A COMPARATIVE STUDY OF PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN WITH GERD IN RELATION TO SERUM ESTROGEN LEVELS

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CERTIFICATE

This is to certify that the dissertation titled "**A comparative study of premenopausal and postmenopausal women with GERD in relation to serum estrogen levels**" is an original work done by **Dr. Praveena M**, Post graduate student, during the period of her post graduation in Physiology in our institution.

This work is done under the guidance of Dr. R. Nagashree, Professor & Head, Department of Physiology, PSG Institute of Medical sciences and Research, Coimbatore.

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CERTIFICATE - II

This is to certify that this dissertation work titled **"A COMPARATIVE STUDY OF PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN WITH GERD IN RELATION TO SERUM ESTROGEN LEVELS"** of the candidate **Dr. Praveena M,** with registration Number **201715402** for the award of **Degree of M.D.** in the branch of **PHYSIOLOGY.** I personally verified the urkund.com website for the purpose of plagiarism Check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **7%** of plagiarism in the dissertation.

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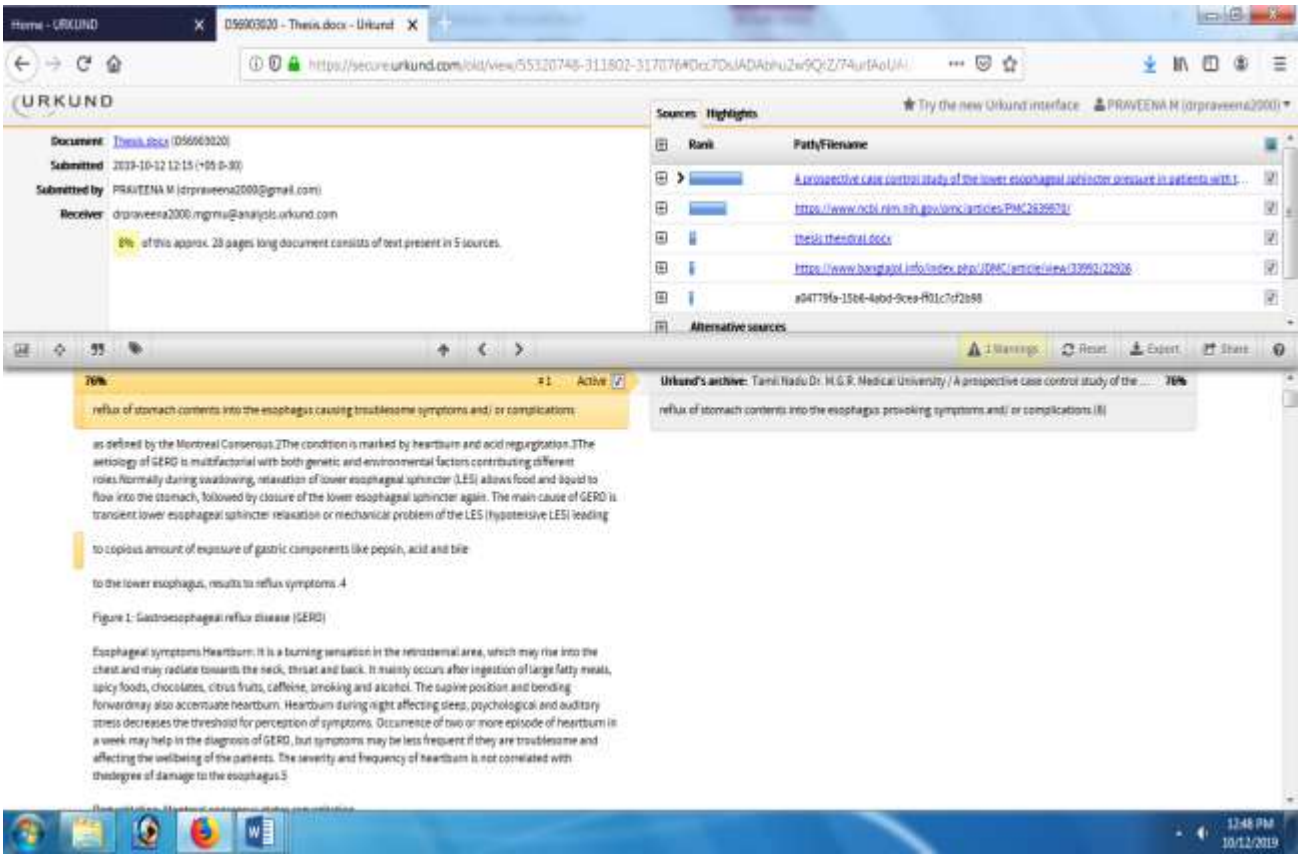
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as defined by the Montreal Consensus.2The condition is marked by heartburn and acid regurgitation.3The aetiology of GERD is multifactorial with both genetic and environmental factors contributing different roles.Normally during swallowing, relaxation of lower esophageal sphincter (LES) allows food and liquid to flow into the stomach, followed by closure of the lower esophageal sphincter again. The main cause of GERD is transient lower esophageal sphincter relaxation or mechanical problem of the LES (hypotensive LES) leading
to copious amount of exposure of gastric components like pepsin, acid and bile
to the lower esophagus, results to reflux symptoms.4
Figure 1: Gastroesophageal reflux disease (GERD)
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DECLARATION

I hereby declare that this dissertation entitled “A comparative study of premenopausal and postmenopausal women with GERD in relation to serum estrogen levels” was prepared by me under the guidance and supervision of Dr.R.Nagashree, Professor & Head, Department of Physiology, PSG IMS&R.

This dissertation is submitted to The Tamilnadu Dr. MGR Medical University in fulfillment of the university regulations for the award of MD Degree in Physiology.

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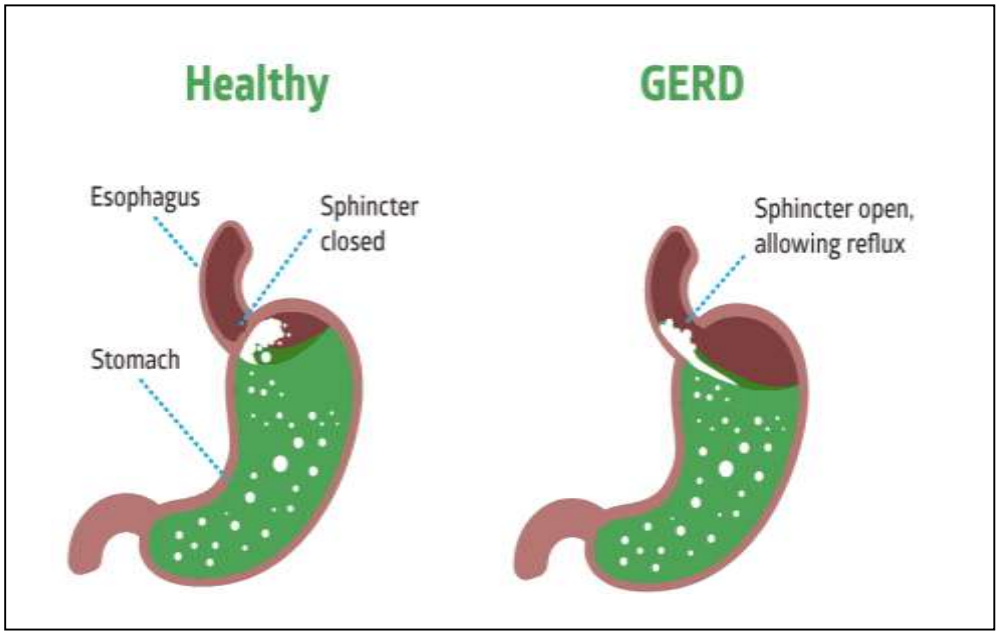
INTRODUCTION

INTRODUCTION

Gastroesophageal reflux disease (GERD), over past 40 years has risen from inconspicuous entity to one of the most important encountered clinical problem. It was first described by Asher Winkelstein in 1935 as peptic esophagitis and hiatus hernia. Later, in 1971, reflux disease was defined as a motility disorder due to transient lower esophageal sphincter relaxation or peristaltic disorder [1]. Now, GERD is evolving as a multifactorial disease.

Gastroesophageal reflux disease is a long term condition which is characterised by reflux of stomach contents into the esophagus causing troublesome symptoms and / or complications as defined by the Montreal Consensus [2]. The condition is marked by heartburn and acid regurgitation [3]. The aetiology of GERD is multifactorial with both genetic and environmental factors contributing different roles. Normally during swallowing, relaxation of lower esophageal sphincter (LES) allows food and liquid to flow into the stomach, followed by closure of the lower esophageal sphincter again. The main cause of GERD is transient lower esophageal sphincter relaxation or mechanical problem of the LES (hypotensive LES) leading to copious amount of exposure of gastric components like pepsin, acid and bile to the lower esophagus, results in reflux symptoms [4].

Figure 1: Gastroesophageal reflux disease (GERD)



Esophageal symptoms

Heartburn: It is a burning sensation in the retrosternal area, which may rise into the chest and may radiate towards the neck, throat and back. It mainly occurs after ingestion of large fatty meals, spicy foods, chocolates, citrus fruits, caffeine, smoking and alcohol. The supine position and bending forward may also accentuate heartburn. Heartburn during night affecting sleep, psychological and auditory stress decreases the threshold for perception of symptoms. Occurrence of two or more episode of heartburn in a week may help in the diagnosis of GERD, but symptoms may be less frequent if they are troublesome and affecting the wellbeing of the patients. The severity and frequency of heartburn is not correlated with the degree of damage to the esophagus [5].

Regurgitation: Montreal consensus states regurgitation as the perception of flow of refluxed gastric contents into the hypopharynx or mouth. Lower esophageal sphincter pressure is usually low in patients with regurgitation. They may also present with gastroparesis and esophagitis making the treatment for regurgitation much more difficult.

Although majority of the GERD patients are symptomatic, some of them may be asymptomatic. This usually occurs in elderly patients as they have decreased acidity of refluxed material and also due to their decrease in pain perception [5]. On the contrary, the complications of GERD like Barrett's esophagus may be more pronounced in the elderly.

Prevalence of Gastroesophageal Reflux Disease in Women:

Globally, the prevalence of GERD is highest among the South Asian countries and South East Europe. In India the prevalence of GERD is about 7.6–18.7% [6]. Genetic factors may be associated with GERD in 0-22%. The genetic risk of GERD is polygenic as no single mutation can be attributed to the cause. In India, the prevalence of GERD is rising, due to several other factors such as changes in lifestyle and dietary habits, socioeconomic status of Indians [7].

The prevalence of the GERD rapidly rises during the postmenopausal period than during the reproductive period [8]. The incidence of reflux esophagitis increases with aging women especially in postmenopausal period compared to male. The frequency and severity of esophageal symptoms was analysed quantitatively and it showed to be significantly higher in women than

in men [9]. According to another study by Lin Gerson et al, [10] showed that the quantitative esophageal symptom scores for heartburn, regurgitation, belching, and nocturnal symptoms that had been analyzed by endoscopy, ambulatory pH, and esophageal manometry, were found to be significantly higher in women than in men. From these results, we can infer that there is a difference in perception and reporting of symptoms between females and males. Women tend to have a higher frequency of GERD symptoms, which lowers their quality of life when compared to men.

Obesity and GERD:

Due to rapid urbanisation and industrialisation, prevailing epidemiological events are changing rapidly in India. The period after the independence, under nourishment was the primary disorder of health but now it is slowly replaced by over-nutrition and obesity. Thus, obesity is rapidly becoming an independent existence of disease. Obesity is an independent risk factor for GERD and hiatus hernia [11]. It is the major environmental risk factor associated with GERD. Obesity may also be associated with Barrett's esophagitis, erosive esophagitis and esophageal adenocarcinoma [4].

