface area of columnar cells which helps in increased sensation and mucus secretion.The goblet cells are present throughout the columnar cells.This cells produces mucus through secretory granules that contain mucin,which gives the elastic

and viscous characteristic of the mucus.The goblet cell has a small opening via which the mucin comes out in the cavity of nose.

By hemidesmosomes basal cells are adhered to BM and are safeguarded from the contaminated air of the sinonasalcavity.This cells acts as a progenitor cell which gives rise to different cells like cilliated columnar or goblet cells.3 types of intercellular junction are present in sinonasal mucosa.

1. Adherens junction-binds the epithelial cell to BM.
2. Tight junction-protective function doesnot allow pathogens to pass.
3. Gap junction-small opening which connects the cytoplasm of neighbouringepithelial cell.It helps in ion exchange and coordination of cillia.Under the thin BM lies the LP which consists of blood vessels, nerves and glands of sinonasal mucosa. LP has 3 layers
4. Deep glandular layer
5. vascular layer
6. Superficial glandular layer

Serous glands keeps the nasal cavity moisturized and are found on the ant. part of nasal septum and lateral wall of nose. Serous as well as mucus is produced by seromucinous glands.The nerves(PS fibres) present in the LP arises from the SSN of brainstem and are carried by Nervus intermedius which is a branch of FN to the GSPN.

From the sympathetic trunk arises the sympathetic fibres which synapses in the sup cervical ganglion and then it is carried by the DPN. The GSPN and DPN combine and form vidian nerve, which carries the ANS supply through the pterygopalatine ganglion, where the PS fibres synapse and finally autonomic fibres reach the mucosa by the 5th cranial nerve(V2).The PS fibres of the LP stimulates the

glandular secretions. The sympathetic fibres of LP plays a important role in constriction of vessels, decongestion and also in nasal secretion regulation.

MUCUS

Cellular debris, pathogens and particulate matter from the air breathed in gets stuck in the mucus overlying the epithelium.It consists of 2 layers

1. The gel phase-outer layer is viscous that slides along the tips of extended cillia but is not continuous.
2. The sol phase- inner layer is continuous which is of less viscosity and is present around the shafts of cilia and is composed of electrolytes like sodium, potassium, calcium, chloride and water.

"Mucus is an immunologically active substance made out of proteins and peptides(2–3%), water (95%), salts (1%), and debris (1%), with a somewhat acidic pH of 5.5 to 6.5.[17] Its liquid substance incorporates fluctuating groupings of plasma exudate, submucosal and challis cell emissions, tears and lacrimal organ discharges, and serous emissions from the olfactory organs of Bowman."

Sinonasal mucosa produces 600-1800 ml of mucous per day nearly.[17] Mucin proteins are glycoproteins which has oligosaccharides side chains and peptides.
Initially it is produced in condensed form but later undergoes hydration and forms a gel.It also has a significant role in protection of nasal airway. " Their carbohydrate side chains seem to bind surface adhesins on microorganisms, and acknowledgment destinations on side chains have been portrayed for adhesins of Pseudomonas aeruginosa, Mycoplasma pneumoniae, influenza, Streptococcus pneumoniae and Escherichia coli.[18] Therefore, mucin-related carbohydrates may serve an extra defensive system by firmly official and successfully clearing microorganisms that regularly colonize the upper airway routes".

Mucins combines to various endogenous proteins like lysozyme and lactoferrin, for defense mechanism.[19] Neutrophils stimulates goblet cells to secrete mucin during acute inflammation. Lysozyme acts as a protective enzyme by helping in the hydrolysis of bacterial cell wall and are most effective against G+ bacteria.

Lactoferrin is a type of protein which has a high affinity towards iron that has an immune modulatory and antimicrobial activities. Lactoferrin deprives local bacteria and fungi by strongly binding to iron. Lipopolysaccharides directly damages the cell wall.[15]

Surfactant proteins (SP-A and SP-D) and mannose-binding protein are collectin proteins which has antimicrobial properties and interact with several bacteria such as Staphylococcus species, P. aeruginosa, Streptococcus species, E. coli, K. pneumoniae, Aspergillus, Mycobacterium tuberculosis, and Salmonella species.[20] These bacteria have pathogen-associated molecular patterns (PAMP) that consist of polynucleotides and polysaccharides , such as lipopolysaccharide (LPS)[20]MALT present in the inferior and middle turbinates generates IgA and IgG which acts as a protective barrier of sinonasal mucus membrane.

IgEplaysa important role in allergic reaction. IgM is present in less concentration. IgM is one of the most important antibody present in sinonasal secretions and its deficiency may lead to repeititive sinus and pulmonary infections. This antibody stimulates the complement pathway .IgG also helps in phagocytosis and has less important role here.

THE CILIARY STRUCTURE AND FUNCTION

Pathogens and debris trapped in the mucus blanket in the URT and LRT are cleared by respiratory cilia by beating in a rhythmic and coordinated manner. Cillia protrudes from the upper part of epithelial cells and are attached to the intracellular basal border.50 to 200 cillia present in per epithelial cell, each cillia measuring 5-7mm (length) and 0.2 to 0.3mm (diameter).[17]Each cilium is made up of a interconnect microtubules bundle known as axoneme .This microtubules are composed of protofilaments, which are made up of alpha and beta dimers.

The significant beta-tubulin present in cilia is the sort 4 isotype, twenty seven which is substantially more bounteous in the cilia than somewhere else in the cell and is a perfect marker for respiratory cilia in the exploration setting. "The axonemes of motile cilia contain 2 central singlet microtubules encompassed by 9 doublet microtubules. Every doublet comprises of 1 alpha-tubule, a total hover of 13 protofilaments, and 1 beta-tubule—a fragmented hover of 10 protofilaments. This structure of axoneme is protected over the motile cilia of the URT epithelium, the oviduct, and the ventricular ependymal cells".The two focal microtubules are appended by matched scaffolds, while the fringe doublets join to the focal pair by means of spiral talked heads. Through inward IDA, ODA, and nexin,each outer doblet interacts with the adjacent outer doblets, each having an significant job in the dynamic movement of cilia bending.[28]

Activation of dynein arms creates a sliding movement of 1 microtubule doublet against the adjacent doublet.

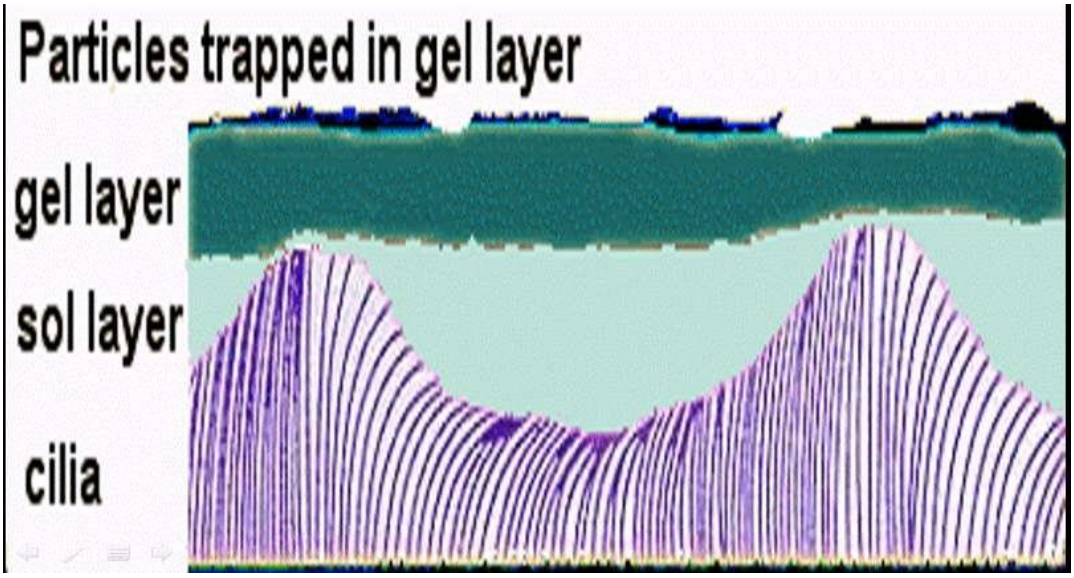


Fig 6:Ciliary Mechanics

In the Chlamydomonas,frequency of cilia beat is regulated by phosphorylation of ODA and the waveform of pattern beating is regulated by the phosphorylation of IDA.

During the ciliary stroke, radial spoke heads restricts the sliding between the microtubules in this way changing over the sliding movement created by the dynein arms into a bowing movement of the axoneme. Every cilium has a forward power stroke followed by a recovery stroke. cilium is completely broadened during the power stroke and at the apogee of the bend, the distal tip reaches the thick external outer mucus layer(gel stage), in this manner transmitting directional power to the overlying mucus blanket. During the recovery stroke, the cilium bends 90 degrees and comes back to its initial point inside the periciliary liquid layer (the sol stage).

The component of ciliary movement relies upon a progression of ATPdependent sub-atomic engines that reason the external doublets of the axonemeto slide in respect to one another, creating avectorial power. The focal pair of microtubule singlets separates the axoneme into two restricting parts.As proposed by the “switch point” hypothesis, "the dynein motors on one side of the axoneme are

predominantly active during the effective stroke, whereas the motors on the other side are mainly active during the recovery stroke. If the microtubules are numbered in a clockwise fashion from 1 to 9,the effective stroke would involve the ODAs on the 9–1-2–3-4 microtubules, and the recovery stroke would involve activity of the dynein in the 5–6-7–8 microtubules.[24] The dynamic force of each power stroke is directly proportional to the number of dynein-microtubule interactions and there is usually a physiologic reserve available to increase the force of the stroke when necessary.[33] The orientation of the stroke is determined by the orientation of the anchoring basal body of the axoneme" .

Once a metachronous wave is established spontaneous beating of cilia is 9-15Hz in humans, velocity is 600 - 1,000 mm/s or 3 - 25 mm/min .In 10 mins cilia can clear the mucus blanket of paranasal sinus and nose.CBF is affected by

1. chemical
2. thermal
3. machanical
4. hormonal stimuli
5. extracellular and intracellular pH

CBF increases with increase in intracellular pH and decreases with deccrease in intracellular pH.Optimum temperature for CBF is 32° to 37°C.Direct mechanical stimulation of cilia increase the CBF due to increase in intracellular calcium.

SINONASAL DISEASE

CHRONIC SINUSITIS

It is the inflammation of the mucosal lining of one or more paranasal sinuses,usually caused by anatomical/pathological obstruction to its drainage,and is characterized by chronic postnasal mucopurulent discharge with or without recurrent headache/facial pain(more than 1 month).

Etiologic factors in Rhinosinusitis

Systemic Host factors

1. Innate Immunity

It exist at the surface of mucosa as defense mechanism against pathogens present in the environment. Activation of Toll-like receptors(TLR)which are pathogen recognition receptors are expressed on dendritic cells, macrophages and epithelial cells stimulates the innate immunity. Various altered expression of innate effectors such as antimicrobial cathelicidin, serum amyloid A, surfactants and beta defensins are present in the sinonasal epithelial cells of patients with CRS with or without polyps.

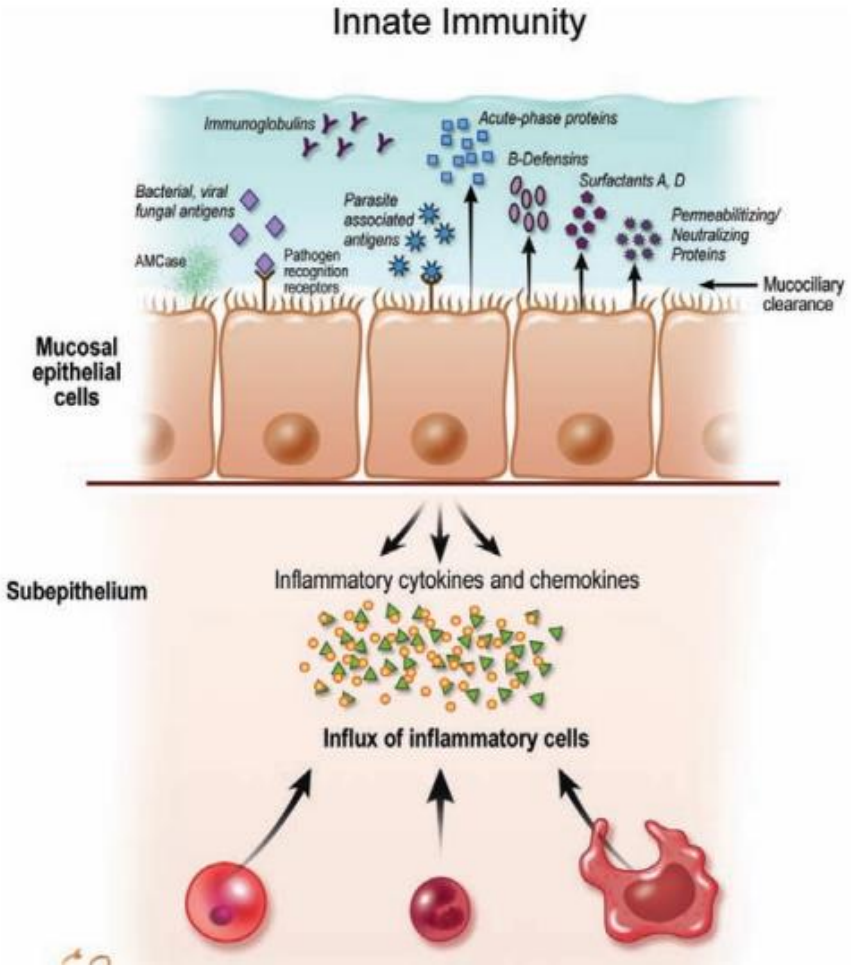


Fig 7:Innate immunity

2. **Acquired/Adaptive Immunity**-It is also a defense mechanism against environment pathogens where dendritic cells ,B cells andT cells are involved.These cells are present near the epithelial cells of the mucosal surface and are in contact with antigenic material.The dendritic cell activate T cells which in turn brings the basophils, eosinophils and neutrophils to the mucosal surface with the help of chemokines and inflammatory cytokines.
3. **Airway hyperactivity**-The irritants in the upper airway and nasal cavity causes bronchoconstriction by nasobronchial reflex. This is mediated by efferent branches derived from 10th cranial nerve and afferent branches derived from 5th cranial nerve.