Therefore, this study focussed on relation between body mass index and GERD symptoms, for its impact on the treatment and to indicate as important risk factor for progression of GERD to adenocarcinoma of esophagus.

Oestrogen and GERD:

According to the World Health Organization (WHO) classification, [12] premenopausal women are those who have experienced regular menstrual

bleeding within the last 12 months, post-menopausal women are those who have not experienced menstrual bleeding for 12 months or more. Natural menopause[13] is defined as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity. Age of onset of natural menopause also varies worldwide, with the international range being 44.6 to 52 years. In India, the mean age ± SD is 45.02 ± 4.35 years [14]. Menopause is a critical period in a women life that not only marks the end of reproductive ability but it is also associated with multiple physical, vasomotor, psychological and sexual complaints, There is considerable variation in reporting of menopausal symptoms by women all over the world [15]. During the menopausal transition, ovarian production of estrogen and progesterone declines. This natural endocrine transition is associated with diminished circulating levels of estrone, estradiol, and sex hormone binding globulin (SHBG) and an associated increase in levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH)[16].

Estrogen is a female steroid hormone synthesized from the ovary that controls the menstrual cycle in females. Three major forms of physiological estrogens are present in females namely estrone (E1), estradiol (E2) or 17β-estradiol and estriol (E3). The three forms of the estrogens are derived from cholesterol by series of reactions. The major product from the whole biosynthesis process is E2 and it is the most potent estrogen. Healthy ovaries in reproductive females are characterised by their ability to synthesize estrogens. During the process of folliculogenesis both thecal and granulose cells are involved in cell-specific estrogen synthesis. These estrogens derived from the

ovary are released into general circulation and they target distal estrogen-responsive tissues which includes both reproductive and non-reproductive organs [17]. The reproductive status of the individual determines the level of estrogen synthesis and it reaches highest during the reproductive years and has a decline during transition and the postmenopausal period. During the menopause, serum E2 (17ß estradiol) levels decrease by 85–90% from mean premenopausal levels [18].

The esophageal barrier function plays an important role in the protection against reflux in GERD. Decrease in estrogen after menopause might be associated with the rise in the incidence and severity of reflux esophagitis. After menopause, a reduction in the levels of E2 can potentially increase epithelial permeability and translocation of microbes [19]. An animal study was conducted by Honda et al [20] to identify the role played by estrogen treatment on the esophageal epithelial barrier function and concluded that 17β-estradiol administration reduced the intercellular space dilation caused by luminal irritants. Moreover, expression of occludin was found to be increased with 17β-estradiol administration. Adhesion between esophageal neighbouring cells is also enhanced by estrogen. The reflux esophagitis can be attributed to lack of these protective effects of estrogen in menopause.

Estrogen has been found to have anti-inflammatory activity which may contribute to tissue resistance in females. Estrogen can target the tissue macrophage inhibitory factor to promote wound healing by inactivation of macrophages. It has been suggested that this anti-inflammatory role associated

with estrogen may be the cause of sex and gender differences in GERD. Estrogen also increases esophageal mucosal resistance by up regulation of the expression of esophageal tight junction protein namely occludin. This explains the estrogen predominance in pathophysiology of GERD. [21,22]

In this study, the electrochemiluminescence immunoassay [23] (ECLIA) is used for the in vitro quantitative determination of estradiol in human serum and plasma.

Patients presenting with GERD symptoms, visiting to outpatient department for the evaluation of reflux disease has been increased recently. In the United States, GERD is considered as most common indication for the upper esophageal endoscopy [24]. Endoscopy was considered in patients who had persistent or progressive GERD symptoms inspite of appropriate medical therapy, dysphagia, odynophagia, persistent vomiting, involuntary weight loss, anemia and Barrett's esophagus. Severity of reflux esophagitis was evidenced by upper esophageal endoscopy as ulceration, erythema, erosion, strictures and Barrett's esophagus are diagnostic with 95% specificity for GERD. Endoscopy with normal findings seen in 50% of patients with uncomplicated GERD. However, degree of esophageal damage does not correlate with the severity of GERD symptoms. [25, 26]

Lower esophageal sphincter pressure is measured by high resolution esophageal manometry [27]. It is the most direct method of assessment of motor function.It better characterizes the mechanisms of gastroesophageal reflux disease and abnormal esophageal motility.The functional anatomy of the

esophago-gastric junction, the segmental character of esophageal peristalsis and the dynamic action of the upper esophageal sphincter can be revealed by high resolution manometry [28].

Therefore, in order to study the lower esophageal sphincter laxity and to assess the severity of damage to the esophageal mucosa and gastro esophageal junction in symptomatic GERD patients, upper esophageal endoscopy was used and to study LES pressure, high resolution esophageal manometry was used in this study in both pre-menopausal and post-menopausal women who presented with heartburn and regurgitation.

The scientific evidence for an association between GERD and circulating endogenous estrogen level is sparse and contradictory. A better understanding of the response of basic serum estrogen hormone levels in premenopausal and postmenopausal women presenting with GERD will help in enhancing the research of examining the disease risk.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

AIM:

To compare the association between GERD and serum estrogen level in premenopausal and postmenopausal women.

OBJECTIVES:

a) To determine the pattern of symptoms and severity of GERD among premenopausal and postmenopausal women in relation to estrogen levels and endoscopy findings.

b) To determine the relation between body mass index and gastroesophageal symptoms.

REVIEW OF

LITERATURE

REVIEW OF LITERATURE

Gastroesophageal reflux disease:

Gastroesophageal reflux is a normal physiological process that occurs several times a day without symptoms or damage of the esophageal mucosa in healthy subjects. Gastroesophageal reflux occurs when excessive or associated impaired clearance of the refluxed gastric juice causes the complex disease called GERD. Gastroesophageal reflux disease is a condition that develops when there is a retrograde flow of stomach contents causing symptoms or complications. The incidence of GERD increases with age, especially after 40 years.

GERD can present as non erosive reflux disease and erosive reflux disease. The typical symptom of GERD occurs in the absence of visible mucosal injury during endoscopy called as non erosive reflux disease. In Erosive reflux disease, patients present with histopathological changes in esophageal mucosa, called as erosive esophagitis or reflux esophagitis [28].

The cardinal symptoms of GERD are heartburn and regurgitation. These symptoms most often manifest 30 minutes to 60 minutes after meals and lying back in a relaxed position. Patients often have a sense of relief on intake of baking soda and antacids. When patients present with these types of symptoms, the diagnosis can be established with a high degree of accuracy. Other symptoms of GERD include dysphagia (difficulty in swallowing), odynophagia (painful swallowing), globus sensation or lump in the throat, and nausea. GERD may also present with certain atypical presentations. These atypical

presentations refer to symptoms that are extra esophageal, which includes chronic cough, recurrent aspiration pneumonia, asthma, non - cardiac chest pain and arrhythmias. The most common complications of GERD include esophagitis, esophageal strictures, and Barrett's esophagus.

The normal gastroesophageal junction integrity is maintained by both the LES tone and the crural diaphragm to prevent GERD. In 90% of patients, transient LES relaxation causes GERD symptoms without hiatus hernia. Thus, the major factor that predisposes to GERD is the tonicity, structural and functional defects in LES. Other predisposing factors such as delayed gastric emptying, hypersecretion of gastric acid, pregnancy also cause GERD [29].

An important role, in the pathogenesis of GERD, is by contact of esophageal mucosa with the refluxate, which are mostly composed of acid, pepsin, bile and duodenal contents. A major role is played by acid in most of the patients affected by GERD. The duration of acid exposure is directly proportional to the severity of reflux esophagitis as well as the prevalence of complications such as Barrett's esophagus. The role of other components like biliary acids or pancreatic enzymes, may also contribute to the pathogenesis of GERD.

Many mechanisms are involved in the pathogenesis of GERD. They include anatomical factor like hiatus hernia, motor abnormalities, such as impaired lower esophageal sphincter (LES) resting tone, transient LES relaxation (TLESR), impaired esophageal acid clearance, visceral hypersensitivity, delayed gastric emptying and impaired mucosal resistance [3].

Hiatus hernia:

Hiatus hernia is frequently noted in patients with GERD. The proximal part of the stomach dislocates through the diaphragmatic hiatus into the chest, and thus the crural diaphragm gets separated from the LES [30]. This is a major factor causing disruption of the integrity of the gastroesophageal sphincter, leading to increased amount of acid exposure to esophagus. Hiatus hernia is the most important factor for chronic GERD by hampering the function of LES [31].

LES pressure abnormalities:

Some of the patients with GERD have a sustained weak, low pressure LES, which causes reflux every time the pressure in the stomach exceeds than that of the LES pressure. This commonly occurs when LES pressure is < 6 mmHg. A chronically decreased LES resting tone may usually be associated with severe esophagitis. LES defects have also been found in many patients with other GERD complications, like esophageal stricture and Barrett's esophagus. Factors decreasing LES tone include medications such as nitrates, calcium channel blockers. Endogenous hormones such as cholecystokinin, progesterone in pregnancy [32] and specific foods like fatty meals, chocolate and habits like smoking, caffeine and alcohol also reduces the tonicity of LES [33].

Transient lower esophageal sphincter relaxation (TLESR):

Transient lower esophageal sphincter relaxation is brief episode of LES relaxation unrelated to swallowing or peristalsis [34]. The TLESR is a visceral reflex with vagal afferent and efferent pathways that transmit information to and from the dorsal nucleus of the vagus [35]. The TLESR is mainly induced by

gastric distension caused by stimulation of proximal gastric tension and stretch receptors [36]. Gastroesophageal reflux occurs normally during swallowing induced LES relaxation and TLESR. In patients with GERD, about 48-73% of reflux episodes occur during TLESR. Fat, chocolate, alcohol and smoking may also affect the frequency of TLESR [29].

Impaired oesophageal acid clearance and visceral hypersensitivity:

The degree and duration of esophageal acid exposure determines the degree of esophageal mucosal injury and frequency and severity of symptoms[37]. The esophageal acid clearance by primary and secondary peristalsis, swallowing of salivary bicarbonate, acts as an important protective mechanism against development of GERD. It has been found that GERD patients have acid clearance time, two to three times longer than the normal subjects. Impaired volume clearance has been identified to be caused by peristaltic dysfunction and re-reflux. Failed peristalsis and low-amplitude contractions (< 30 mmHg), causes peristaltic dysfunction, leading to incomplete esophageal emptying. Hiatal hernias cause re-reflux, as the cleared fluid trapped in the hernia returns into the esophagus after LES relaxation. Prolongation of acid clearance is also induced by a reduced salivary rate or by decreased salivary capacity to neutralize acid. Reduced salivation during sleep accounts for marked prolongation of acid clearance, which is a major causative factor in serious form of GERD [38].

Delayed gastric emptying:

During the post-prandial period, delayed gastric emptying leads to increased retention of acidified gastric contents in the stomach, which may increase the risk of GERD. Delay in gastric emptying has a role in the pathogenesis of GERD in a small number of patients, mainly by increasing the available refluxate amount and thereby leading to gastric distension [39].

Impaired mucosal resistance:

The ability of the oesophageal mucosa to resist injury is an important factor in the development of GERD. The mucosa of oesophagus contains several structural and functional components, which protects against noxious substances. The mucosa is protected by strong epithelium, which has a rich blood supply and a weak pre-epithelium. The pre-epithelial defence system consists of a small layer of bicarbonate derived from the submucosal glands secretions in the oesophagus with less buffering capacity and also from swallowed salivary secretions. The epithelial defence system mainly consists of three components. They are the cellular and intercellular buffers like bicarbonate, phosphate and proteins that helps in neutralising back-diffusing luminal acid, cell membranes and the intercellular junctional complex, which helps by limiting the rate of hydrogen ion penetration into the intercellular space or cell cytosol, and ion transporters present in the cell membrane serve to release acid from the cytosol of cell when intracellular pH falls to acidic levels [40].

With these precise information in mind it is vital to study the basic anatomy and physiology of the upper gastrointestinal system to understand the pathogenesis of GERD.

Anatomy of Gastroesophageal junction

Anatomically, esophagus is a long muscular tube with length of about 18 to 25 cm. The food travels from mouth to stomach, through the food conduit, which consists of the oral cavity, pharynx, and esophagus. The esophagus serves as a dynamic tube, pushing the food forward from mouth towards the stomach. The esophageal mucosa secretes mucous to provide lubrication and easy passage of food. The peristaltic contractions actively propel food particles from the esophagus into the stomach. There are three distinct regions of esophagus namely cervical, thoracic and abdominal [41]. Gastroesophageal junction is a part of the abdominal esophagus. The extension of abdominal esophagus is from the diaphragmatic hiatus to the orifice of the cardia of the stomach. By forming a truncated cone, about 1 cm long, the base of the esophagus makes a transition smoothly into the cardiac orifice of the stomach. The abdominal esophagus located in the esophageal groove, which is present on the posterior surface of the left lobe of the liver. The esophagus is collapsed in between swallows to accommodate a swallowed bolus, as the lumen distends upto 2 cm anterio - posteriorly and laterally upto 3 cm [42].

Structurally, the wall of the esophagus is composed of four layers from inner to outermost namely mucosa, submucosa, muscularis propria, and adventitia. Unlike the rest of gastrointestinal tract, the esophagus has no serosal

layer. The esophagus is divided into three parts namely the upper esophageal sphincter, middle esophageal body and lower esophageal sphincter. Upper part of esophagus contains striated muscle fibres, lower part of esophagus contains smooth muscle fibres and middle body of esophagus contains both types of muscle fibres. The two high pressure zones namely upper and lower esophageal sphincter prevent the backflow of food. The limits of the sphincters are not clearly demarcated anatomically [43].

Lower esophageal sphincter (LES)

The lower esophageal sphincter is a high-pressure zone located where the esophagus merges with the stomach. It is also called as cardiac sphincter or gastroesophageal sphincter. It is a specialized region of the esophageal circular smooth muscle that allows passage of swallowed bolus to the stomach and prevents reflux of gastric contents into esophagus.The LES is a functional unit, which is composed of an intrinsic and an extrinsic component. The intrinsic structure of LES mainly consists of esophageal muscle fibers and is under neurohormonal influence. The extrinsic component mainly consists of the diaphragm muscle, which functions as an adjunctive external sphincter that raises the pressure in the distal esophagus related to the movements of respiration. Malfunction in any of these two components is the major cause of gastroesophageal reflux and its subsequent symptoms and mucosal changes. LES is 3-4 cm high pressure zone of muscular activity in distal esophagus. Normal LES pressure in healthy individuals is about 10 - 30 mm Hg above the intragastric pressure and this account for ninety percent of the basal pressure at

the gastroesophageal junction.When LES relaxes, stomach contents wash up into esophagus repeatedly and irritate the lining of esophagus [44].

The intrinsic component of the LES consists of circular layers of the esophagus, clasp-like semi-circular smooth muscle fibers on the right side, and sling-like oblique gastric muscle fibers on the left side. The circular muscles of the LES are thicker than the adjacent esophagus. The clasp-like semi-circular fibers has significant myogenic tone but are not very responsive to cholinergic stimulation, whereas the sling-like oblique gastric fibers have little resting tone but contract vigorously to cholinergic stimulation.

The extrinsic component of the LES consists of the crural diaphragm, which forms the esophageal hiatus, and it represents a channel through which the esophagus enters into the abdomen. The crural diaphragm encircles the proximal 2 to 4 cm of the LES, and determines inspiratory spike-like increases in LES pressure as measured by esophageal manometry.[42,44]

The LES is innervated by both parasympathetic (vagus) and sympathetic (primarily splanchnic) nerves. The vagal pathways are essential for reflex relaxation of LES. Vagal sensory afferents from the LES and distal esophagus end in nucleus tractus solitarius of the hindbrain. The motor innervation of the LES is provided topographically through preganglionic fibers from the dorsal motor nucleus of the vagus. The dorsal motor nucleus along with the tractus solitarius nucleus forms a dorsal vagal complex in the hindbrain that coordinates reflux control of the sphincter [45].

Lower esophageal sphincter is mainly controlled by vagus, as it plays a vital role in controlling tonicity, relaxation during swallowing and transient LES relaxation. The TLES relaxation linked with reflux of acid and belching during secondary peristalsis. Effect of sympathetic innervation of the LES found to increase the LES pressure.

The lower oesophageal sphincter plays an essential role in maintaining antireflux barrier between stomach and oesophagus. Myogenic tone of LES is maintained by a specialized thickened circular smooth muscle present around it. Excitatory and inhibitory motor neurons, which carry neurotransmitters, supply circular smooth muscle and finally join with vagal preganglionic neurons. Combination of physical, myogenic and neurogenic mechanisms maintains the closure of LES. Activation of inhibitory motor neurons causes LES relaxation to facilitate swallowing, belching, and gastroesophageal reflux episodes. Preganglionic neurons of vagus present in LES, through nicotinic cholinergic receptors linked to enteric motor neurons, which is inhibitory in function. Other receptors like serotonin (5-HT) receptors and muscarinic (M1) also involved in synaptic transmission of vagus. Relaxation of LES is also maintained by recruiting enteric inhibitory motor neurons by intrinsic reflexes[46].

Jaswant et al distinguished two types of vagal fibers according to its discharge patterns in animal experiments. The two types of fibers estimated with unusual latency gradients. The findings in the study concluded that vagal

fibres of short latency projected to the myenteric inhibitory neurons and the long latency vagal fibers projected to the excitatory myenteric neurons [47].

Blackshaw et al, explained experimentally the mechanism of neurotransmitters involved in extrinsic neural influences are primarily adrenergic inhibition over excitation reaction to the sympathetic nerve stimulation, and the release of tachykinin which is inhibitory to the stimulation of peripheral nerve. These mechanisms are essential in LES control both in normal and disease state. In normal physiological condition, collaterals from afferent fibre does not release tachykinins, but occur in certain conditions like inflammation, noxious stimulation and reflux disease due to excitation of local axon collateral reflexes [48].

The transient relaxation of lower esophageal sphincter is also neurochemically mediated by nitric acid (NO) and vasoactive intestinal polypeptide (VIP) [49]. The release of nitric oxide and vasoactive intestinal polypeptide from the interneuron causes LES relaxation [50]. The LES contraction is controlled by the discharge of acetylcholine from vagal nerve endings. The contraction of the chest and abdominal muscles during respiration is controlled by phrenic a nerve that helps in the maintenance of tone of the lower esophageal sphincter [44].

Physiology of esophageal phase of Peristalsis:

The bolus of food is propelled from the pharynx to the stomach through a tubular structure called as esophagus. There are two types of peristalsis occurs in esophagus known as primary and secondary peristalsis. The food material

enter the esophagus by relaxation of upper esophageal sphincter, primary peristalsis begins which helps in propelling the food downwards towards the stomach. Reflex contraction of upper esophageal sphincter prevents regurgitation of food bolus back into the pharynx [49].

When bolus of food enters the esophagus, a ring of contraction is formed cephalad to the food bolus, which helps in propelling the bolus forward to reach the stomach. The waves of peristalsis travels at about 2-6 cm/sec it can even take up to 10 seconds to propel the food through the entire esophagus down into stomach. Secondary peristalsis initiated by presence of bolus of food in the esophagus due to incomplete primary peristaltic wave. During quiescent period, the LES remains tonically contracted normally and it relaxes when peristaltic wave move towards the sphincter, which helps in propelling the food without any resistance to enter the stomach [44]. The inhibition of circular muscles of sphincter by vagal neurons, causes relaxation of LES. It is mediated by activation of cholinergic vagal fibres that secrete nitric oxide and vasoactive intestinal polypeptide. During digestion in stomach, the local hormone gastrin serves to keep the LES tightly closed by increasing the tone of the sphincter. Hence, LES prevents reflux of food from stomach back into the esophagus.[44,48] The movement of the bolus of food is carried out by coordinated contractions of striated muscle layer in cephalic region and smooth muscle layer in thoracic region of esophagus. In upright position, the gravity allows the food bolus to pass at a faster rate through the esophagus [48].

Kronecker et al, experimentally observed that there was a pressure change in esophagus during the late swallow of bolus of liquid, not when the bolus entered the stomach. Hence, it was concluded that esophagus act as a passive channel during transport of food bolus in upright position[41].

Ingelfinger et al, concluded that in head down position, barium swallow transport of bolus under fluoroscopy guidance is carried by the peristaltic waves towards the stomach from the esophagus.[48,51]

The major portion of esophagus lies in the thoracic region. Due to lower pressure than pharynx and stomach, it can combat the passage of gastric contents and air. The upper and lower sphincter on either protects the esophagus by being closed in between the swallows. During swallowing, the pressure at various levels of esophagus can be monitored by pressure sensing devices.[49,52]

The mechanism of peristalsis in the striated muscle of the esophagus is experimentally mediated by the motor neurons present in the swallowing centre of brain stem in vitro. On electrical stimulation, there was a tetanic contraction of the striated muscle and immediate termination of contraction followed by removing the stimulus. Efferents of vagus on electrical stimulation in vivo generated a non-peristaltic tetanic contraction [53].

Christensen et al observed that the esophageal circular smooth muscle contracts on termination of stimulus due to rebound phenomenon [54]. Further, this study suggested that peristalsis in smooth muscle of esophagus is mediated by local inhibitory neurons.

Weisbrodt et al observed that the walls of the smooth muscle of the esophagus had inherent property of latency gradient. This study revealed that, the longest latency of contraction was in the proximal part of the esophagus and the shortest latency was towards the distal end of the esophagus [55].

The upper esophageal sphincter is a physiological sphincter. The contraction of cricopharyngeal muscle and elastic nature of sphincter causes closure of the upper esophageal sphincter. The continuous activity of vagal neurons and the release of neurotransmitter of acetylcholine from nucleus ambiguous maintain the tonicity of the upper esophageal sphincter. The relaxation of the upper esophageal sphincter is coordinated with the pharyngeal muscles of contractions. The upper esophageal sphincter relaxation is brought by suppression of nerve impulses from the swallowing centre through the activity of the nucleus ambiguous [49].

The prevalence of GERD is more common in women compared to men. The symptoms of GERD like heartburn, regurgitation, dysphagia, belching, extra esophageal symptoms more seen in women than men. The female steroid hormone namely estrogen is closely related to prevalence of GERD in elderly[56].

Masakaet al observed in experimental rat model, that severity of damage to the mucosa of the esophagus is related to the gender difference in GERD. The findings concluded that estrogen plays a beneficial role in preventing the risk of esophageal damage in reproductive years of women [57].

Asanuma et al reported that, the protective mechanism of estrogen delays the progression of GERD to the development of Barrett's esophagus,

adenocarcinoma of esophagus especially in postmenopausal women. This study also explains that mucosa of lower part of esophagus express TRPV1 in increased levels in GERD patients. Hence, this factor increases the sensitivity of pain to gastric reflux in women [8]. The decline in estrogen level in postmenopausal women might play an important role in increasing the symptoms of GERD.