4. **Allergy**- "In 1992,Suzuki and colleagues demonstrated an increased prevalence of allergy among CRS patient and also found higher levels of eosinophils and inflammatory cytokines in the sinus fluids of allergic patients who concurrent had CRS."[44]
5. **Aspirin Intolerance**-Samter's triad which is a combination of aspirin sensitivity,asthma and nasal polyposis.It may be due to disorder in arachidonic acid metabolism which leads to overproduction of leukotrienes which produces bronchoconstriction.
6. **Mucociliary Dysfunction**-Primary ciliary Dyskinesia like Kartagener syndrome which is a clinical triad of sinusitis,bronchiectasis and situs inversus(dextrocardia).
7. **Immunodeficiency**like HIV, IgA deficiency, Transporter associated with presentation deficiency syndrome.[47]
8. **Granulomatous diseases**-Wegener Granulomatosis,sarcoidosis and churgstrauss syndrome can cause chronic nasal inflammation.Wegener involves the paranasal sinuses, nose, lung and kidney.
9. **Cystic fibrosis**-Mucous is viscous in patient with cystic fibrosis which results in impaired mucociliary clearance, mucosal edema and local inflammation of tissue.[49]

Local host factors

1. Odontogenic inflammation like dental implants,periodontal diseases ,dental extraction and dental abscess may lead to chronic sinusitis
2. Anatomic Abnormalities One of the major cause of chronic sinusitis is structural and anatomical anomalies of the sinonasal cavity.[51]For proper sinus functioning,the outflow tract should be patent which allow mucociliary

function and clearance of mucus from particulates and pathogen. So any anatomical variants like DNS, conchabullosa, Hallercells, craniofacial abnormalities, traumatic obstruction or narrow outflow tracts lead to chronic sinusitis.

3. Bone inflammation -Recent studies have suggested that CRS may be due to constant inflammation of mucosa which is secondary to inflammation of the underlying bone.[55]
4. Acquire mucociliary Dysfunction
5. GERD

Environmental factors

1. Viruses-for eg Rhinovirus
2. Bacteria like staph epidermis,staphaureus,alpha -gamma streptococci and propionibacterium acne
3. fungi-plays a prominent role in CRS
4. Air pollution
5. Smoking

PATHOGENESIS

Usually Rhinogenic and unresolved acute sinusitis

Any form of rhinitis which leads to mucosal odema in OMC and ultimately results in pathological obstruction

Any anatomical variation which leads to anatomical obstruction Stagnation and secondary chronic sinusitis

Primary mucociliary dysfunction

*Anterior ethmoids is the key area for causation of chronic anterior group sinusitis because

Ostiomeatal complex is situated within it which acts as reservoirs of infection

Bacteriology

Mixed infection streptococcus pnuemoniae, haemolyticus, Staphaureus,gram negative bacteria etc

Anaerobic infections> foul smelling discharge

Clinical features

Mucopurulent/purulent post nasal discharge

Cachosmia

Headache/facial pain-depending on the site and type-usually dull aching

Nasal obstruction

Aural and throat symptoms

SIGNS

Discharge in the middle meatus on anterior rhinoscopy

Mucosal changes in the middle meatus

Discharge in middle meatus/superior meatus on posterior rhinoscopy

Postnasal drip

INVESTIGATIONS

Plain radiographs "Water's view"

Mucosal thickening, haziness, opacity, polyp

CT scan of OMC/paranasal sinuses(coronal cuts)

Diagnostic nasal endoscopy

Treatment when refractory to medical line of treatment then FESS is done

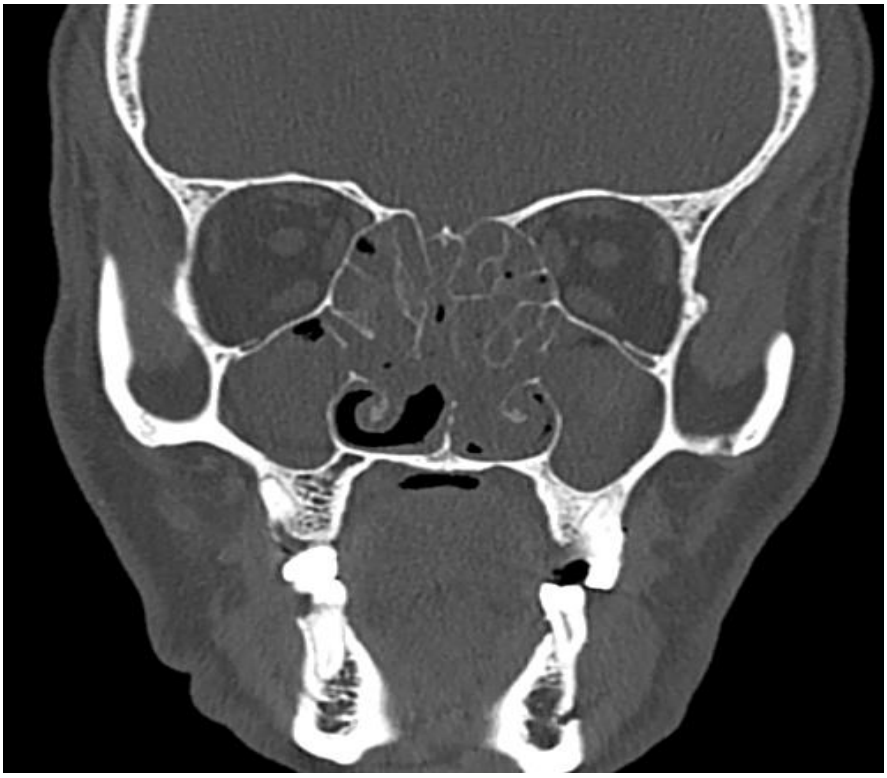


Fig 8:CT PNS coronal section showing bilateral ethmoid and maxillary sinusitis

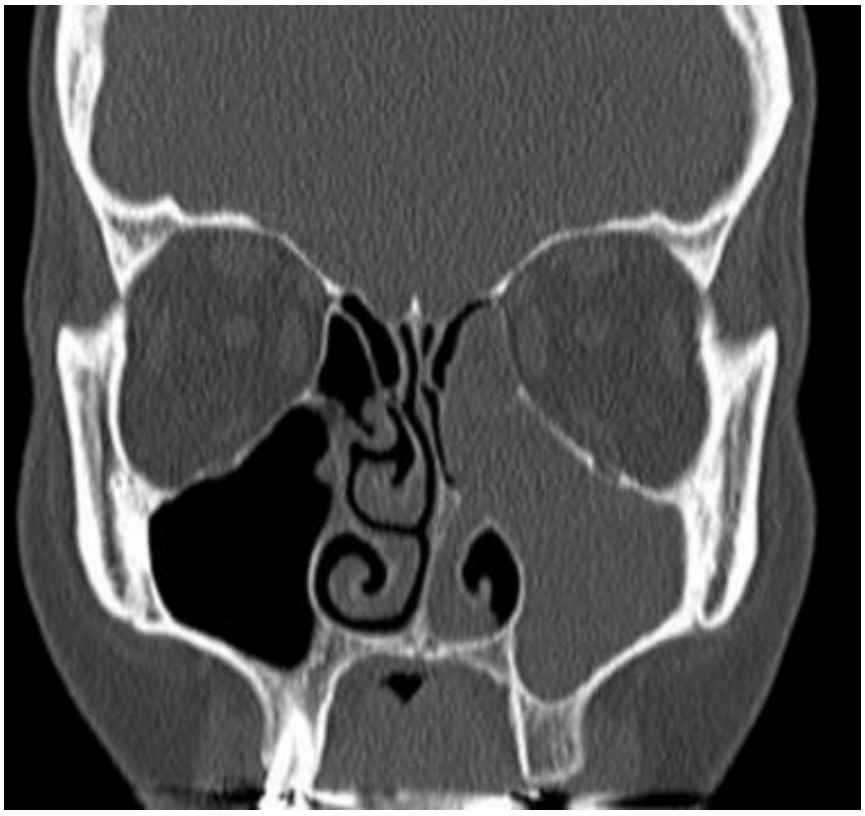


Fig 9:CT PNS coronal section showing left Antrochoanal Polyp

MATERIALS AND METHODS

SOURCE OF DATA:

Patients satisfying the inclusion and exclusion criteria of patients admitted to the department of otorhinolaryngology, _____

_____

PERIOD OF STUDY:

November 2017 to June 2019.

STUDY DESIGN:

Cross sectional study.

SAMPLE SIZE: 55 cases

STATISTICAL ANALYSIS:

Data will be analyzed by

Dependent‘t’ test

One way ANOVA

Post hoc test

Correlation coefficient.

INCLUSION CRITERIA:

Patients diagnosed with any sinonasal diseases like chronic or recurrent rhinosinusitis , nasal polyposis(antrochoanal polyp),atrophic rhinitis .Patients who are in the age group of 18 to 65 years.

Patients who fulfills the indications for endoscopic sinus surgery.

EXCLUSION CRITERIA:

Previous sinus operations.

Asthma or frank allergy.

Acetylsalicylic acid intolerance.

Diabetes, HIV or any immunocompromised state.

Allergic polyposis.

At the time of enrollment to the study if patient is pregnant.

Habits of smoking

Proven malignancy of nose.

METHOD OF COLLECTION OF DATA

Pre-operative examination of the patient including the complete clinical history of patient was taken.

Detailed examination of the patient with emphasis on detailed diagnostic nasal endoscopic (DNE) findings was done .

Patients were subjected to investigations such as urine routine and blood routine examinations. And especially CT scans of the PNS was done to evaluate the status of the PNS.

Two samples of sinonasal mucosa biopsies taken from the middle turbinate (preoperatively and postoperatively at 1st, 3rd and 6 month) and sent for histopathological study in 10% formalin and ELISA test in 0.9%NaCl .

Concentration of MMP-9 was measured by using commercially available ELISA kit.

EQUIPMENTS USED

1. Nasal endoscope: Endoscopes used were of 4mm diameters with view of 0,30,and 70 angles .Karl StorzHopkins rod optical with cold light source and fibre optic light delivery system.
2. Karl StorzEndovision Telecam deluxe camera system with monitor.
3. 4% Xylocaine with 1:100,000 adrenaline as topical decongestant and anaesthetic agent

4. Savlon as Antifog solutions.
5. Blakesley forceps, Suction apparatus and cannula,.

Position:

Patient is put on supine position withslightly elevated head which is turned towards the examiner standing at the right side of the patient.

Anaesthesia:

Topical decongestant 4% Xylocaine with 1:100,000 adrenaline solution using applicators like cottonoid strips.

Procedures:

Endoscopy was performed by three passes

1. **First pass:** To visualize the status of inferior turbinate and meatus,, nasopharyngeal mucosa, eustachian tube orifice ,nasolacrimal duct orifice and any pathology along the floor of nasal cavity towards nasopharynx .
2. **Second pass:** Scope was inserted along the superior surface of inferior turbinate. As the endoscope was withdrawn the sphenoid ostium, spheno-ethmoidal recess,and superior nasal meatus visualized.
3. **Third pass:** Middle meatus is examined in detail.

ELISA(Immunoassay)

1. For homogenization the tissue should be thawed, weighed and 1 ml of 0.9% NaCl was added per 0.1gm of tissue.
2. At 1000 rpm the tissue was then homogenized for 5 min.
3. The homogenates were centrifuged at 1500gm for 10 min at 40C.

4. Supernatants were a liquot in 250 UL and stored at -80 degree Celcius until analysis after centrifugation.
5. MMP-9 conc.were determined in supernatants using commercially available ELISA kits.

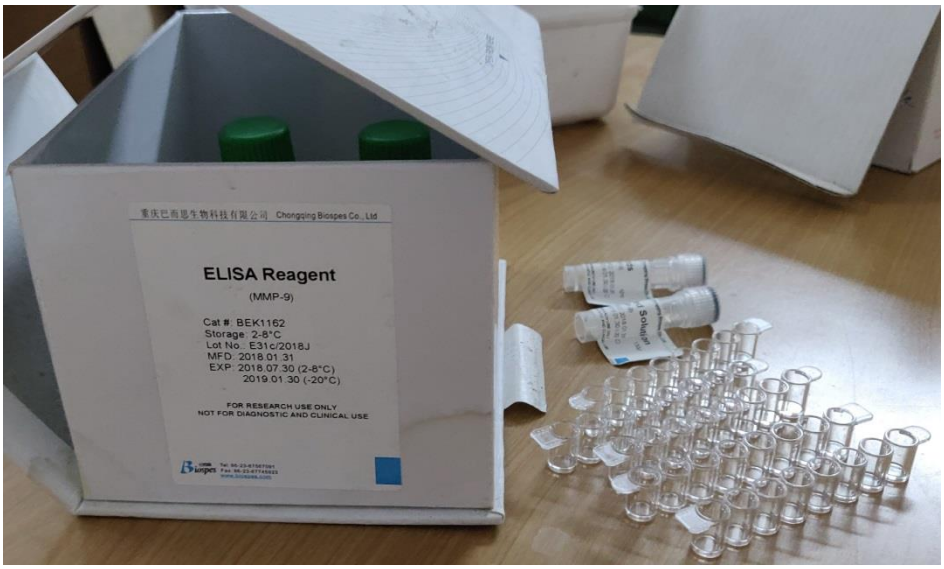


Fig 10: Showing MMP-9 ELISA kit which was used in the study

STATISTICAL ANALYSIS

Statistical analysis

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean±standard deviation (SD) were used. For categorical data, the number and percentage wereused in the data summaries and diagrammatic presentation. Chi-square (χ^2) test was used for association between two categorical variables. The formula for the chi-square statistic used in the chi square test is:

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

The subscript "c" are the degrees of freedom. "O" is observed value and E is expected value.

The difference of the means of analysis variables between two independent groups was tested by unpaired t test.

The t statistic to test whether the means are different can be calculated as follows:

$$t = \frac{(\overline{x_1} - \overline{x_2}) - (\mu_1 - \mu_2)}{\sqrt{\frac{{s_1}^2}{n_1} + \frac{{s_2}^2}{n_2}}}$$

where $\bar{x}_1$ = mean of sample 1

$\bar{x}_2$ = mean of sample 2

n_1 = number of subjects in sample 1

n_2 = number of subjects in sample 2

${s_1}^2$ = variance of sample 1 = $\frac{\Sigma(x_1 - \bar{x}_1)^2}{n_1}$

${s_2}^2$ = variance of sample 2 = $\frac{\Sigma(x_2 - \bar{x}_2)^2}{n_2}$

If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analyzed using SPSS software v.23.0. and Microsoft office 2007.

RESULTS

TABLE 1: DISTRIBUTION OF CASES ACCORDING TO AGE

AGE(YRS)	N	%
≤20	16	30.2
21-30	9	17
31-40	9	17
41-50	7	13.2
51-60	9	17
61-70	3	5.7
Total	53	100

	RANGE	Mean	SD
AGE(YRS)	18-65	36.4	16.5

GRAPH 1: DISTRIBUTION OF CASES ACCORDING TO AGE

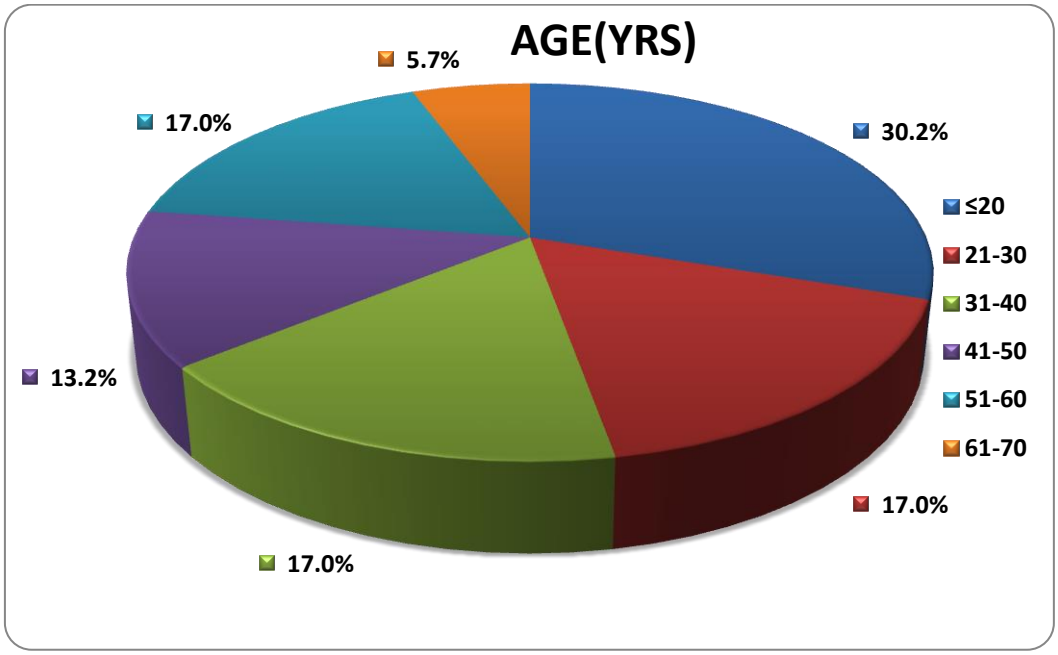


TABLE 2: DISTRIBUTION OF CASES ACCORDING TO SEX

SEX	N	%
MALE	21	39.6
FEMALE	32	60.4
Total	53	100

GRAPH 2: DISTRIBUTION OF CASES ACCORDING TO SEX

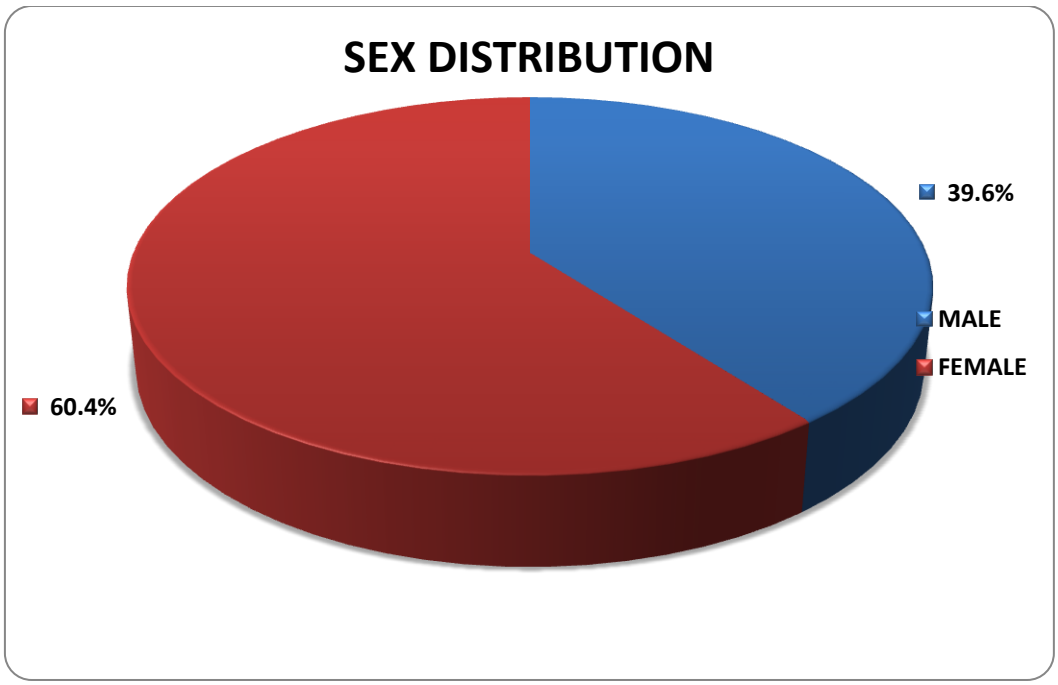


TABLE 3: ASSOCIATION OF AGE AND SEX

AGE(YRS)	MALE		FEMALE		p value
	N	%	N	%	
≤20	4	19.0%	12	37.5%	
21-30	4	19.0%	5	15.6%	
31-40	5	23.8%	4	12.5%	
41-50	3	14.3%	4	12.5%	0.755
51-60	4	19.0%	5	15.6%	
61-70	1	4.8%	2	6.3%	
Total	21	100.0%	32	100.0%	

GRAPH 3: ASSOCIATION OF AGE AND SEX

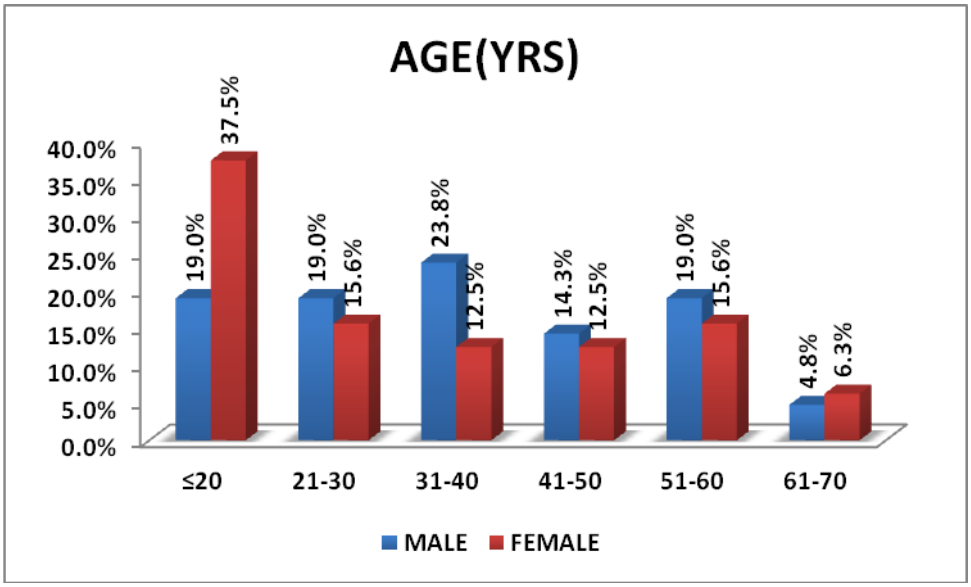


TABLE 4: DISTRIBUTION OF METAPLASIA ACCORDING TO TIME

METAPLASIA	PRE OP		1st mnth		3rd mnth		6th mnth	
	N	%	N	%	N	%	N	%
ABSENT	38	71.7	42	79.2	46	86.8	50	94.3
PRESENT	15	28.3	11	20.8	7	13.2	3	5.7
Total	53	100	53	100	53	100	53	100

GRAPH 4: DISTRIBUTION OF METAPLASIA ACCORDING TO TIME

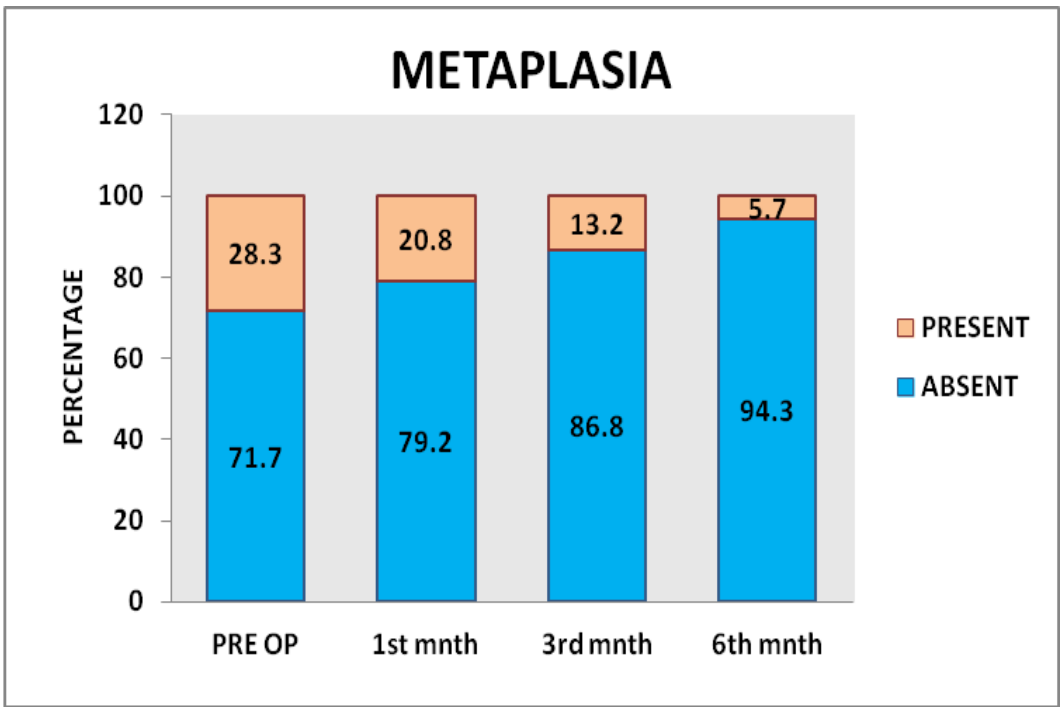


TABLE 5: DISTRIBUTION OF GOBLET CELLS ACCORDING TO TIME

GOBLET CELLS	PRE OP		1st mnth		3rd mnth		6th mnth	
	N	%	N	%	N	%	N	%
ABSENT	9	17	8	15.1	13	24.5	34	64.2
DECREASED	3	5.7	18	34	30	56.6	14	26.4
NORMAL	26	49.1	27	50.9	10	18.9	5	9.4
HYPERPLASIA	15	28.3	0	0	0	0	0	0
Total	53	100	53	100	53	100	53	100

GRAPH 5: DISTRIBUTION OF GOBLET CELLS ACCORDING TO TIME

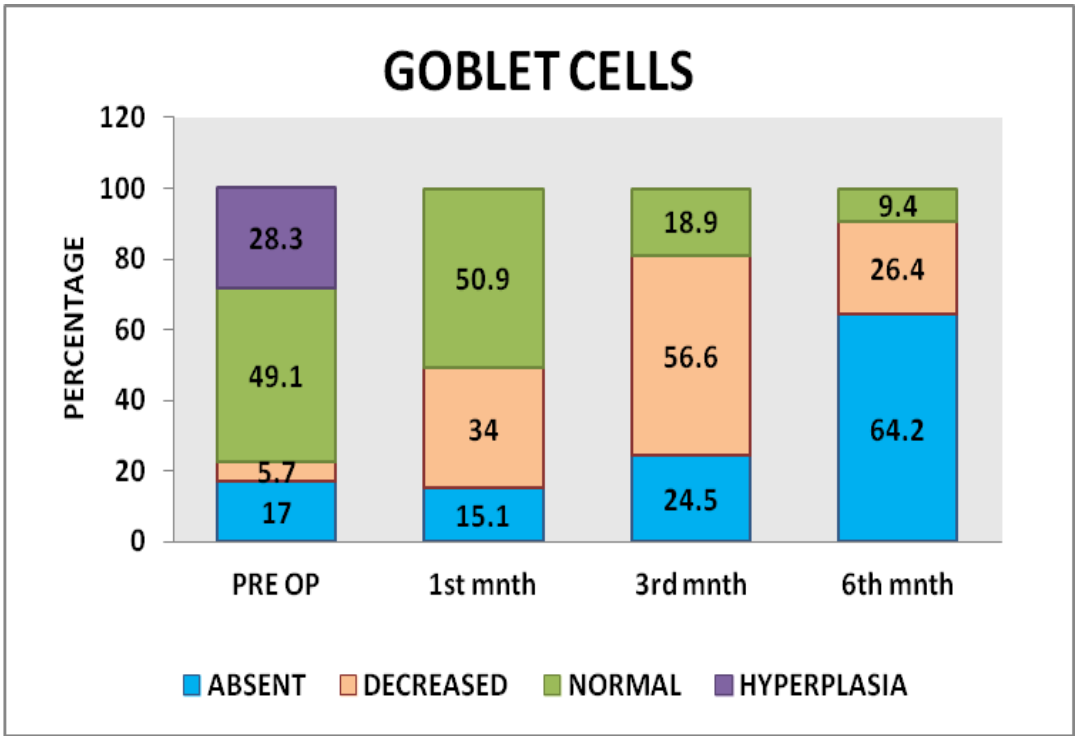


TABLE 6: DISTRIBUTION OF THICKNESS ACCORDING TO TIME

EPI THICKNESS	PRE OP		1st mnth		3rd mnth		6th mnth	
	N	%	N	%	N	%	N	%
THIN	3	5.7	3	5.7	4	7.5	10	18.9
NORMAL	16	30.2	42	79.2	47	88.7	43	81.1
THICK	34	64.2	8	15.1	2	3.8	0	0
Total	53	100	53	100	53	100	53	100