Lagergren et al and Vaezi et al found that estrogen executes an anti – inflammatory action which may protect the mucosa of esophagus in reproductive women against esophageal carcinoma when compared to postmenopausal women. Therefore, the female sex hormone, estrogen plays a significant role in the pathogenesis of GERD in women.[58,59]

The knowledge of obesity causing GERD is widespread. Hashem et al found a positive relation between symptoms and frequency of GERD and obesity among older women. The factors, which increased risk of GERD by obesity, are consumption of high amount of fatty diet, distribution of body fat in abdomen, humoral factors like insulin, leptin. Abdominal obesity increases intragastric pressure, relaxation of TLES frequency and hiatus hernia formation[60].

Nilsson M et al did a study to evaluate the relation between body mass and gastroesophageal reflux symptoms and to determine how this relation was influenced by estrogen. The positive association among women seemed to be augmented by postmenopausal hormone therapy, suggesting a role of female sex hormones in the etiology of reflux disease. It was

concluded that there is a significant association between body mass and symptoms of gastroesophageal reflux. The association was stronger among premenopausal women under hormonal therapy. This study also highlighted that estrogen mediates increased nitric oxide synthesis leading to LES relaxation [61].

Georgios Kouklakis et al evaluated the presence of GERD in a Greek cohort in relation to the Body Mass Index (BMI) using the 3-hr postprandial esophageal pH monitoring. They concluded that there was a strong correlation between obesity and severity of the gastroesophageal reflux. Patients who were obese and overweight had significantly higher distal esophageal acid exposure time. They found that a significantly decreased lower esophageal sphincter pressure was evident when the BMI increased [62].

Bergstrom et al found that, there was no positive relation between reflux symptoms and obesity when compared to normal BMI individuals. Heartburn and regurgitation symptoms were assessed by using GERD scoring questionnaire. This study revealed that obesity plays an independent role in causing Barrett's esophagus and esophageal carcinoma. The reduction of body weight does not found to decrease the severity, frequency of heartburn and regurgitation [63]. Hence, the scientific knowledge about the association between the obesity and GERD is not adequate and the results are contradictory.

Assessment of LES:

The typical symptoms caused by GERD are heartburn and regurgitation. These symptoms can be further assessed by diagnostic testing. Ambulatory pH

monitoring considered as a gold standard diagnostic tool for monitoring the reflux in GERD patients. Esophageal manometry is commonly used to diagnose esophageal motility disorders [64]. It is also considered to be used in the diagnosis of reflux disease. The breeching of anti-reflux barrier along with peristalsis disturbances in GERD are not diagnostic findings since there is no pathognomic reflux manometric design [65].

The basis of esophageal manometry is used to measure and record the pressures patterns with the use of a catheter from the lumen of esophagus that drive a transport of bolus during a specific time [27]. In late 19th century, balloon tipped catheters was used in animals and humans for measurement of the intra – esophageal pressure. To record the volume change, water manometer was connected to the intraluminal balloon. The balloon attached to the open tipped catheter caused obstacle to liquid and solid swallow. They observed that the pressure changes are measured were from balloon. So, the balloons kymograph method was found imprecise in detecting pressure changes. To overcome this drawback, miniature balloons were placed at the tip of the catheter or over the micro transducer to measure the intraluminal pressures. To obtain accurate pressure measurements, finally they used non distensible polyester balloon to maintain the original diameter and shape of the balloon during recording [28].

In initial period of 20th century, Brody et al used non-perfused, open-tipped catheters to record peristalsis and pressure changes. To obtain highly accurate recording, they used catheters, which had holes at the side known as pneumo-hydraulic perfusion method [66].

The basic principle used to record the data is computer digitized and analyzed in many types of manometry devices. To measuring the intra-luminal and wall contact pressure in an accurate method, orientation and spacing of the pressure sensors, accuracy in measuring the pressure changes, and the digitization of the pressure signal being transmitted to a computer are the various the components need to be taken in account [67]. The features of manometry recording are temporal resolution which denotes the degree of measurement of pressure, spatial resolution refer to the distance between the recording points and the precision of readings obtained.

There are two types of manometric recording systems based on the placement of sensors inside or outside the catheter. In the latter system, where the sensors located externally the pressures are transmitted along a column of water perfused slowly through the catheter. In both the systems, the data are computer digitized and recorded.

The water perfused external transducers are preferred than intraluminal transducers due to easy maintenance, cost effective, excellent dynamic performance. The catheters are stiff, delicate, expensive and show fluctuations in pressure due to changes in temperature than water perfused catheters. Since these catheters are temperature sensitive, individual baseline recording is affected.

In the water perfused external transducer system, from the pneumo-hydraulic pump water moves through the external transducers the rate of flow of water inside each channel is recorded. This movement of water is regulated

by capillary resistance present in opposite end of the flexible thin catheter thus ensuring the patient to feel comfortable during recording the pressure by manometry. The pressure measured by manometry is not continuous so the pressure recorded at a distance of 1-3 cm at a frequency 25 Hz or higher by manometry within the body of esophagus gives temporal resolution [67].

The factors, which determine the pressure recordings, are transducer placement, pressure artefacts, pressure changes that initiate pneumo-hydraulic pump perfusion bubbles effect in the lumen of catheter, transducer drift. All these factors produce alteration in baseline pressure recording by the transducer, which is caused by change in electrical activity and temperature changes.

The device is calibrated by introducing the transducers inside the water bath to simulate as located inside the gastrointestinal tract. To the transducer a known pressure is introduced, in each transducer there should be same elevation of pressure. During recording, the rise in pressure must remain constant for about 20-30 minutes. The reasons for the artefacts, during recording of the pressure may be due to compression by liver and adjacent blood vessels, compression of the catheter on wall of the gastrointestinal tract due to bending and finally electrical disturbances caused by faulty transducers. To avoid all the above mentioned artefacts and to obtain an accurate recording the catheter should be repositioned to the required demand.

During visualizing the vital area on the computer screen, the lower esophageal sphincter appears as high pressured area. The presence of the lower esophageal sphincter, position of the diaphragm can be heightened during

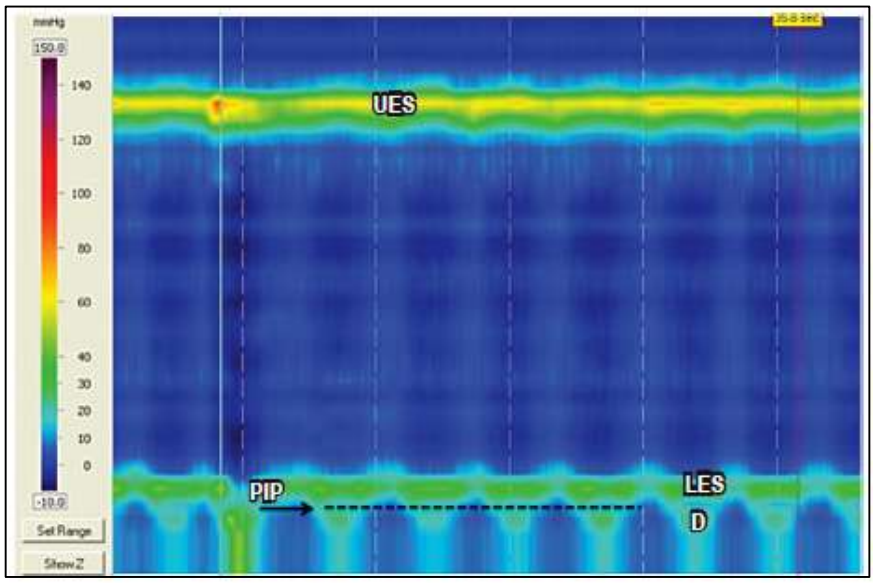
swallowing and deep breathing. The recordings can be visualized as a line plot or a spatiotemporal topographic color plot. The software measures the pressure events of the observed area of the gastrointestinal tract especially during swallowing [67].

The high fidelity manometry system was introduced first in 1970 by Wyle Jerry Dodds and Ron Arndorfer. After 20 years, in 1990 Ray Clouse introduced high-resolution manometry (HRM) system with certain modifications. In the conventional manometry, five pressure sensors located at a 3-5cm distance but in HRM, the long catheter has 36 pressure sensor placed at a distance of 1cm to view upper and lower esophageal sphincters during each swallow. Both spatial and temporal resolution of the esophageal motor function is visualized and recorded [32]. The conventional manometry has two dimensional plots with pressure plotted along x-axis and time along y-axis whereas in HRM z-axis is added as third plot which assembled the gastric pressure waves in front and pharyngeal pressure on the back of topographic graph. The pressure plotting axis was changed to the y-axis and time along the x-axis. In the conventional system is of low cost and difficulty in placing the catheter in exact location of LES but there is no difference in accuracy of recording when compared to HRM. Clouse synchronized the pressures and presented as three dimensional color contours. The high pressures were of red and yellow and the low pressures were blue and green. The data are flashed through esophageal pressure topography (EPT) or Clouse plots which generated colorful spatiotemporal topography in contrast to conventional line tracings.[68,69] The Chicago

Classification categories motility disorders of esophagus which corresponds to high resolution manometry topographic plots [70].

Hani et al concluded that, HRM made systematic visualization of the upper esophageal sphincter, the esophageal body and the lower esophageal sphincter. HRM also forecasted motor function of esophagus, elements of anti-reflux barrier and reflux events. The resting pressures of the upper and lower esophageal sphincter has been evaluated by normal color change to pink and red shades respectively on the screen. The pressure inversion point (PIP) denotes in which the negative intrathoracic pressure changes to positive intragastric pressure, it is the point which depicts the diaphragm separating the chest and abdomen [71].

Figure 2: Upper esophageal sphincter (UES), Lower esophageal sphincter (LES), Pressure inversion point (PIP) and crural diaphragm (D) in HRM



Basal lower esophageal sphincter pressure:

Niebisch et al in his study concluded that the basal LES pressure was to be 27.9 ± 11.5 mmHg. The pressure range was 12.3 mmHg to 52.2 mmHg. In normal individuals, the high pressure zone accounts for 90 percent of the basal pressure marked at the gastroesophageal junction which is above the intragastric pressure [72]. Bogte et al in his study found that, the basal pressure to be around 29.35 mmHg and the mean EGJ relaxation pressure was 16.79 mmHg [44]. In our institute the normal basal LES pressure value taken in the range from 10 – 35 mmHg. Basal LES pressure below 10 mmHg is noted as reduced and elevated LES pressure is marked above 35 mm of Hg. Reduced lower esophageal pressure was found to be in patients with abnormal esophageal acid exposure.

Basal inspiratory and Basal expiratory pressure:

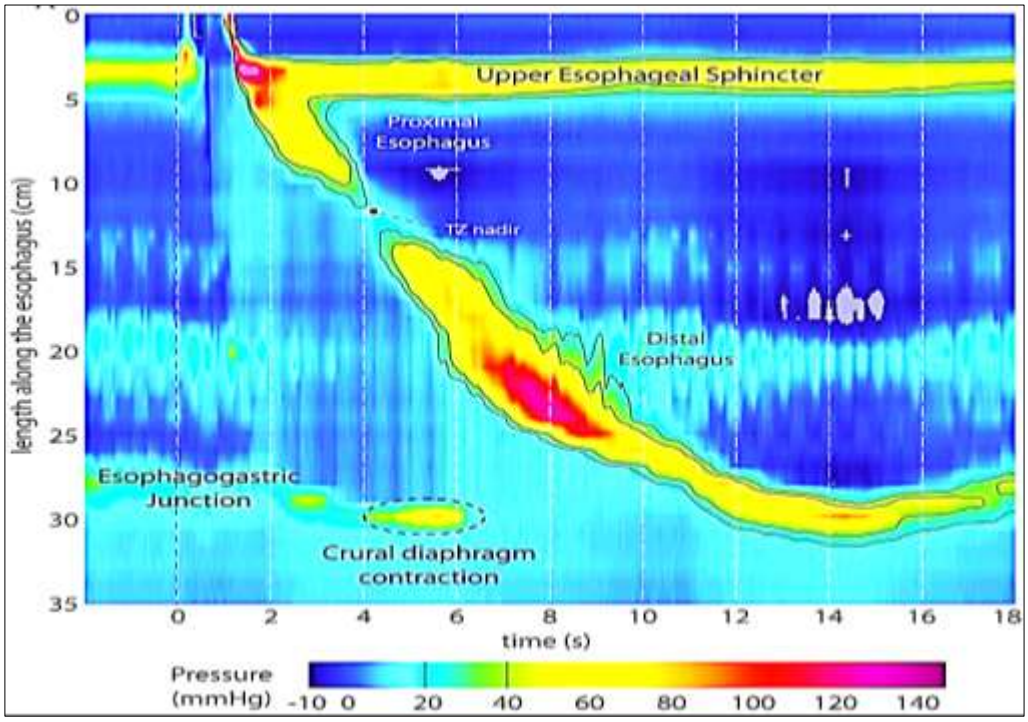
The basal expiratory pressure or basal LES pressure considered more accurate than mid respiratory lower esophageal sphincter pressure due to respiratory artefacts [73]. The rise in pressure during inhalation is due to the contraction of the diaphragm around the esophagus is characterized as LES pressure [74]. So the normal basal expiratory pressure range is considered as basal LES pressure of 10-35 mm of Hg.