GRAPH 6: DISTRIBUTION OF THICKNESS ACCORDING TO TIME

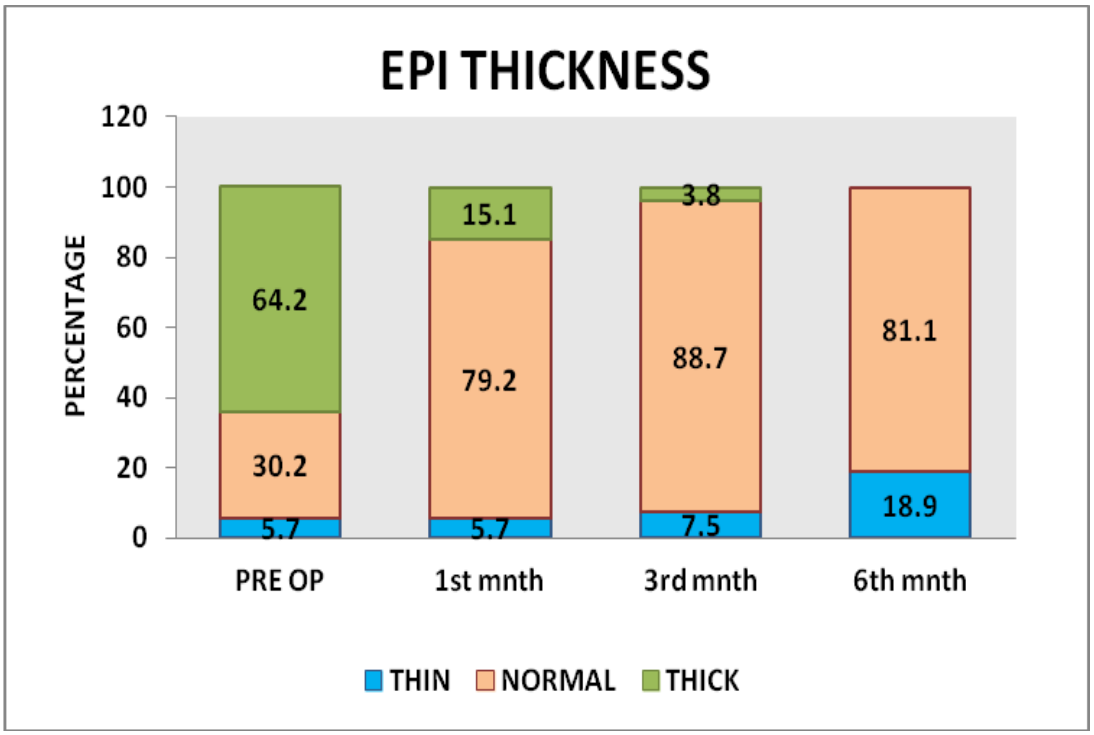


TABLE 7: DISTRIBUTION OF INFLAMMATORY CELLS ACCORDING TO TIME

INFLAMMATORY CELLS	PRE OP		1st mnth		3rd mnth		6th mnth	
	N	%	N	%	N	%	N	%
ABSENT	0	0	1	1.9	13	24.5	48	90.6
FOCAL /MILD	6	11.3	39	73.6	39	73.6	5	9.4
PATCHY /MODERATE	45	84.9	13	24.5	1	1.9	0	0
EXTENSIVE/MARKED	2	3.8	0	0	0	0	0	0
Total	53	100	53	100	53	100	53	100

GRAPH 7: DISTRIBUTION OF INFLAMMATORY CELLS ACCORDING TO TIME

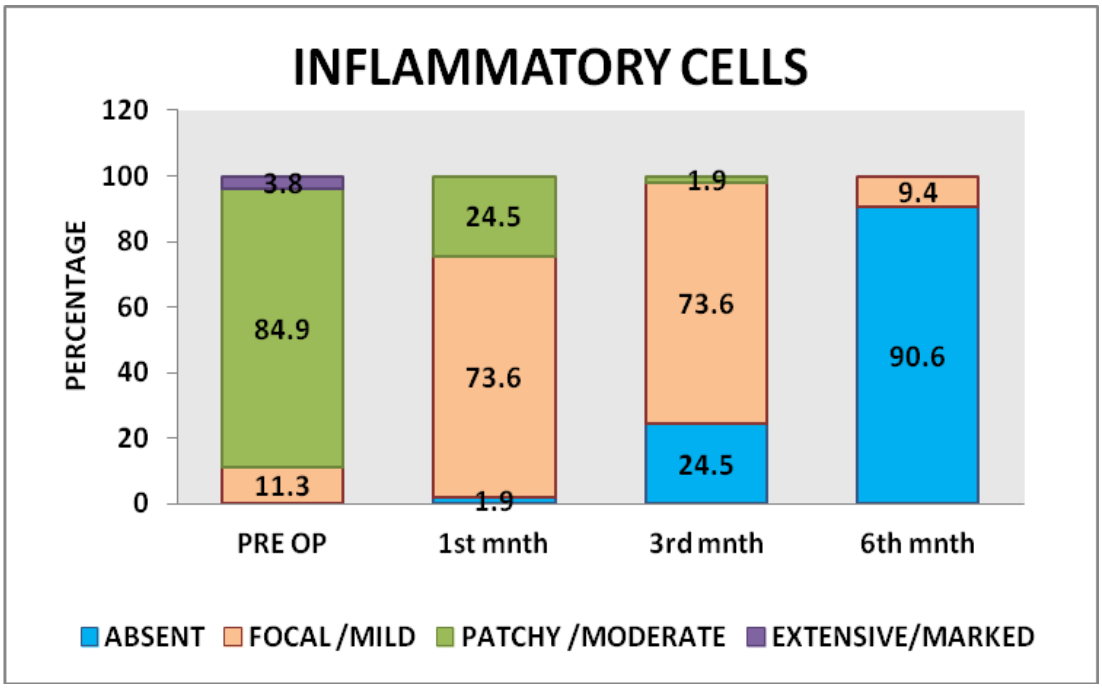


TABLE 8: DISTRIBUTION OF STROMAL EDEMA ACCORDING TO TIME

STROMAL EDEMA	PRE OP		1st mnth		3rd mnth		6th mnth	
	N	%	N	%	N	%	N	%
ABSENT	0	0	2	3.8	21	39.6	47	88.7
MILD	14	26.4	46	86.8	32	60.4	6	11.3
MODERATE	37	69.8	5	9.4	0	0	0	0
MARKED	2	3.8	0	0	0	0	0	0
Total	53	100	53	100	53	100	53	100

GRAPH 8: DISTRIBUTION OF STROMAL EDEMA ACCORDINGTO TIME

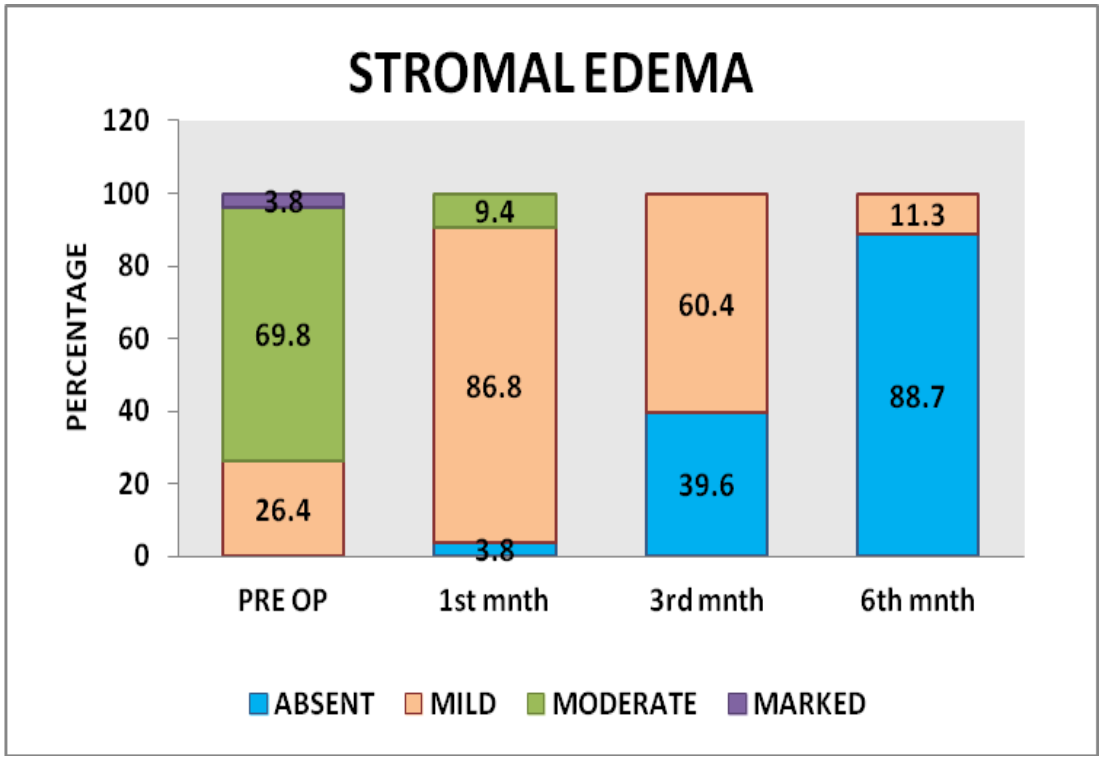


TABLE 9: DISTRIBUTION OF STROMAL FIBROSIS ACCORDING TO TIME

STROMAL FIBROSIS	PRE OP		1st mnth		3rd mnth		6th mnth	
	N	%	N	%	N	%	N	%
ABSENT	6	11.3	4	7.5	7	13.2	22	41.5
MILD	36	67.9	49	92.5	44	83	27	50.9
MODERATE	11	20.8	0	0	2	3.8	4	7.5
Total	53	100	53	100	53	100	53	100

GRAPH 9: DISTRIBUTION OF STROMAL FIBROSIS ACCORDING TO TIME

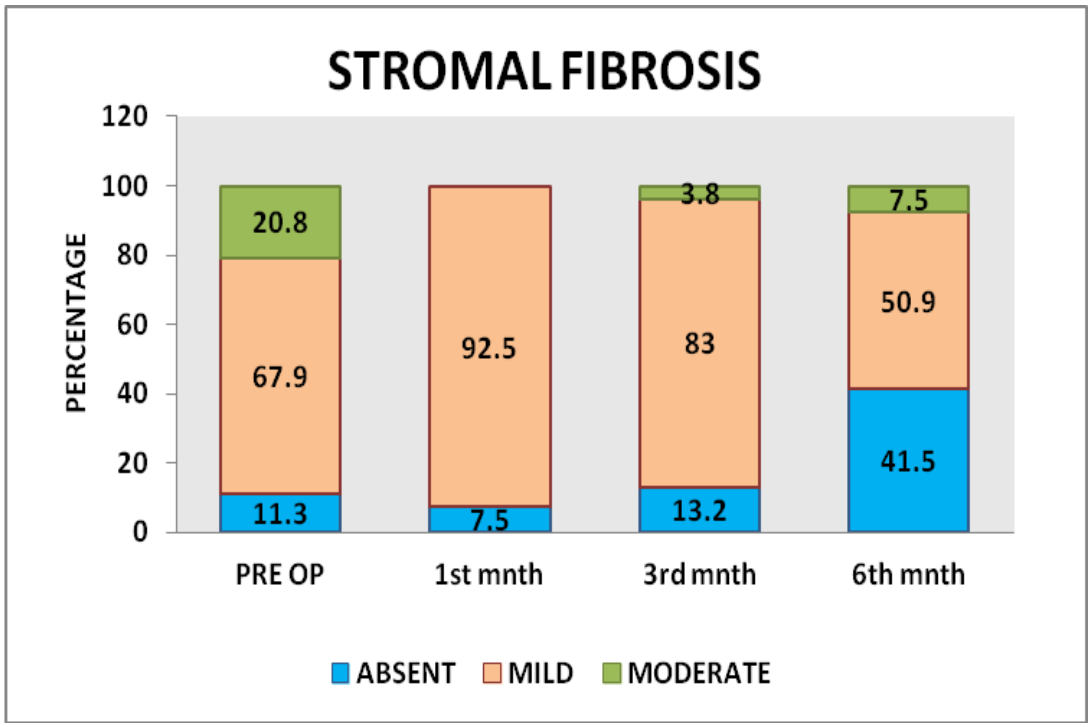


TABLE 10: DISTRIBUTION OF ANGOGENESIS ACCORDING TO TIME

ANGOGENESIS	PRE OP		1st mnth		3rd mnth		6th mnth	
	N	%	N	%	N	%	N	%
ABSENT	1	1.9	8	15.1	23	43.4	48	90.6
MILD	26	49.1	41	77.4	29	54.7	5	9.4
MODERATE	25	47.2	4	7.5	1	1.9	0	0
MARKED	1	1.9	0	0	0	0	0	0
Total	53	100	53	100	53	100	53	100

GRAPH 10: DISTRIBUTION OF ANGOGENESIS ACCORDING TO TIME

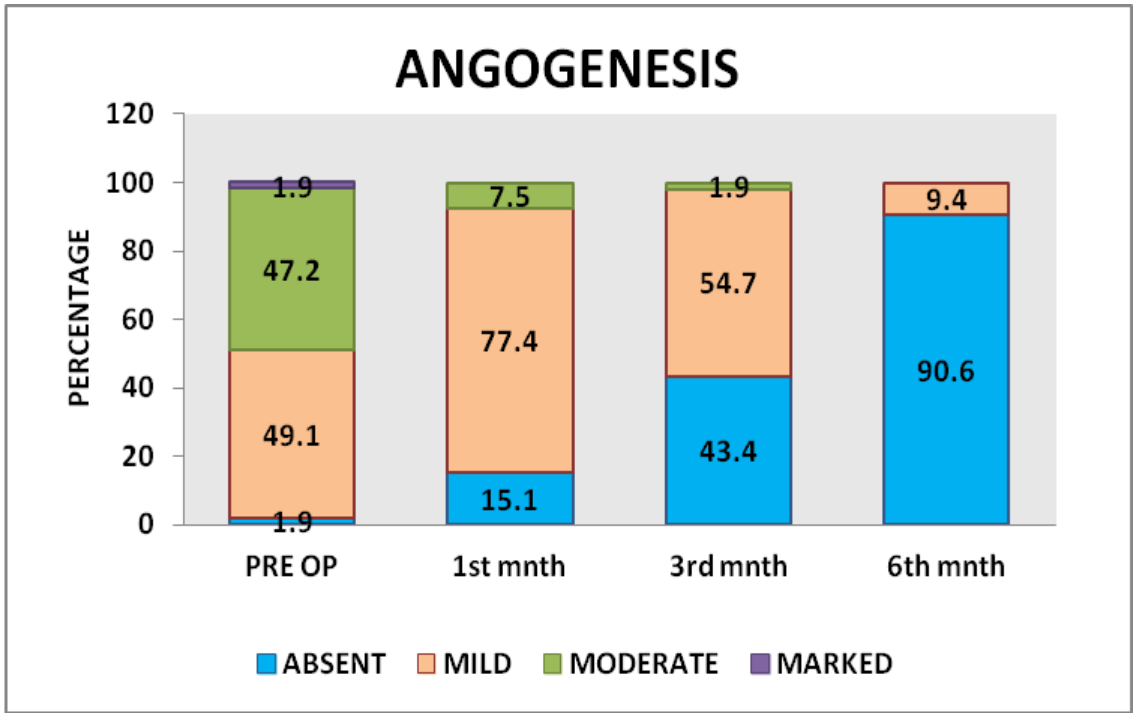


TABLE 11: CHANGE IN MEAN MMP-9

FOLLOWUP TIME	MMP-9		ANOVA p value
	Mean	SD	
PRE OP	1459.2	1323.4	<0.001*
1st mnth	796.9	465.3	
3rd mnth	513.2	219.6	
6th mnth	339.8	182.7	
Total	777.3	829.2	