Esophagogastric junction contractile integral (EGJ –Cl):

It represents the function of EGJ barrier function on HRM. By means of measuring distal contractile integral, mean: esophagogastric junction contractile integral value (mmHg.cm) is calculated. The average of both inspiratory and expiratory values for three respiratory cycles gives the EGJ

pressure. The esophagogastric junction possesses its function vitally at a pressure of about 20 mmHg. The pressure range over 2-5 cm considered as short peristaltic defect and pressure more than 5 cm considered as long peristaltic defect [7].

Figure 3: Esophagogastric junction in HRM



Median integrated relaxation pressure (IRP):

The EGJ junction is located before recording the mean (IRP). Deglutition window of 10 seconds is created in the region of the EGJ following the relaxation of upper esophageal sphincter [67]. The median IRP measures the relaxation of LES. The software provides the minimum mean pressure for 4 seconds inside the deglutition window by excluding the pressure generated by the bolus and crura of the diaphragm. The Chicago classification denotes the upper margin for IRP is 15 mmHg. The median integrated pressure value

greater than 15 mmHg denotes high resistance to bolus transit at EGJ. This pathological conditions can be seen in achalasia, strictures or neoplasm at the esophagealgastric junction.[71,67]

Distal contractile integral (DCI):

The distal contractile integrity denotes the strength of distal portion of esophagus contraction and it is calculated as amplitude x duration x length (mmHg-s-cm) of the contraction of distal section of esophagus. The pressure value greater than 20 mmHg from proximal to distal illustrated in the graph as troughs [67]. DCI explains the propagation of contractile length, mean contraction amplitude of the esophagus and the esophageal contraction duration.

Contractile deceleration point (CDP):

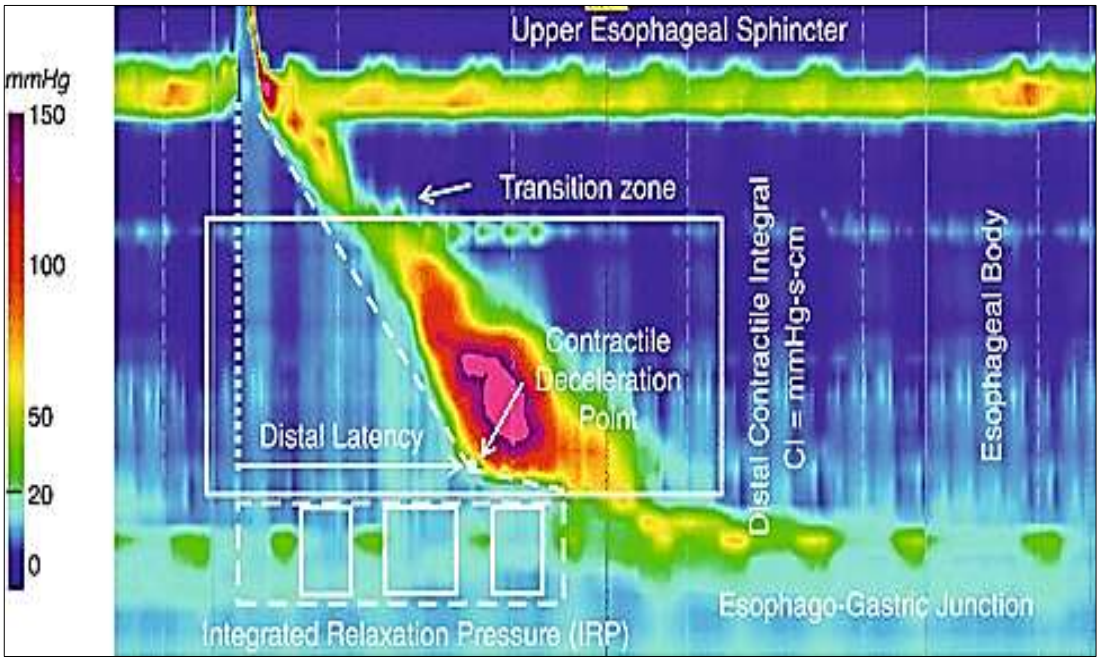
During emptying of the bolus from the esophagus into the stomach, the EGJ resistance is reduced in the distal esophagus, which lowers the velocity of peristaltic waves of contraction. This point is referred as contractile deceleration point. The speed of inclination of the line present between the transition zone and CDP called as contractile front velocity (CFV).The normal contractile front velocity value is computed by software to be lower than 9cm/sec [67].

Distal latency (DL):

It is the period between the relaxation of the upper esophageal sphincter to the contractile deceleration point. The distal latency gives information about the initiation of peristalsis and deglutition inhibition time. The lower margin for the DL is 4.5 seconds. The Chicago classification reveals that short distal

latency of the contraction of the esophagus, is considered as distal esophageal spasm [70].

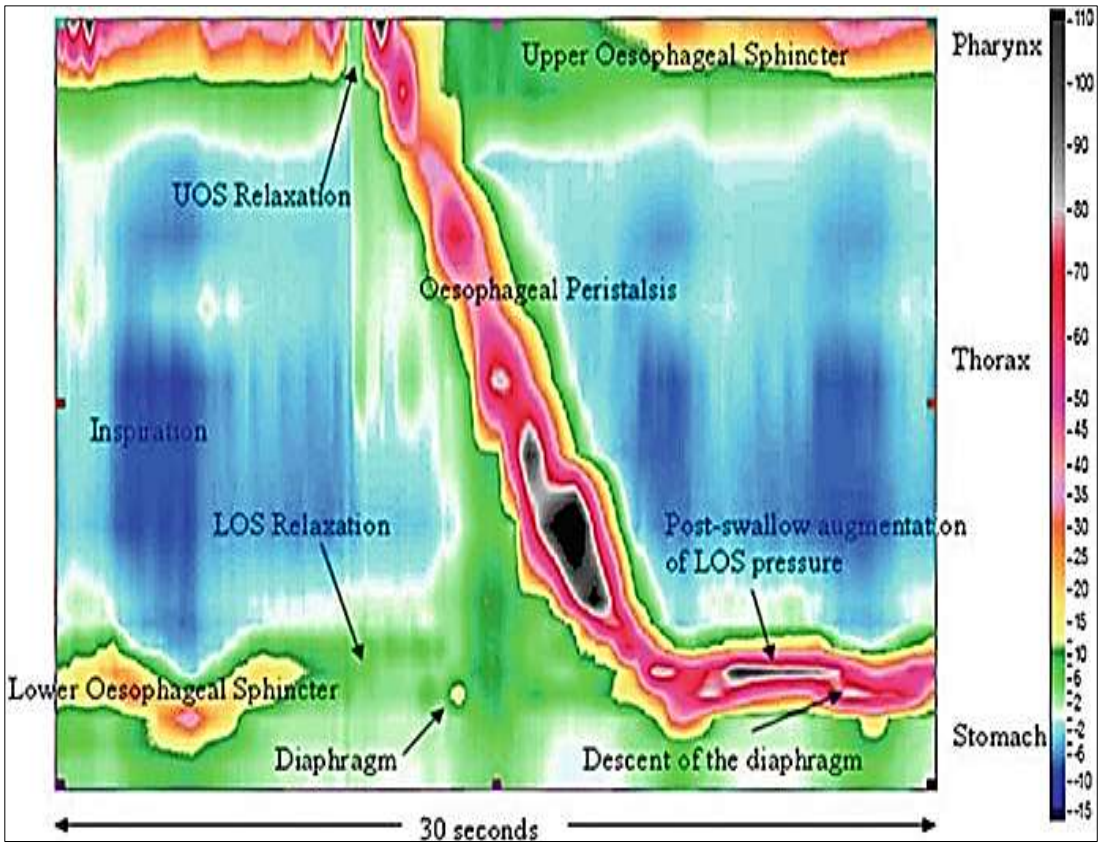
Figure 4: Median integrated relaxation pressure, Distal contractile integral, Distal latency, Contractile deceleration point in HRM



Esophageal body motility or peristalsis:

The esophageal body motility can be normal, propagative, or ineffective peristalsis. The normal peristalsis begins with relaxation of upper esophageal sphincter followed by transport of food bolus within the esophageal body and ends with relaxation of the lower esophageal sphincter thereby food enters the stomach. In HRM, ineffective esophageal motility is categorized as weak contraction when DCI value of < 450 mmHg/s/cm and failed contraction when DCI <100 mmHg/s/cm. Chen et al in his study revealed that ineffective esophageal peristalsis is common in GERD patients presented with heartburn and dysphagia [76].

Figure 5: Normal esophageal peristalsis in HRM



Esophageal gastro duodenoscopy (EGD) role in GERD:

In 1868, the first gastroscopy was invented by Kussmaul. The illumination in the endoscopy was worked out by Thomas Edison in 1878. Later, in 1911, Hoffmann designed the endoscopy with prisms and lenses. After about two decades, Wolf launched semiflexible gastroscope. Hopkins and Curtiss in 1954, invented fibreoptic flexible endoscope. Later years, the charge coupled device (CCD) was assimilated into endoscope which created a digital image took new era in endoscopy technique [77].

The endoscope consist of a control head incorporated with valves for suction and insufflation of air, a shaft which is flexible in nature has light guide with single or many service channels and a tip contains bending section. The

endoscope is connected to the processor, light source, suction and air supply by light guide connecting tube. The charge coupled device captures the image, transmits the captured image electronically and displays on the screen of a video monitor. The CCD chip contains photocells called as pixels, which works based on the light and dark degrees. There are two types of color assignment system. The system in which individual photocells are organized by a chain of color filter stripes called as color charge coupled device. The other method used the color filter, which is fixed in a rotating wheel to illuminate the pixel with primary color known as monochrome CCD. This system considered expensive due to implementation of advanced mechanical device and image processing tool. External source of high intensity delivers illumination trough light carrying fiber bundles.

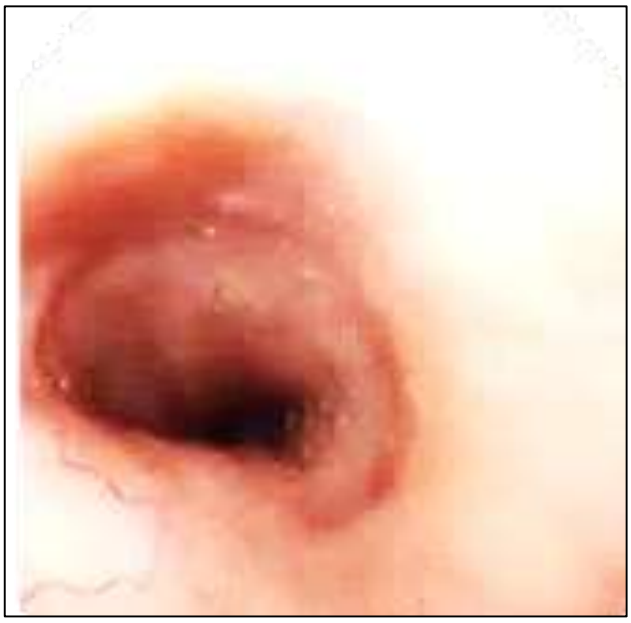
The shaft consists of suction or biopsy channel, which starts from biopsy port to the endoscope tip. The suction channel pushes the aspirated secretion through the suction valve by means of the external suction pump, which is connected, to the light guide connecting tube. The pump in the light source supplies the air, which passes through small channel to distend the organ to be examined. This air channel is also controlled by another valve. The air channel also allows stream of water to clean the distal lens by pressurizing the water bottle. The shaft of the endoscope maintains the stability of remaining straight when the rotation of the control head transmitted to the tip of the endoscope. The diameter of routine upper endoscopy instrument is 8 to 11mm this enables the shaft to incorporate and to safeguard the wires, tubes, bundles.

The endoscope tip and the bending section, which is 10cm in length, is turned in either planes upto 180°or more degrees. The pull wires present beneath the outer sheath of the tip travels back of the entire length of the shaft of endoscope until the two angulations control wheels on the control head maintains the tip control. The tip can be fixed in any desired position by a control wheel that contains braking friction device [78]. The origin of high resolution magnification endoscopes with new CCD, yielded high resolution images which accounts to detect 80% of lesion and to take biopsy in condition like Barrett's esophagus with dysplastic changes. The images are magnified from 1.5 to 150 times without increasing resolution. The contrast can be augmented by using dyes like Congo red, methylene blue, Indigo carmine that produces color change by reacting with the components of cell. Another technique called as equipment based technique, improved color contrast by using narrow band imaging which increased the blue and green light elements of a digital image. The changes like tissue with abnormal morphology and vascularity can be distinguished easily from normal tissue [79]. This method is time effective when compared with dye based technique.

On endoscopy, the lumen of esophagus appears as a smooth, pale pink tube with visible submucosal blood vessels. The transition from esophageal to gastric mucosa is called as the Z-line and consists of an irregular circumferential line between two areas of different colored mucosa. The gastric mucosa is darker as compared to the pale pink colour of esophageal mucosa. Peristaltic waves can be visualised during endoscopic examination [80].

Figure 6: Endoscopy view of mucosa of esophagus

NORMAL



GERD

Manometry is performed for localisation of LES, which adds to the time, expense and discomfort to the procedure. Majority of the patients undergo EGD prior to manometry, which may help in the localisation of LES. The localization of the LES endoscopically is different from that of manometric localization.

The endoscopic localization of the LES is presumed to be determined by changes in the esophageal mucosa colour owing to transition from non-stratified squamous epithelium of esophagus to the gastric mucosa, changes being known as the Z-line. A study correlating manometric and endoscopic localization of the LES (Z-line) concluded that the functional location of LES was 3 cm distal to the Z-line. Therefore it is necessary to localize the lower esophageal sphincter for the diagnostic purpose [41].

Sonnenburg et al suggested that there is ongoing rise in prevalence of GERD. It has streamed to evolve as one of the most important disease of upper gastrointestinal tract.

The role of upper endoscopy or esophageal gastro duodenoscopy has been raised in diagnosing GERD than peptic ulcer and gastric carcinoma [81].

GERD is classified into erosive reflux esophagitis (ERD) and non-erosive reflux esophagitis (NERD) by endoscopy. The Los Angeles system classified the erosive esophagitis by endoscopy into four grades. Grade A- mucosal break more than one, not more than 5mmwhich do not extend between the top of two folds of mucosa, Grade B - mucosal break more than one, more than 5mm which do not extend between the top of two folds of mucosa, Grade C - mucosal break more than one extending between the top of two or more fold of mucosa involving not more than 75% of circumference of the esophagus, Grade D- mucosal break more than one involving at least 75% of circumference of esophagus [11].

Asanuma et al concluded in the study that the postmenopausal females suffer from erosive reflux esophagitis in higher rate when compared to males. The Barrett's esophagus and esophageal carcinoma tend to increased prevalence in postmenopausal females. The non-erosive reflux esophagitis is seen most commonly in premenopausal age group. The anti-inflammatory action of estrogen during the premenopausal period prevents the development of metaplasia of esophagus and its progression to adenocarcinoma of esophagus. Hence esophageal gastro duodenoscopy plays a vital role in diagnosing and evaluating the GERD patients [8].

Electrochemiluminescence Immunoassay (CIA):

Immunoassay method is used for the in vitro quantitative estimation of estradiol in human serum. There are many methods of immunoassay. They include fluoro-immunoassay, bioluminescence, enzyme linked immunoassay and chemiluminescence technique.

The chemiluminescence (CIA) works on the principle of production of light by the chemicals, which is measured by luminometer. The steroid hormones like estrogen are labelled with chemiluminescent substances. CIA is a non-radioisotope sensitive method. CIA is considered as convenient, time conserving, and an easy technique to perform than radio immunoassay technique [82].

The electrochemiluminescence immunoassay (ECLIA) method is further proposed to use on Elecsys and cobas e immunoassay analyzers. ECLIA works by using competition test principle. The competitive test includes two

specific monoclonal antibodies reacts with 17beta estradiol, which is biologically most active estrogen. The derivative of estradiol labeled ruthenium complex is added to the test sample containing endogenous estradiol, which is released by mesterolone. The endogenous estradiol present in the sample competes with ruthenium complex to bind on the estradiol biotinylated antibodies binding sites.

The microparticles in the reaction mixture are transferred to the measuring cell attaches to the surface of the electrode by magnetic force. The chemiluminescence reaction is produced when the voltage is applied to the electrode. The photomultiplier measures the chemiluminescent emission. The Elecsys and cobas e immunoassay analyzer automatically calculates the estrogen concentration in the sample, which gives accurate results in pg /ml. The results are delivered by instrument derived specific two point calibration master curve which is read through reagent barcode [23].

MATERIALS

AND

METHODOLOGY

METHODOLOGY

Inclusion criteria:

1. Females belonging to the age intervals between 35 years to 70 years.
2. Females presenting with symptoms or investigations suggestive of GERD.

Exclusion criteria:

1. Females less than 35 years of age and more than 70 years.
2. Females who does not possess the symptoms of GERD.
3. Females who underwent hysterectomy.
4. Pregnant women.
5. Carcinoma of the upper gastrointestinal tract.
6. History of gall stones.

Materials and Methodology:

The study was conducted in the Gastrointestinal Motility Laboratory in the Department of Gastroenterology, PSGIMS&R, Coimbatore, after obtaining clearance from the Institutional Human Ethics Committee (IHEC). Informed and written consent was obtained in the regional language before initiation of the study from the participants who were involved in this study.

It is a cross sectional observational study. Participants with GERD were subjected for selection based on history followed by a preliminary questionnaire(GERD HRQL), which were further down streamed differentiating among premenopause (n = 30) and postmenopause (n = 30)

women. All participants were assessed for body weight and height. BMI was calculated based on objective measurements as

$$\text{Body mass index (BMI)} = \frac{\text{Weight (kg)}}{\text{Square of height (m)}}$$

The body mass index was classified into three categories according to WHO classification of body mass index [83].

1. Normal (18.5 -24.99 kg/m^2)
2. Overweight (25.0 -29.99 kg/m^2)
3. Obese (>30 kg/m^2)

The study participants were analyzed for their gastroesophageal reflux disease based on their symptoms, severity was assessed endoscopically by Los Angeles classification of esophagitis, [11] and the study participants were subjected to high resolution esophageal manometric study. The level of serum estrogen was checked using electrochemiluminescence immunoassay (ECLIA)[23] to scrutinize and evaluate the influence of estrogen in the causation of gastroesophageal reflux disease among the women.

Assessment of symptoms and severity of GERD:

The study participants diagnosed as GERD were subjected to assess the symptoms and severity by GERD Health Related Quality of Life Questionnaire (HRQL) [84,85] scoring system. This validated questionnaire was established to evaluate the symptoms like heartburn and regurgitation. The scoring system has a scale from 0 to 5.

Scale 0 - no symptoms

Scale 1- not bothersome noticeable symptoms

Scale 2 - bothersome noticeable symptoms and not seen daily

Scale 3 - bothersome symptoms present daily

Scale 4 - symptoms affecting every day activities

Scale 5 - incapacitated symptoms not able to do every day activities

The each question of heartburn score greater than 2 indicates typical heart burn scores. Similarly typical regurgitation question scores indicate each question scoring greater than 2.The scores are calculated by summating the individual scores. The summated score of $\geq$ 12 is considered as total heartburn and regurgitation score individually. This scoring system is further classified as score 0 indicating no symptoms and score 30 as worst symptoms of heart burn and regurgitation.

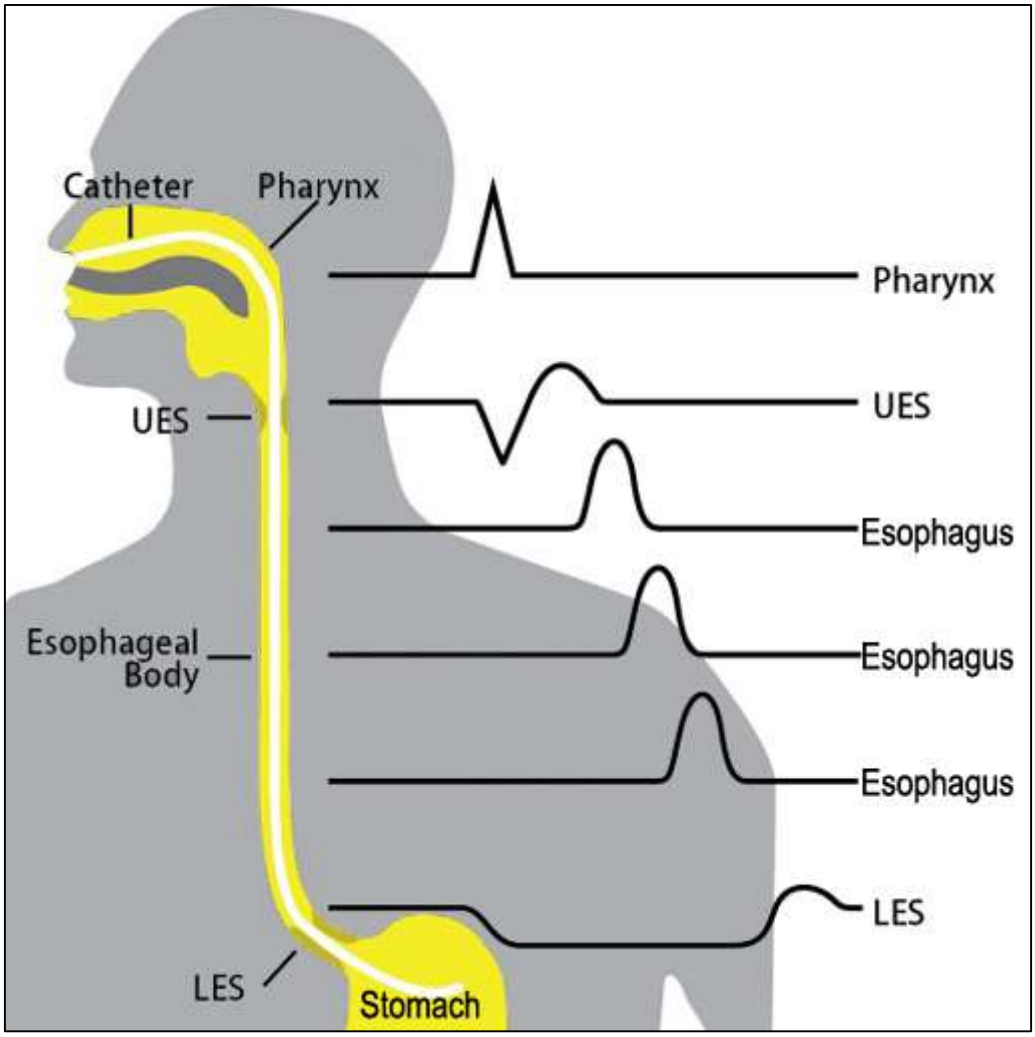
High resolution esophageal manometry:

The study participants were asked to come for esophageal manometry with overnight fasting and were advised not to take anything orally until the procedure was complete. The water perfused catheter, manufactured by Dent sleeve, Canada CE mark. The 16 channel water perfused manometry catheter was used. The upper 8 channels are placed at a distance of 3cm gap and lower 8 channels are placed at a distance of 1cm. The perfusion pressure maintained at 0.2 ml / minute. The water based perfusion HRM system, manufactured by Royal Melbourne hospital (RMH), Australia.