Note: * significant at 5% level of significance (p<0.05)

GRAPH 11: CHANGE IN MEAN MMP-9

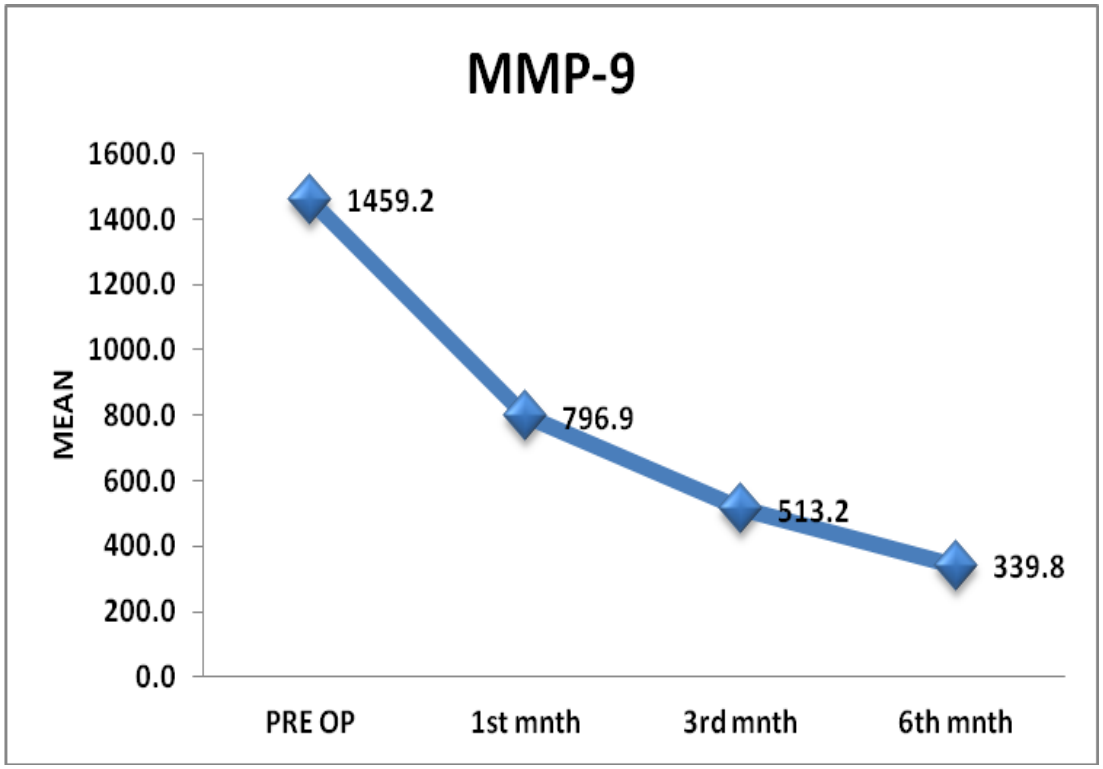


TABLE 12: CHANGE IN MEAN MMP-9 BY HYPERPLASIA OF GOBLET CELLS

FOLLOWUP TIME	HYPERPLASIA				p value
	PRESENT		ABSENT		
	Mean	SD	Mean	SD	
PRE OP	1882.5	1525.5	1292.1	1216.4	0.145
1st mnth	-	-	796.9	465.3	-
3rd mnth	-	-	513.2	219.6	-
6th mnth	-	-	339.8	182.7	-

GRAPH 12: CHANGE IN MEAN MMP-9 BY GOBLET CELLS

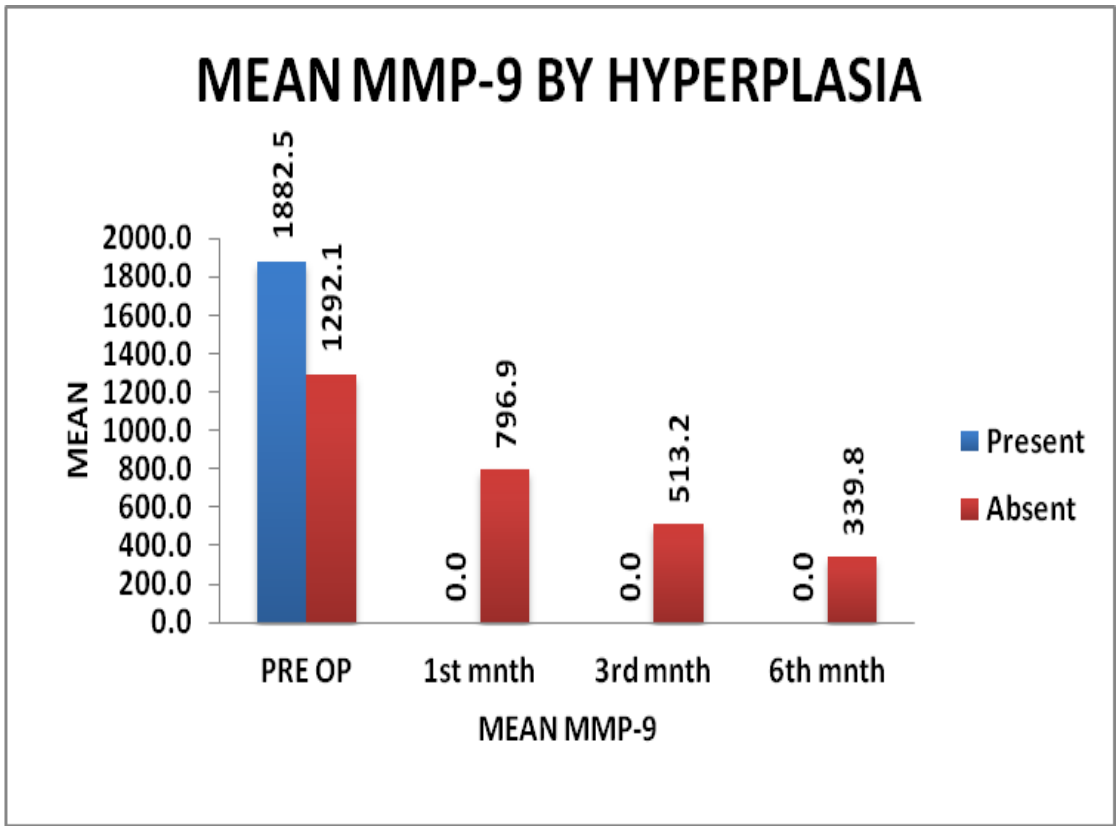


TABLE 13: CHANGE IN MEAN MMP-9 BY INFLAMMATORY CELLS

FOLLOWUP TIME	INFLAMMATORY CELLS				p value
	PRESENT		ABSENT		
	Mean	SD	Mean	SD	
PRE OP	1459.2	1323.4	-	-	-
1st mnth	806.9	464.0	275.2	0.0	0.262
3rd mnth	513.5	216.8	512.1	237.2	0.984
6th mnth	388.0	0.0	334.8	191.4	0.54

GRAPH 13: CHANGE IN MEAN MMP-9 BY INFLAMMATORY CELLS

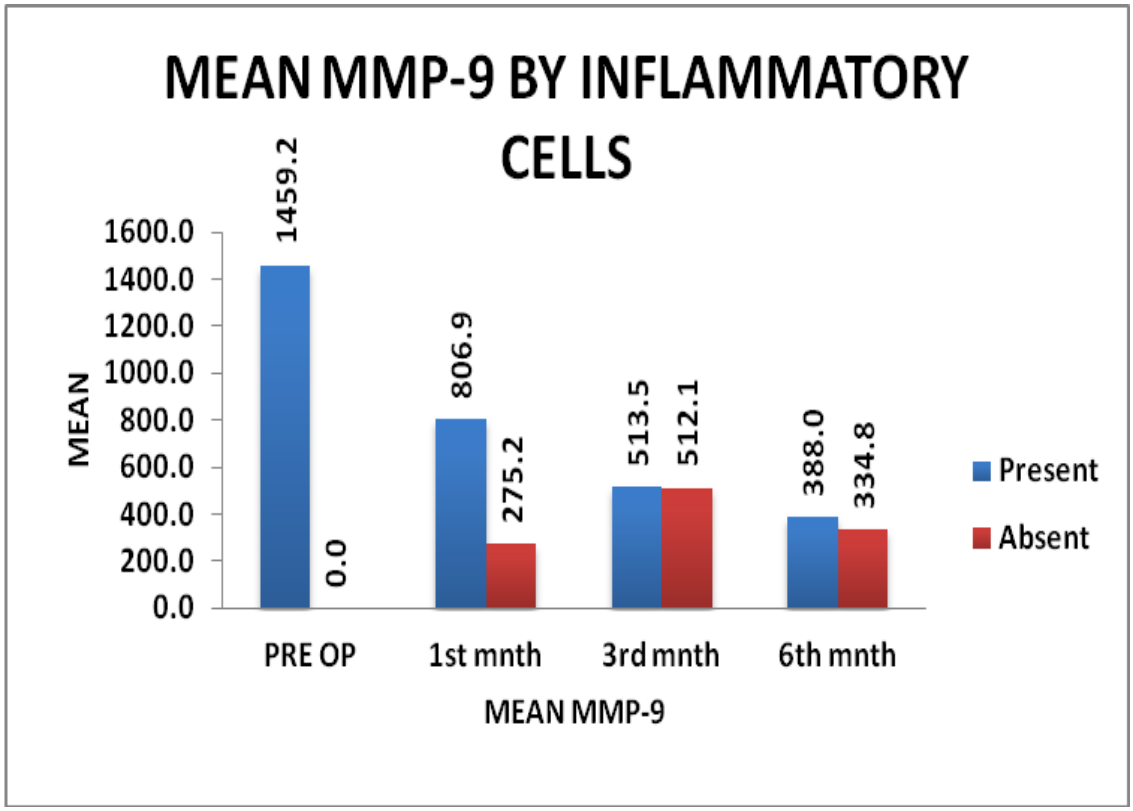


TABLE 14: CHANGE IN MEAN MMP-9 BY STROMAL EDEMA

FOLLOWUP TIME	STROMAL EDEMA				p value
	PRESENT		ABSENT		
	Mean	SD	Mean	SD	
PRE OP	1459.2	1323.4	-	-	-
1st mnth	807.9	469.3	516.5	289.2	0.39
3rd mnth	536.3	233.7	478.0	196.5	0.349
6th mnth	344.7	38.9	339.2	193.8	0.945

GRAPH 14: CHANGE IN MEAN MMP-9 BY STROMAL EDEMA

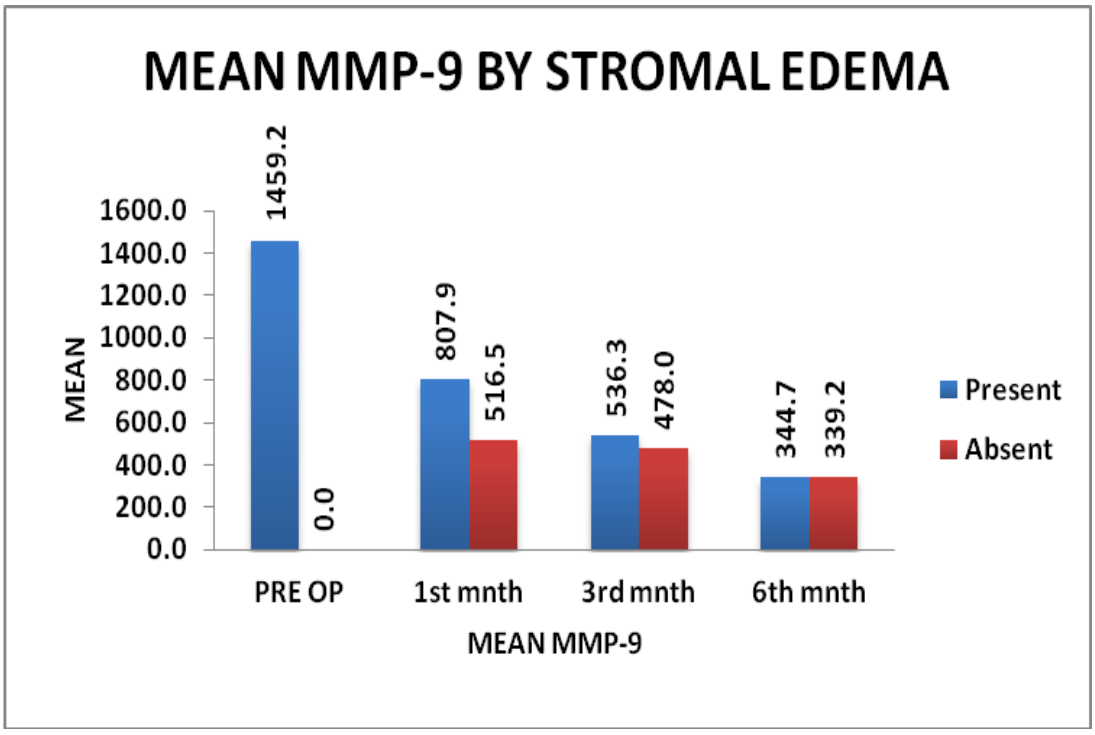


TABLE 15: CHANGE IN MEAN MMP-9 BY STROMAL FIBROSIS

FOLLOWUP TIME	STROMAL FIBROSIS				p value
	PRESENT		ABSENT		
	Mean	SD	Mean	SD	
PRE OP	1081.0	423.3	1507.5	1392.6	0.463
1st mnth	796.0	476.1	807.2	354.3	0.964
3rd mnth	392.6	214.1	531.5	216.9	0.12
6th mnth	289.5	126.3	375.5	208.6	0.092