The study participants were made to lie flat in supine position. Prior to the insertion of the probe or catheter, the nasal cavity of the patients were examined with the help of sufficient light to rule out any nasal septal deviation or polyp, which could hinder the entry of the catheter. Lignocaine gel was applied on the probe for anesthetic and lubricative purpose and the probe was inserted into the esophagus through the nasal cavity.

The study participants were asked to swallow the tube, like swallowing saliva. Since the catheter was small and flexible, it was easily passed into the esophagus. The study participants' complete cooperation and patience for doing the procedure was highly essential for the effective completion of the procedure. The probe enters the nasal cavity, pharynx, upper esophageal sphincter, esophageal body, lower esophageal sphincter. The physiologist monitored the observed pressure changes by passage of probe into the lower esophageal sphincter.

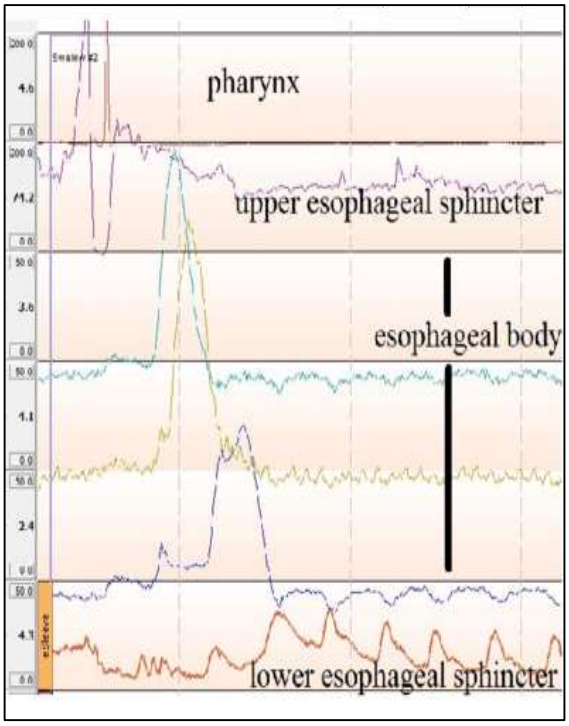
Figure 8: High resolution manometry pressure catheter and trace



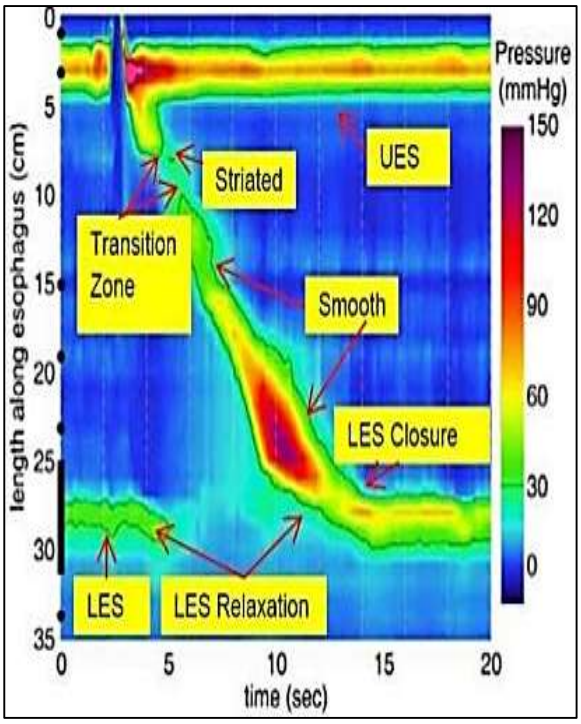
The pressure changes observed was initially plotted on a line plot, which was then converted into a color graph on a computer screen. The identification of lower esophageal sphincter was made by change in color on the color graph and rise in pressure.

Figure 9: Normal high resolution esophageal manometry

Line plot



Color contour plot



Initially, for one minute a basal reading was recorded. The study participants were instructed to swallow about 5ml of water (wet swallow) slowly at once. The study participants were clearly instructed not to swallow the saliva in between the swallow of water. The wet swallow was given ten times at an interval of 30 seconds to the study participants, the motility and pressure changes recordings were made between two wet swallow. Finally, at the end of ten wet swallow, the nasal catheter was removed safely from the esophagus.

Using high resolution esophageal manometry the following parameters were recorded and analyzed using Trace 1.2.3 Chicago classification (Geoffrey S. Hebbard, RMH, Australia) [67].

1. Basal lower esophageal sphincter (LES) pressure - The basal LES pressure was recorded after identifying the lower esophageal sphincter as band of high pressure zone. The tracing runs for at last three inspiration and expiration and no swallows in that period. The basal LES pressure recorded for one minute. The normal basal LES pressure is 10 – 35 mm Hg [86,67].

2. Esophagogastric junction contractile integral (EGJ-CI) – The mean basal pressure was found to be 20.5 mm Hg in water perfused HRM assembly [87].

3. Body Motility

4. Basal inspiratory pressure

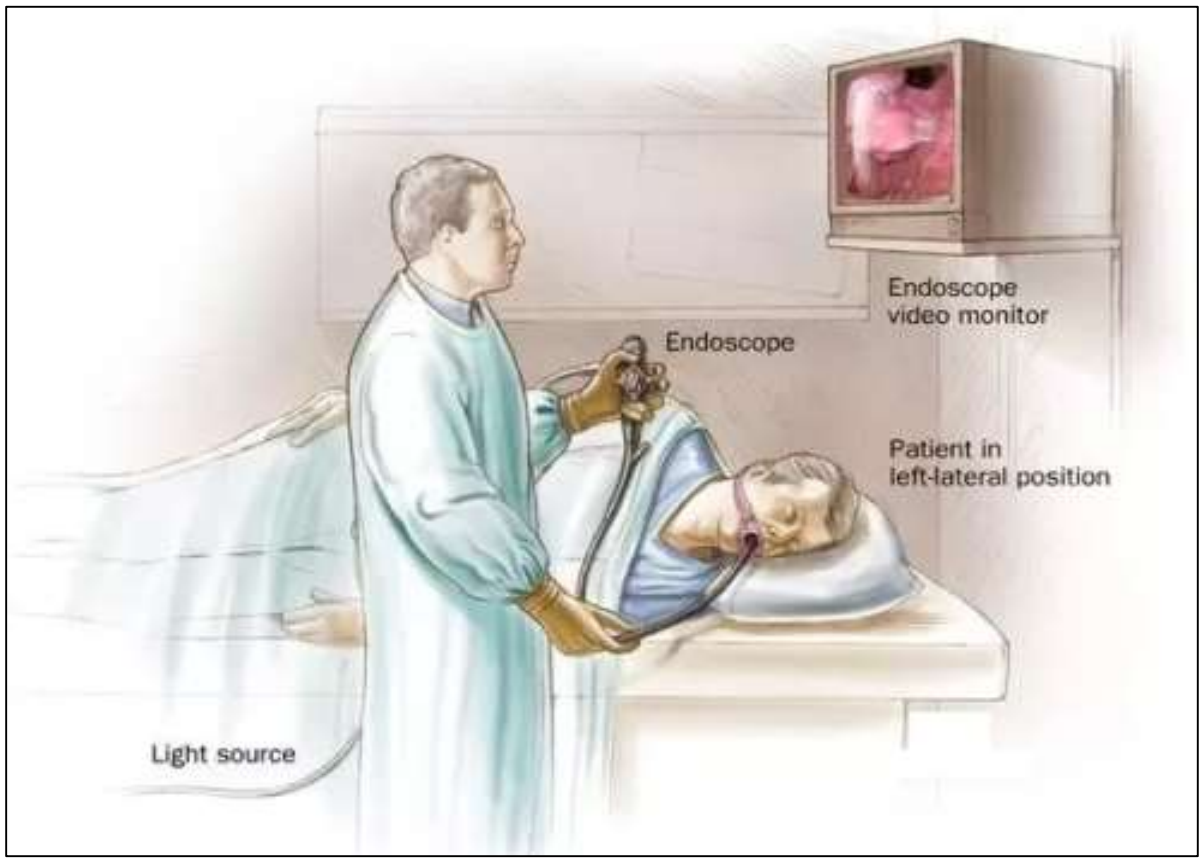
5. Mean integrated relaxation pressure

Upper esophageal endoscopy:

The study participants were advised not to take orally for about 6 hours, mostly overnight. The participants were well briefed about the endoscopic procedure and a written consent was obtained. The patients were advised to wear loose fitting gowns, to remove dentures and spectacles prior to the procedure. They were examined and the vital signs were recorded. A good intravenous access was secured for sedation for anxious patients.

To prevent the gag reflex occurring during endoscopy, local anaesthetic spray (lignocaine) was used just before the procedure. The patient was made to lie on the examination table in the left lateral position. A small, firm pillow was used to support the patient head and the neck was slightly flexed. A bite guard was used to prevent inadvertent biting of the endoscope by the patient. The endoscopist stands t o the left of the patient and passes the endoscope under direct vision. The left hand controls the head of the endoscope while right hand was used to hold the endoscopic at the 30 cm mark and insert the scope. The tube was advanced gently while the patient was asked to swallow to open up the cricopharyngeal sphincter. The patients were encouraged to take deep breaths during the procedure. This ensured smooth entry of the endoscope into the esophagus.The endoscopic view of the mucosa and laxity of the esophageal sphincter was assessed. Care was taken to survey the mucosa during withdrawal of the tube [78]. After a short break, the participants were advised to do esophageal manometry if warranted.

Figure 10: Upper esophageal Endoscopy Procedure



Hormone estrogen assay:

Blood samples were collected from the study participants to estimate the serum estrogen levels (17beta estradiol). In menstruating females with regular cycle, morning blood samples were taken between 8^{th} and 10^{th} day of the menstrual cycle after the esophageal manometry. The collected in vitro blood samples are quantitatively analyzed by electrochemiluminescence Cobas e 411immunoassay analyzers.

After collecting the sample, 25µL of the sample was incubated with two estradiol specific biotinylated antibodies, which lead to the formation of immune complexes. The streptavidin coated microparticles and a derivative of

estradiol labeled with a ruthenium complex was added and the mixture was incubated again, which leads to the formation of antibody hapten complex. These antibody hapten complexes conquer the vacant sites of biotinylated antibodies. The complex entirely bounded to the solid phase are aspirated and transferred to the measuring cell and measured by electrochemiluminescence method. The unbound substances are removed with ProCell M/ ProCell. The duration of first step was nine minutes, second step was nine minutes. The total duration of immunoassay of estrogen was 18 minutes .[88,89]

The results of premenopausal and postmenopausal women were subjected to statistical analysis and significance was determined.