GRAPH 15: CHANGE IN MEAN MMP-9 BY STROMAL FIBROSIS

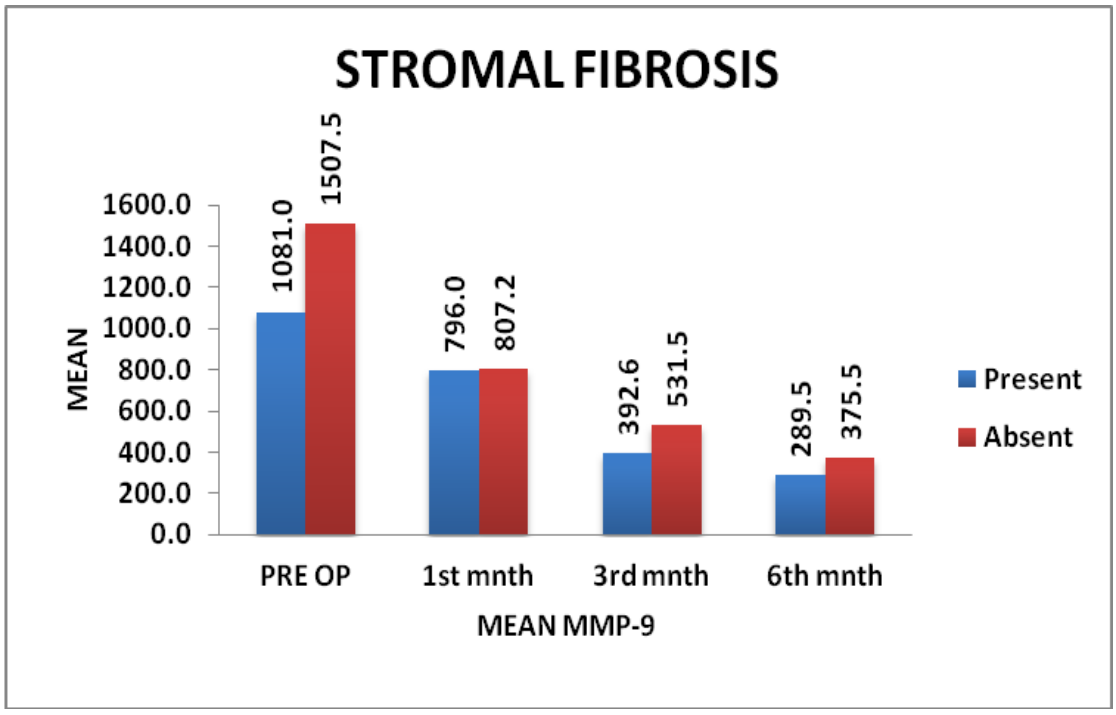
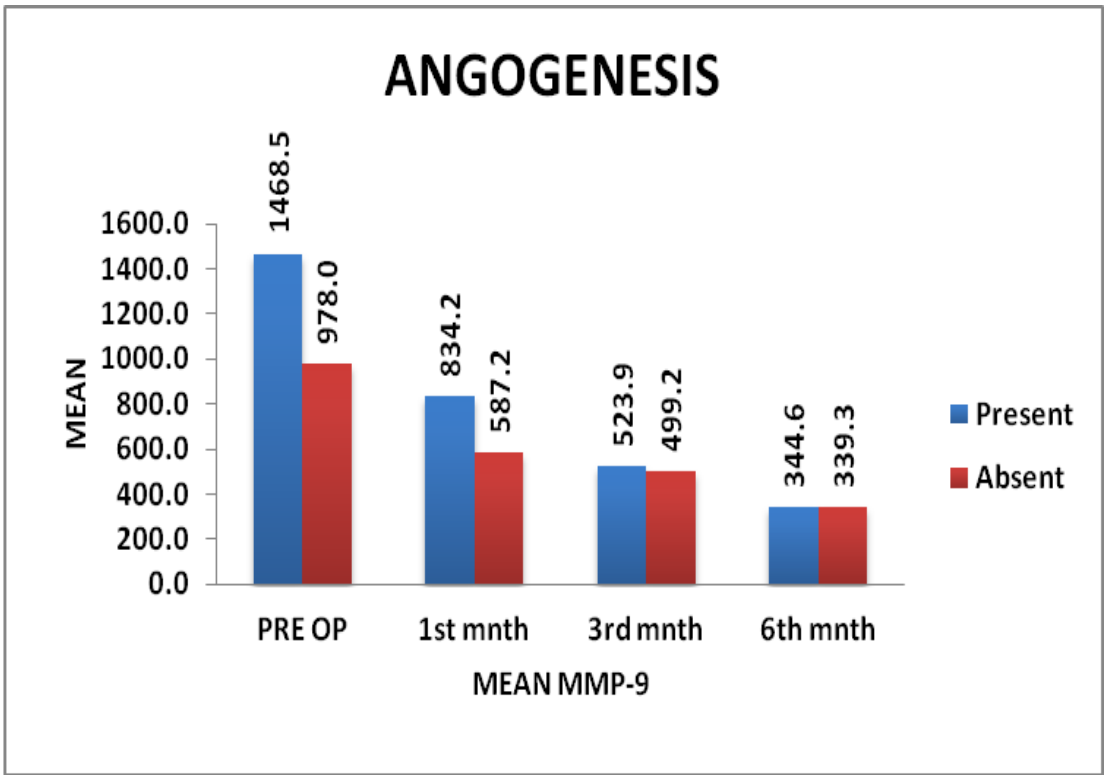


TABLE 16: CHANGE IN MEAN MMP-9 BY ANGOGENESIS

FOLLOWUP TIME	ANGOGENESIS				p value
	PRESENT		ABSENT		
	Mean	SD	Mean	SD	
PRE OP	1468.5	1334.6	978.0	0.0	0.717
1st mnth	834.2	456.7	587.2	488.1	0.169
3rd mnth	523.9	235.1	499.2	202.1	0.69
6th mnth	344.6	43.5	339.3	191.7	0.951

GRAPH 16: CHANGE IN MEAN MMP-9 BY ANGOGENESIS



DISCUSSION

Argyro J bizaki et al., (2016) after a RCT study on sinonasal mucosa suggest that chronic rhinosinusitis reveals findings consistent with inflammation. Thickened epithelium, metaplasia of epithelium, angiogenesis, increased inflammatory cells and hyperplasia of mucosal glands were observed in most of the preoperative samples. Hypertrophy of the mucosal glands, mucosal edema and hyperplasia of blood vessels decreased after balloon sinuplasty and uncinectomy. In epithelium expression of MMP-9 was strongly and significantly correlated with a higher number of inflammatory cells in nasal epithelium and mucosa.[10]

In our study also it was found Epithelium thickening and metaplasia of upper airway epithelium to transitional epithelium was present. There was increase number of goblet cells and inflammatory cell found in the mucosa with fibrosis and angiogenesis in the mucosa. Mucous and serous gland hypertrophy in the mucosa was present.

A thickened epithelium was associated with high no.of inflammatory cell and formation of new blood vessels (angiogenesis).

A past report has demonstrated that there is no change in mucociliary clearance after treatment. This is reliable with the finding that shedding of epithelium was not reestablished after treatment.

As there was more inflammatory cells in the epithelium it resultant in more number of goblet cells which ultimately lead to increase in sinonasal secretion.

There was high number of inflammatory cells in the mucosa and hyperplasia of blood vessels present with edema of the mucosa due invasion of intravascular fluid exudates in the extracellular space during active inflammation.

Degree of fibrosis was less in the sinonasal mucosa during inflammation. As inflammation starts subsiding the degree of fibrosis increases. More fibrosis is present in chronic and less active inflammation.

MMP-9 levels in the epithelium was positively correlated with a increase number of inflammatory cells in sinonasal mucosal epithelium.

J.B watelet et al., (2006) did a study on 23 patients, biopsies were collected from patients undergoing FESS for CRS and nasal polyposis.MMP-9 expression was correlated with healing quality. Conclusion was MMP-9 predicts healing quality after sinus surgery.[5]

This study shows that concentration of MMP-9 in sinonasal mucosa reflect the expression of MMP-9 inside the extracellular matrix after the endoscopic sinus surgery.MMP-9 was highly correlated with healing quality. This results confirm the predictive role of MMP-9 in the healing outcome of sinonasal mucosa after ESS. Increased secretion of matrix metalloproteinase-9 leads to ECM damage and decrease the healing quality. Therefore a link is established between the inflammatory reaction and MMP-9 expression in tissue.

Several cells like monocytes, eosinophils, macrophages and epithelial derived cells actively express MMP-9 which neutrophil stores. BM, fibronectin, collagen fibres and elastin is degraded by MMP-9.At various level MMP-9 action is controlled, the gene under control of cytokines or interaction of cells, proenzyme activation by serine protein or various other MMP's. ultimately regulation of activity is by TIMP's.

MMP-9 is not only an effector but also a leukocyte regulator. Granules of mature neutrophils stores MMP-9 and is a specific marker of neutrophil maturation

Cytokines. viral bacterial products and cellular interaction stimulates transcription of gelatinase B gene .The neutrophil is in charge of the fast production of MMP-9 as

result of preformed enzymes which is stored in the granules in reponse to LPS. Release of gelatinase-B by degranulation of neutrophils happens inside the primary hour when these cells are invigorated by chemotactic factors.

The relationship between neutrophils and macrophages present in tissues during wound healing suggests the amount of MMP-9 are high and that a combination of rapid discharge and continuous production is needed. MMP-9 clips numerous cytokines or chemokines, for example, IL- 1B or IL-8. On the other hand, the binding of MMP-9 to the plasma layer of neutrophils enables it to be inhibited by TIMPs and accordingly may adjust the pericellular proteolytic balance in favour of degradation of ECM .

CONCLUSION

MMP-9 levels in epithelium and histopathology of the epithelium was checked and compared preoperatively,1st month,3rd month and 6 month. In this study MMP-9 was found to be in increased concentration before the endoscopic sinus surgery and starts decreasing after the endoscopic sinus surgery. We can draw a conclusion that as the inflammation of the sinonasal mucosa decreases the level of MMP-9 in the epithelium also decreases because the number of inflammatory cells(eosinophils,neutrophils) in the sinonasal mucosa decreases which are the source for MMP-9.

Histopathological study of the epithelium preoperatively showed presence of metaplasia, increase number inflammatory cells ,increase thickness of the epithelium with goblet cell hyperplasia, stromal edema, angiogenesis with sparse fibrosis .After 1 month metaplasia was absent in most of the cases, there was decrease number of inflammatory cells, goblet cells, stromal edema, angiogenesis and increase fibrosis with normal thickness of epithelium. After 3 month the epithelium starts healing and coming back to its normal conture with increase fibrosis. After 6 month the epithelium is similar to a normal epithelium with fibrosis.

A decrease in hypertrophy of the mucous glands , decline in the number of inflammatory cells, indicate a positive effect of treatment on the inflammatory process, which may also account for a more functional nasal epithelium and lower nasal airway resistance. This may also account for the post treatment improvement of symptoms and in quality of life.

Matrix metalloproteinase-9 (MMP-9) expression in the mucosal epithelium was positively and statistically significantly correlated with a higher number of inflammatory cells in nasal epithelium and mucosa.

Therefore MMP-9 can be considered as a predictor of healing quality of the sinonasal mucosa after endoscopic surgery.

SUMMARY

This study "Study of MMP-9 and histopathology in sinonasal diseases before and after endoscopic surgery" was done in _____ _____ during the period of November 2017 to June 2019.

A total of 55 patients were selected based on the inclusion criteria for the study of MMP-9 with histopathology of Sinonasal mucosa.

Of the 55 cases the age group of patients varied from 18 to 65 years. There were 21 males and 32 females. 2 cases expired after 1 month of endoscopic sinus surgery .All patients underwent FESS for chronic sinusitis .Sinonasal mucosa sample was collected before and after FESS at an interval of 1mnth, 3month and 6 month.

In 53 cases of our study it was found that metaplasia was present in 28.3% cases preopratively, 20.8% cases at 1st month, 13.2% cases at 3rd and 5.7% cases at 6th month.

Goblet cell hyperplasia was present in 28.3 % cases preoperatively, 50% cases showed normal number of goblet cells at 1st month, 18.9% cases showed decreased number of goblet cells at 3rd month.

Epithelium thickness was present in 64.2% cases preopratively, 15.1 % at 1mnth, 3.8% at 3rdmnth and was normal at 6 mnth.

Inflammatory cells was moderately found in 84.9% cases preopratively, 24% cases at 1st mnth,1.9% at 3rd month and was absent at 6 mnth.

Stromal edema was found moderately in 69.8% cases preoperatively, 9.4% cases at 1stmnth and was absent at 3rd mnth and 6th mnth.

Angiogenesis was moderately present in 47.2% cases preopratively,7.5% cases at 1st mnth,1.9% cases at 3rd mnth and was absent at 6th mnth.

The present study was conducted in 55 patients presenting with sinonasal diseases who underwent FESS. It was found that MMP-9 value significantly decreased over time($P<0.05$).The fall of mean MMP-9 was maximum (53%) from pre-op to 1month which subsequently subsidized from previous value. Histopathological findings like presence goblet cell hyperplasia ,inflammatory cells, stromal edema and angiogenesis were found to be positvely correlated with higher level of MMP-9 values. These findings were consistent over the follow up time of 1st,3rd and 6th month.

It was also found that the presence .of fibrosis was negatively correlated with the level of MMP-9

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ANNEXURES-1

ETHICAL CLEARANCE CERTIFICATE

PROFORMA

SCHEME OF CASE TAKING

1) NAME: CASE NO:

2) AGE: IP NO:

3) SEX: DOA:

4) RELIGION: DOS:

5) OCCUPATION: DOD:

6) RESIDENCE:

7) CHIEF COMPLAINTS:

8) HISTORY OF PRESENTING ILLNESS:

9) PAST HISTORY:

10) FAMILY HISTORY:

11) GENERAL PHYSICAL EXAMINATION:

Pallor: Present/Absent

Icterus: Present/Absent

Clubbing: Present/Absent

Generalized Lymphadenopathy: Present/Absent

Build: Poor/Medium /Well

Nourishment: Poor / Medium / Well

12) VITALS

PR:

BP:

13) OTHER SYSTEMIC EXAMINATION:

- Respiratory System
- Cardiovascular System
- Central Nervous System
- Per Abdomen examination

14) LOCAL EXAMINATION

- NOSE
  - EXTERNAL NOSE
  - Skin
  - Osteocartilaginous framework
  - Vestibule
  - ANTERIOR RHINOSCOPY
  - Nasal passage
  - Nasal septum
  - Floor of nose
  - Roof of nose
  - LATERAL WALL
  - Turbinates and meatuses
  - Colour of mucosa
  - Size of turbinates
  - Discharge
  - Mass
  - POSTERIOR RHINOSCOPY

- PATENCY OF NOSE
- Cold spatula test
- Cottonwool test

PARANASAL SINUSES EXAMINATION

- FRONTAL MAXILLARY ETHMOID
- INSPECTION
- PALPATION

DNE FINDINGS:-

- EAR
- ORAL CAVITY AND OROPHARYNX

15) INVESTIGATION:

BLOOD ROUTINE: URINE ROUTINE:

HPR FINDINGS:-

	SCORING	PRE-OP	AT 1 MONTH	AT 3 MONTH	AT 6 MONTH
1)EPITHELIUM EVALUATION A)METAPLASIA B)GOBLET CELLS C)THICKNESS	 0-absent 1-present 0-not present 1-decreased 2-normal 3-hyperplasia 0-thin 1-normal 2-thick				
2)INFLAMMATORY CELLS	0-not present 1-focal/mild 2-patchy /moderate 3-extensive /marked				
3)STROMAL EDEMA	0-not present 1-mild 2-moderate 3-marked				
4)STROMAL FIBROSIS	0-not present 1-mild 2-moderate 3-marked				
5)ANGIOGENESIS	0-not present 1-mild 2-moderate 3-marked				
HISTOPATHOLOGICAL DIAGNOSIS					

ELISA FINDINGS OF MMP-9:-

- Pre Operative-
- At 1 month-
- At 3 month-
- At 6 month-

CT PNS FINDINGS

LUND AND MACKAY SCORING SYSTEM (0-no abnormality,1-partial opacification,2-complete opacification)

SINUS SYSTEM	PRE-OP	POST-OP
MAXILLARY(0,1,2)		
ANTERIOR ETHMOID(0,1,2)		
POSTERIOR ETHMOID(0,1,2)		
SPHENOID(0,1,2)		
FRONTAL(0,1,2)		
OSTEOMEATAL COMPLEX(0 OR 2)		
TOTAL		

16) FINAL DIAGNOSIS:

17) SURGERY: **Intra operative findings**:-

19) INFERENCE:

20) COMMENTS:

INFORMED CONSENT FORM

TITLE OF THE PROJECT:

STUDY OF MATRIX METALLOPROTEINASE 9 (MMP-9) AND HISTOPATHOLOGY IN SINONASAL DISEASES BEFORE AND AFTER ENDOSCOPIC SURGERY.

PG STUDENT -

PG GUIDE -

All aspects of this consent form are explained to the patient in the language understood by him/her.

1) PURPOSE OF RESEARCH:

I have been informed about this study. I have also been given a free choice of participation in this study.

2) PROCEDURE:

I am aware that in addition to routine care received I will be asked series of questions by the investigator. I have been asked to undergo the necessary investigations and treatment, which will help the investigator in this study

3) RISK AND DISCOMFORTS:

I understand that I may experience some pain and discomfort during the examination or during my treatment. This is mainly the result of my condition and the procedure of this study is not expected to exaggerate these feelings that are associated with the usual course of treatment.

4) BENEFITS:

I understand that my participation in this study will help to patients survival and better outcome.

5) CONFIDENTIALITY:

I understand that the medical information produced by this study will become a part of Hospital records and will be subject to the confidentiality and privacy regulation. Information of a sensitive personal nature will not be a part of the medical records, but will be stored in the investigator's research file and identified only by a code number. The code-key connecting name to numbers will be kept in a separate location.

If the data are used for publication in the medical literature or for teaching purpose, no name will be used and other identifiers such as photographs and audio or videotapes will be used only with my special written permission. I understand that I may see the photographs and videotapes and hear the audiotapes before giving this permission.

6) REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at anytime. _____is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of the study, which might influence my continued participation.

If during the study, or later, I wish to discuss my participation in or concerns regarding this study with a person not directly involved, I am aware that the social worker of the hospital is available to talk with me. A copy of this consent form will be given to me to keep for careful reading.

7) REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and that I may refuse to participate or may withdraw consent and discontinue participation in the study at any time without prejudice to my present or future care at this hospital. I also understand that_____may terminate my participation in the study after she has explained the reasons for doing so and has helped arrange for my continued care by my own physician or physical therapist, if this is appropriate.

8) INJURY STATEMENT:

I understand that in the unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, the appropriate treatment would be available to me, but no further compensation would be provided. I understand that by my agreement to participate in this study I am not waiving any of my legal rights.

I have explained to _____the purpose of the research, the procedures required and the possible risks and benefits to the best of my

ability in patient's own language.

_____ _____

Date

(Investigator)

STUDY SUBJECT CONSENT STATEMENT

I confirm that _____ has explained to me the purpose of research, the study procedures that I will undergo, and the possible risks and discomforts as well as benefits that I may experience in my own language. I have read and I understand this consent form. Therefore, I agree to give consent to participate as a subject in this research project.

_____ _____

Participant / Guardian Date

_____ _____

Witness to signature Date

MICROSCOPIC PICTURES

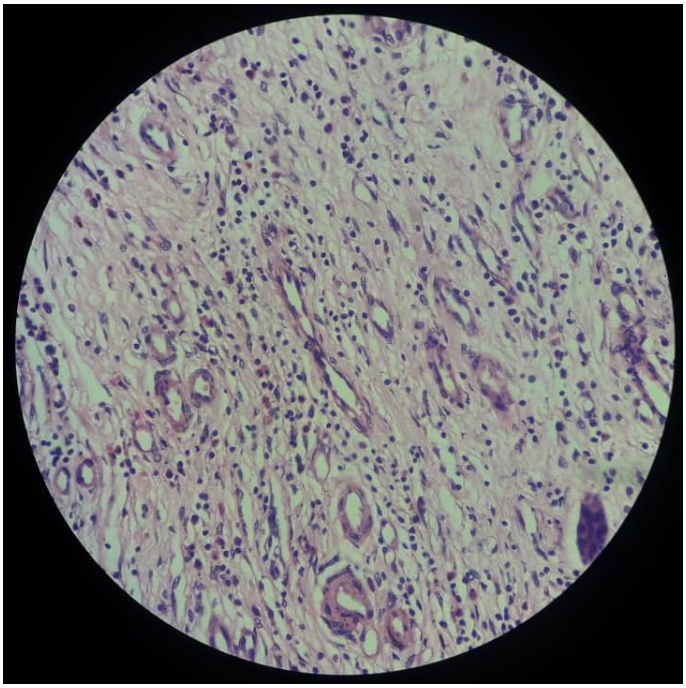


FIG 11: ANGIOGENESIS

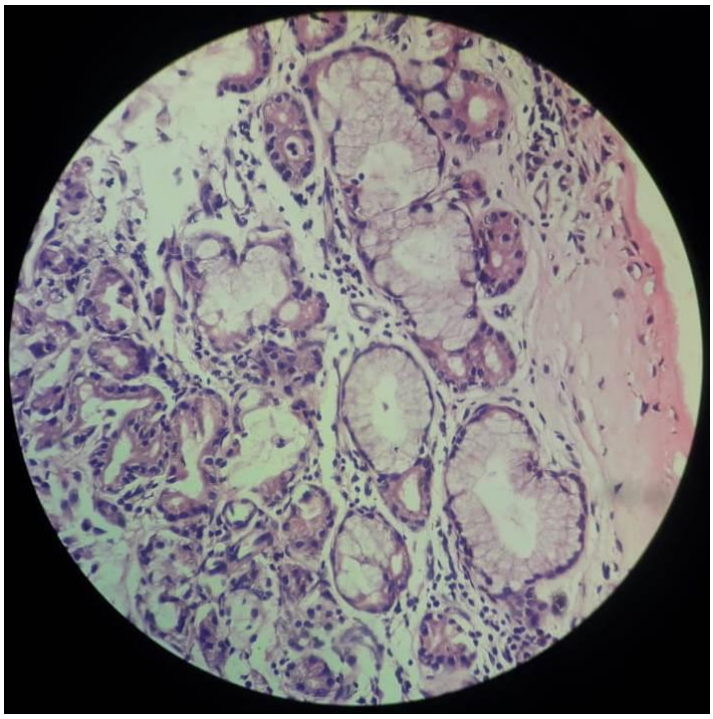


FIG 12: GOBLET CELL HYPERPLASIA

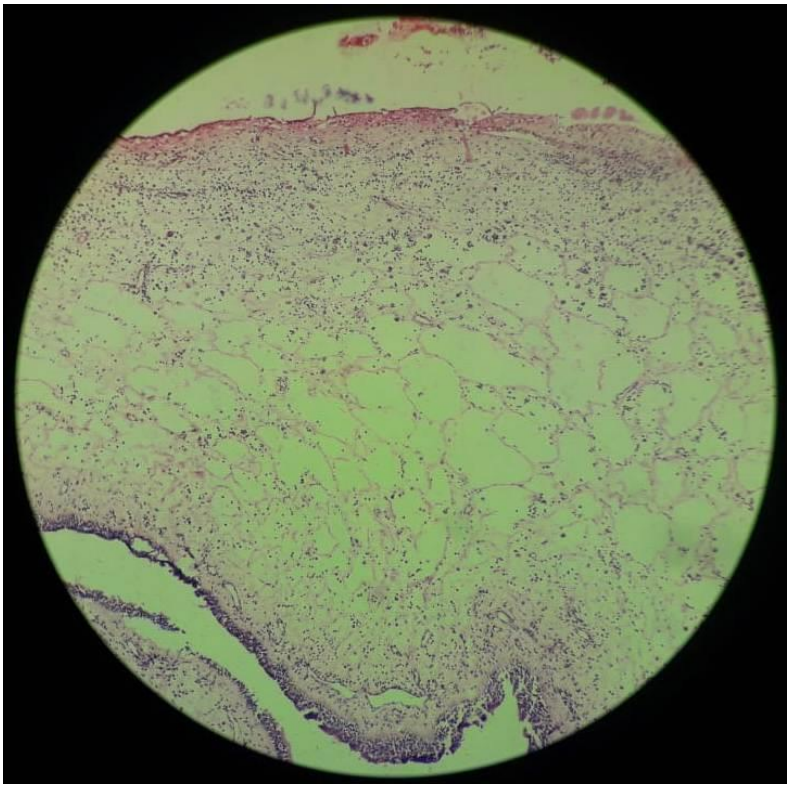


FIG 13:STROMAL EDEMA

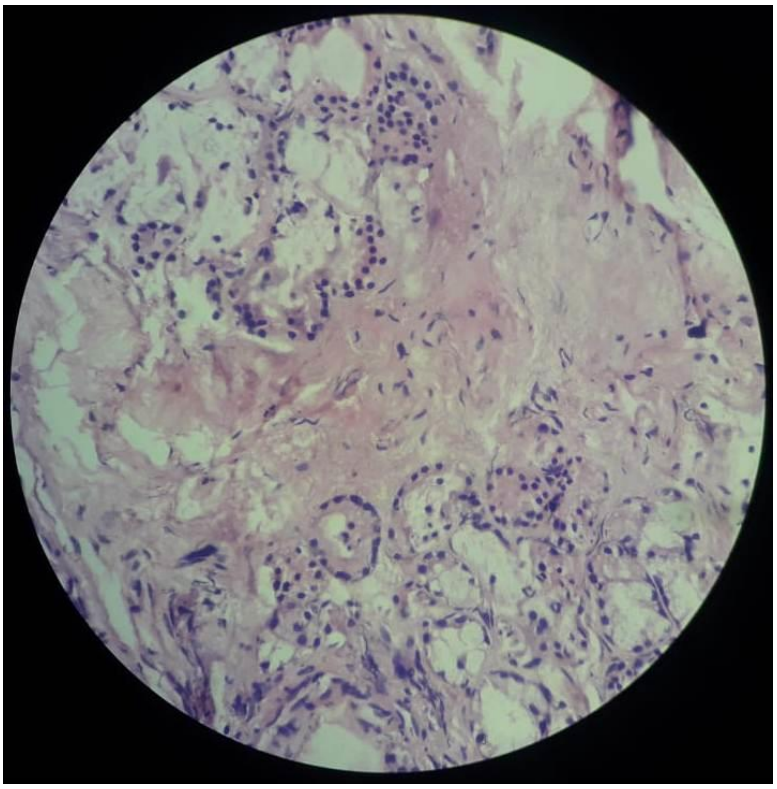


FIG 13:STROMAL FIBROSIS

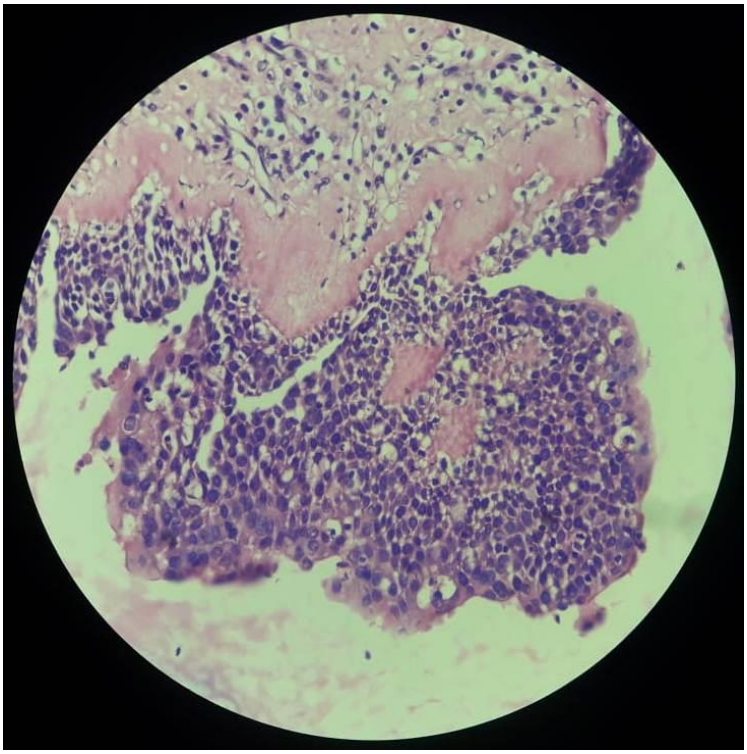


FIG 15:METAPLASIA

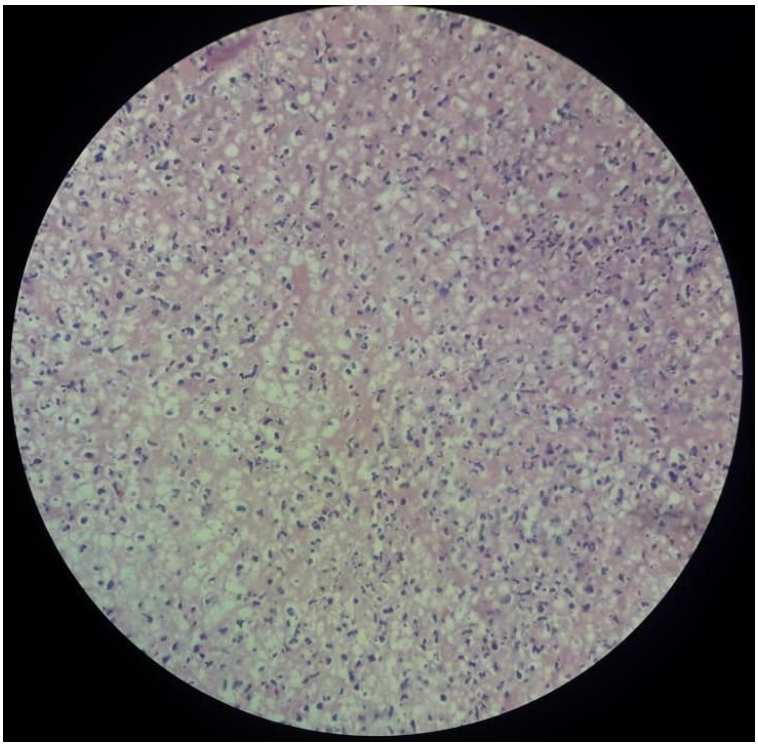
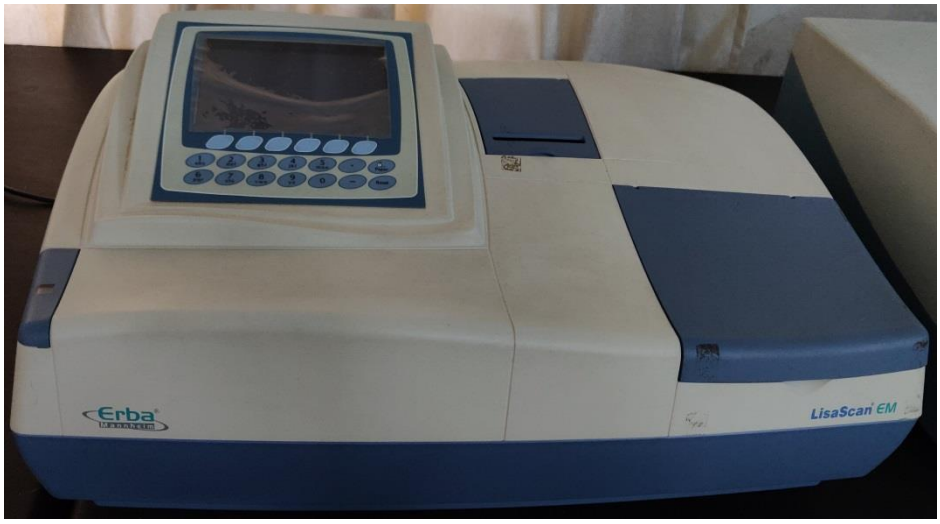


FIG 16:INFLAMMATORY CELLS

PHOTOGRAPHS



KEY TO MASTER CHART

SL.No.	-	Serial Number
IP No.	-	Inpatient Number
MMP-9	-	Matrix metalloproteinase-9
HP	-	Histopathology
MT	-	Metaplasia
		0-absent
		1-present
GB	-	Goblet cells
		0-not present
		1-decreased
		2-normal
		3-hyperplasia
ET	-	Epithelium thickness
		0-thin
		1-normal
		2-thick
IC	-	Inflammatory cells
		0-not present
		1-focal /mild
		2-patchy /moderate
		3-extensive/marked
SE	-	Stromal edema
		0-not present
		1-mild

2-moderate

3-marked

SF - Stromal fibrosis

0-not present

1-mild

2-moderate

3-marked

AG - Angiogenesis

0-not present

1-mild

2-moderate

3-marked

MASTERCHART

					MMP-9				PRE						
Sl.no	IP No	Name	Sex	Age	Pre	1st Mnth	3rd Mnth	6th Mnth	MT	GB	ET	IC	SE	SF	AG
1	12507	Mahadevappa	M	18	1383.8	325.2	312	300	0	2	1	2	1	1	1
2	15465	Vittal	M	32	1155.4	275.2	312	302	0	2	0	1	1	2	2
3	9036	Sushilabai	F	47	1164.2	1140.2	460	345	1	1	2	2	2	0	1
4	18463	Sanyawwa	F	60	1100	1056	850	476	0	1	1	2	2	1	2
5	20729	Bouramma	F	60	1500	812	312	388	1	2	2	2	2	1	2
6	21839	Priyanka	F	20	312	302	300	212	0	0	1	3	2	1	2
7	20639	Sanju	M	18	1000	312	852	880	0	2	1	2	2	2	1
8	22447	Rekha	F	28	1000	3064	972	614	0	0	0	1	2	1	3
9	22411	Mahadevi	F	35	4958	391	312	302	0	3	1	2	1	1	1
10	27502	Ashok	M	28	312	302	289	250	0	2	1	2	3	1	2
11	28395	Sheela	F	40	391	312	304	388	1	1	2	3	1	1	2
12	29011	Parvati	F	40	1000	922.6	852	462	0	2	1	2	2	1	2
13	34003	Basamma	F	26	8100	312	852	1112	0	2	1	2	2	1	2
14	9037	Sulabai	F	47	1231	990	600	230	0	3	2	2	2	1	2
15	34361	Shaila	F	25	6000	890	712	206	0	3	2	2	2	1	2
16	33695	Dharmalinga	M	60	1092	612	312	388	0	3	2	2	2	1	1
17	20739	Leelavati	F	60	1500	812	312	388	1	2	2	2	2	1	2
18	36186	Ravikant	M	27	960	440	312	388	0	3	2	2	2	1	2
19	37545	Sharif	M	25	448	384	302	222	0	2	1	2	1	0	1
20	38637	Rehanna	F	23	850	550	312	345	0	3	3	2	2	1	1
21	39694	Sadanand	M	30	1572	602	312	256	0	3	2	2	2	1	2
22	40381	Rajendra	M	18	1205	952	809	689	1	3	2	2	1	1	1
23	40230	Maruthi	M	35	1012	890	460	330	0	2	2	1	1	1	1
24	40307	Santosh	M	40	1970	847	312	201	0	3	2	2	2	1	2
25	40702	Irraya	F	18	1813	927.4	300	240	0	3	2	2	2	1	1
26	41804	Gouramma	F	45	1808	1744.2	464.4	338	0	0	1	2	1	1	1
27	43953	Sampat	M	36	2100	1815.6	758.5	150	1	3	2	2	2	1	1
28	2067	Geeta	F	18	1465	1086	860	420	1	0	2	2	3	0	1
29	3494	Laxmamma	F	60	608	312	289	201	0	0	0	1	1	2	1
30	4723	Gangaram	M	45	2422	1108	978	521	1	2	2	2	2	1	1
31	42354	Kalappa	M	55	1941	609	233.2	140	0	0	3	2	2	1	2
32	5421	Kasturibai	F	58	966	721	406	234	0	3	1	1	1	1	1
33	8466	Mahadevi	F	20	1599	481.6	312	166	1	0	2	2	2	0	2
34	9133	Ramarao	M	60	1557	688	433	299	0	3	1	2	1	1	2
35	12448	Priya	F	18	1092	678	441	289	1	2	2	2	2	2	1
36	7894	Shivanna	M	59	947	654	426	356	1	0	2	2	1	1	1
37	8174	Shilpa	F	20	1082	986	756	454	0	3	2	2	1	1	1
38	9323	Chandubai	F	50	919	724	563	388	0	0	1	2	2	1	1
39	10654	Sangappa	M	40	1823	1563	876	667	0	2	2	2	2	1	2
40	10982	Basanna	M	50	882	720	540	345	0	3	2	2	2	1	2
41	10832	Suma	F	19	956	877	681	208	0	2	2	2	2	2	2
42	11339	Pandu	M	48	1278	1002	658	378	1	2	2	2	2	2	2
43	12446	Priyanka Ramesh	F	18	1092	678	441	289	1	2	2	2	2	2	1
44	11471	Somibai	F	33	1126	986	784	230	0	2	2	2	2	1	1
45	17948	Pavithra	F	18	894	604	550	150	0	2	2	2	2	2	2
46	12092	Basappa	M	65	874	578	320	180	0	2	1	2	2	1	1
47	12321	Hulgamma	F	18	976	790	388	320	0	2	2	2	2	2	1
48	14688	Parvati	F	65	1067	880	660	340	1	2	2	2	2	2	2
49	17942	Pallavi	F	18	894	604	550	150	0	2	2	2	2	2	2
50	16668	Laxmi	F	30	864	772	633	264	1	2	1	2	2	1	1
51	15397	Dundawwa	F	65	1267	984	455	238	0	2	2	2	2	1	2
52	22813	Sushant	M	18	832	645	366	210	0	2	2	2	2	0	1
53	22728	Anjana	F	19	978	521	343	170	0	2	1	1	1	0	0

	1st Month							3rd Month							6th Month						
Sl.No	MT	GB	ET	IC	SE	SF	AG	MT	GB	ET	IC	SE	SF	AG	MT	GB	ET	IC	SE	SF	AG
1	0	1	1	1	1	1	1	0	1	1	1	0	1	1	0	1	0	0	1	0	1
2	0	2	0	0	1	1	1	0	2	0	0	1	1	1	0	2	0	0	1	1	1
3	1	1	1	1	2	0	1	0	1	1	1	1	0	1	0	1	1	0	1	0	0
4	0	1	1	2	1	1	2	0	1	1	1	1	1	1	0	1	1	0	0	0	0
5	0	2	1	2	1	1	1	0	1	1	1	0	1	1	0	0	1	1	0	1	0
6	0	0	1	2	2	1	1	0	0	0	1	1	1	1	0	0	0	0	0	1	0
7	0	2	1	2	2	1	0	0	1	1	1	1	1	0	0	1	1	0	0	1	0
8	0	0	0	1	1	1	2	0	0	0	0	1	1	1	0	0	0	0	0	1	0
9	0	2	1	1	1	1	0	0	1	1	1	1	1	0	0	1	1	0	0	1	0
10	0	2	1	1	2	1	1	0	1	1	1	1	0	1	0	0	1	0	0	0	0
11	0	1	1	2	1	1	1	0	1	1	2	0	1	0	0	1	0	1	0	1	0
12	0	1	1	2	1	1	2	0	1	1	1	1	0	1	0	0	1	0	0	0	0
13	0	2	1	2	1	1	0	0	1	1	1	1	1	0	0	0	1	0	0	1	0
14	0	2	1	1	1	1	1	0	2	1	1	1	1	1	0	1	1	0	0	1	0
15	0	2	1	1	1	1	1	0	2	1	1	1	1	1	0	1	1	0	0	1	0
16	0	2	2	2	1	1	1	0	1	2	1	1	1	1	0	1	1	1	1	1	1
17	0	2	1	2	1	1	1	0	1	1	1	0	1	1	0	0	1	1	0	1	0
18	0	2	2	1	1	1	1	0	2	1	1	1	1	1	0	2	1	1	1	1	1
19	0	2	1	1	1	1	0	0	1	1	1	1	0	1	0	2	1	0	0	0	0
20	0	2	2	1	1	1	1	0	2	2	1	1	1	1	0	2	1	0	1	1	1
21	0	2	2	1	1	1	1	0	2	1	1	1	1	1	0	2	1	0	0	0	0
22	0	2	1	1	1	1	1	0	1	1	1	1	1	1	0	0	0	0	0	0	0
23	0	2	1	1	1	1	1	0	2	1	0	1	1	0	0	1	0	0	0	2	0
24	0	2	1	1	1	1	1	0	1	1	0	1	1	1	0	0	1	0	0	2	0
25	0	2	1	1	1	1	1	0	1	1	1	0	0	0	0	0	1	0	0	0	0
26	0	0	1	1	1	1	0	0	0	1	0	1	2	0	0	0	1	0	0	2	0
27	1	2	1	1	1	1	1	0	2	1	1	0	1	1	0	1	1	0	0	1	0
28	1	0	2	2	2	0	1	1	0	1	1	1	1	1	1	0	1	0	0	1	0
29	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
30	1	1	1	1	1	1	1	0	1	1	0	1	1	1	0	0	1	0	0	1	0
31	0	2	2	1	1	1	1	0	2	1	1	0	0	0	0	1	1	0	0	0	0
32	0	2	1	1	0	1	0	0	1	1	1	0	1	0	0	0	1	0	0	0	0
33	1	0	2	1	1	0	1	1	0	1	1	1	0	1	1	0	0	0	0	1	0
34	0	2	1	1	1	1	2	0	1	1	1	1	1	2	0	0	1	0	0	1	0
35	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0	1	0	0	0	0
36	1	0	1	1	1	1	1	1	0	1	1	0	1	0	1	0	0	0	0	0	0
37	0	2	2	2	1	1	1	0	1	1	1	0	2	0	0	0	1	0	0	1	0
38	0	0	1	1	1	1	1	0	0	1	1	0	1	0	0	0	1	0	0	1	0
39	0	1	1	1	1	1	1	0	1	1	1	0	1	0	0	0	1	0	0	2	0
40	0	2	1	1	1	1	1	0	1	1	1	0	1	1	0	0	1	0	0	1	0
41	0	1	1	1	1	1	1	0	1	1	1	0	1	1	0	0	1	0	0	0	0
42	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	0	0	1	0
43	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0	1	0	0	0	0
44	0	1	1	2	1	1	1	0	0	1	1	0	1	0	0	0	1	0	0	1	0
45	0	1	1	1	1	1	1	0	1	1	1	1	1	0	0	1	1	0	0	0	0
46	0	1	1	2	1	1	1	0	1	1	1	1	1	0	0	0	1	0	0	0	0
47	0	1	1	1	1	1	1	0	1	1	0	0	1	0	0	0	1	0	0	0	0
48	1	2	1	1	1	1	1	1	1	1	0	1	1	1	0	0	1	0	0	0	0
49	0	1	1	1	1	1	1	0	1	1	1	1	1	0	0	1	1	0	0	0	0
50	1	1	1	1	1	1	1	0	1	1	1	0	1	0	0	0	1	0	0	0	0
51	0	2	1	1	1	1	1	0	2	1	0	0	1	0	0	0	1	0	0	1	0
52	0	2	1	1	1	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0
53	0	1	1	1	1	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0

****Out of 55 cases 2 cases expired during the study period.**