RESULTS

RESULTS

Statistical Analysis:

In this study, 30 premenopausal and 30 postmenopausal women, diagnosed with GERD over a period from March 2018 to March 2019 were recruited from gastroenterology department. The data thus collected was subjected to appropriate statistical analysis using an SPSS Software Version 24.0.

To compare the normally distributed continuous variable between the premenopausal and postmenopausal GERD women the unpaired independent student 't' test was used. The values of were presented as mean ± SD. The parameters like basal LES pressure, EGJ – CI, esophageal motility pattern and endoscopy findings between the two groups are compared in relation to estrogen level. To find out the association between the severity of symptoms of gastroesophageal disease and BMI, estrogen level, BMI and estrogen level of both the groups, Pearson's correlation coefficient test was used.

p value < 0.05 was considered as statistically significant.

p value < 0.001was considered highly significant.

p value > 0.05 was considered as not statistically insignificant.

In Pearson correlation coefficient, + 1 denotes positive correlation, 0 denotes no correlation, - 1 denotes negative correlation.

Table 1 and Chart 1 : Comparison between premenopausal and postmenopausal GERD women Normal Basal Lower Esophageal Sphincter Pressure (LES) with estrogen level.

The normal Basal Lower Esophageal Sphincter Pressure (LES) falls in a range of 10-35 mm of Hg. In this study, among 30 premenopausal GERD women 18 subjects presented with normal mean LES pressure of 20.48± 5.50 and mean estrogen value was found to be151.57 ± 39. In 30 postmenopausal GERD women, 12 subjects presented with normal mean LES pressure of 13.77 ± 2.56 and mean estrogen value found to be 16.15 ± 5.28.The normal mean basal LES pressure and mean estrogen value was found to be higher in premenopausal than postmenopausal GERD women. By using independent 't' test both the groups were compared and the p value (< 0.001) was found to be highly statistically significant which denotes estrogen plays a vital role in maintaining normal LES pressure.

Table 2 and Chart 2: Comparison between premenopausal and postmenopausal GERD women Reduced Basal Lower Esophageal Sphincter Pressure (LES) with estrogen level.

The reduced LES pressure means when the value falls below 10 mm of Hg. The basal LES pressure was found to be reduced in 12 premenopausal GERD women with mean of 8.48± 1.02 and mean estrogen value of 73.37 ± 17.90. In postmenopausal GERD women, 18 subjects were found to have mean reduced LES pressure of 6.81 ± 1.31and mean estrogen value of 6.38 ± 3.58. The reduced mean basal LES pressure and mean estrogen value was found to

be lower in postmenopausal than premenopausal GERD women. The p value (< 0.001) was found to be highly statistically significant which indicates that reduced estrogen level was the cause for reduced basal LES pressure in postmenopausal groups than premenopausal GERD women.

Table 3 and Chart 3: Comparison between premenopausal and postmenopausal GERD women Normal EGJ – Cl with estrogen level.

In this study, 16 premenopausal GERD women presented to have normal esophageal junction contractile integral (EGJ – Cl) with mean of 45.31± 16.30 and mean estrogen value of 152.14 ±43.35.Among the postmenopausal GERD women, 12 subjects were found to have with mean normal esophageal junction contractile integrity of 26.86 ± 4.17 and mean estrogen value of 15.66 ± 6.31. The normal mean EGJ – Cl and mean estrogen value was found to be higher in premenopausal than postmenopausal GERD women. By applying independent 't' test both the groups were compared and the p value (< 0.001) was found to be highly statistically significant. This finding explains that estrogen has a significant role in maintaining normal esophagealgastric junction barrier.

Table 4 and Chart 4: Comparison between premenopausal and postmenopausal GERD women Reduced EGJ – Cl with estrogen level.

The esophageal junction contractile integrity was found to be reduced in 14 premenopausal GERD women with mean of 10.74 ± 4.75 and estrogen value of 85.67±25.56. The mean reduced EGJ – Cl of 6.26 ± 1.39 was found to be presented in 18 postmenopausal GERD women with mean estrogen value of 6.11 ± 2.82. The mean reduced EGJ – Cl and mean estrogen value was found

to be lower in postmenopausal than premenopausal GERD women. The p value (<0.001) found to be highly statistically significant between two groups denoting that reduced estrogen level could not able to maintain the EGJ – Cl function significantly in postmenopausal women.

Table 5 and Chart 5: Comparison between premenopausal and postmenopausal GERD women Normal Motility pattern with estrogen level.

In this study, 18 premenopausal GERD women were found to have normal motility pattern in esophageal manometry with mean estrogen value of 128.34 ± 43.59. The normal motility pattern was observed in 10 postmenopausal GERD women with mean estrogen value of 17.30 ± 5.43.The normal motility pattern and mean estrogen value was found to be higher in premenopausal than postmenopausal GERD women. The calculated p value (<0.001) showed highly statistically significance by comparing both the groups using independent 't' test. This finding reveals that estrogen influences normal motility pattern significantly in premenopausal groups than postmenopausal GERD women.

Table 6 and Chart 6: Comparison between premenopausal and postmenopausal GERD women Motility disorders with estrogen level.

The motility disorders was observed in 12 premenopausal GERD women by esophageal manometry and the mean estrogen value was found to be 110.30 ± 56.43. Among the postmenopausal women 20 subjects were found to have motility disorders with mean estrogen value of 6.25 ± 2.82. The motility

disorders and mean estrogen value was found to be increased in postmenopausal than premenopausal GERD women. The calculated p value (< 0.001) showed highly statistically significance which denotes that esophageal motility disorders observed to be significantly higher in postmenopausal women due to low estrogen level.

Table 7 and Chart 7: Comparison between premenopausal and postmenopausal GERD women Normal Endoscopy findings with estrogen level.

In this study, 17 premenopausal GERD women observed to have normal endoscopy finding with mean estrogen value of 150.43 ± 40.80. The normal endoscopy finding was observed in 9 postmenopausal GERD women with mean estrogen value of 17.22 ± 5.76. The normal endoscopy findings and mean estrogen value was found to be higher in premenopausal than postmenopausal GERD women. The calculated p value (< 0.001) showed highly statistically significance when compared between two groups by performing independent student 't' test. This finding indicates that estrogen has relation with increased normal endoscopy finding in premenopausal groups than postmenopausal GERD women.

Table 8 and Chart 8: Comparison between premenopausal and postmenopausal GERD women Abnormal Endoscopy findings with estrogen level.

Among the premenopausal GERD women 13 subjects observed to have abnormal endoscopy findings with mean estrogen value of 82.80 ± 27.91. The

abnormal endoscopy finding was observed in 21 postmenopausal GERD women with mean estrogen value of 6.80 ± 3.76.The abnormal endoscopy findings and mean estrogen value was found to be increased in postmenopausal than premenopausal GERD women. The p value (< 0.001) was found to be highly statistically significant, which denotes that low estrogen level in postmenopausal GERD women associated with increased abnormal endoscopy findings.

Table 9 and Chart 9: Comparison between premenopausal and postmenopausal GERD women Normal Lower esophageal sphincter (LES) findings in Endoscopy with estrogen level.

In this study, 17 premenopausal GERD women were found to have normal LES endoscopy findings with mean estrogen value of 147.54 ± 43.48.Among postmenopausal GERD women 11 subjects was found to have normal LES endoscopy findings with mean estrogen value of 16.63 ± 5.60. The normal LES endoscopy findings and mean estrogen value was found to be higher in premenopausal than postmenopausal GERD women. The p value (<0.001) was found to be highly statistically significant by performing independent student 't' test between two groups. This finding suggests that estrogen maintains normal LES significantly in premenopausal groups than postmenopausal GERD women.

Table 10 and Chart 10: Comparison between premenopausal and postmenopausal GERD women Lax LES findings in Endoscopy with estrogen level.

Among premenopausal GERD women, 13 subjects were observed with Lax LES endoscopy findings with mean estrogen value of 86.56 ± 31.46. The Lax LES endoscopy findings were observed in 19 postmenopausal GERD women with mean estrogen value of 6.05 ± 2.75.The Lax LES endoscopy findings and mean estrogen value was found to be increased in postmenopausal groups than premenopausal GERD women. The p value (< 0.001) was found to be highly statistically significant which denotes that low estrogen level associated with significantly increased Lax LES endoscopy findings in postmenopausal GERD women.

Table 11 and Chart 11: Comparison between premenopausal and postmenopausal GERD women Non-erosive reflux findings in Endoscopy with estrogen level.

In this study, 21 premenopausal GERD women were found to have non – erosive reflux endoscopic findings with mean estrogen value of 136.79 ± 46.53. Among post-menopausal women, 10 subjects presented with non – erosive reflux endoscopic findings with mean estrogen value of 17.30 ± 5.43. The non – erosive reflux endoscopic findings and mean estrogen value was found to be higher in premenopausal than postmenopausal GERD women. The calculated p value (< 0.001) was found to be highly statistically significant by using independent student ‘t’ test for comparing both the groups.

This finding denotes that increased estrogen level in premenopausal women significantly increased non–erosive reflux endoscopic finding in premenopausal groups compared with post menopausal GERD women.

Table 12 and Chart 12: Comparison between premenopausal and postmenopausal GERD women Reflux esophagitis finding in Endoscopy with estrogen level.

The reflux esophagitis endoscopy finding was observed in 9 premenopausal GERD women with mean estrogen value of 84.55 ± 33.87. Among the postmenopausal GERD women, 20 subjects presented with reflux esophagitis endoscopic finding with mean estrogen value of 6.25 ± 2.82. The reflux esophagitis endoscopy findings and mean estrogen value was found to be higher in postmenopausal than premenopausal GERD women. By performing independent student 't' test between two groups the p value (<0.001) was found to be highly statistically significant which denotes that reflux esophagitis observed to be significantly higher in postmenopausal GERD women due to low estrogen level.

Table 13 : Comparison between premenopausal and postmenopausal GERD women Normal Endoscopy findings with GERD - HRQL score.

In this study, 17 premenopausal GERD women were presented with normal endoscopy findings, with mean GERD-HRQL score value of 19.41±7.24. Among the postmenopausal GERD women,9 subjects presented with normal endoscopic findings with mean score value of 20.77 ± 7.44 higher than premenopausal GERD women. The calculated p value was found to be

0.655, which was not statistically significant. This implies that severity of symptoms of GERD has no significant association with normal endoscopy findings.

Table 14: Comparison between premenopausal and postmenopausal GERD women Abnormal Endoscopy findings with GERD - HRQL score.

Among premenopausal GERD women, 13 subjects presented with abnormal endoscopy findings with mean GERD-HRQL score value of 23.92 ± 6.48. The abnormal endoscopy findings was observed in 21 postmenopausal GERD women with mean GERD-HRQL score value of 19.47 ± 5.74 lower than premenopausal GERD women. The calculated p value was found to be 0.045, which was not statistically significant between the two groups, which denote that severity of symptoms of GERD does not significantly influence the mucosal tear of the esophagus.

Table 15 : Correlation between Normal BMI and GERD- HRQL score between premenopausal and postmenopausal GERD women.

Pearson's correlation coefficient between Normal BMI and GERD - HRQL score yielded a weak positive correlation (r = 0.378, p = 0.460) and (r = 0.219, p = 0.603) in premenopausal and postmenopausal GERD women. The calculated p value in both the groups was not statistically significant.

Table 16 : Correlation between Overweight and GERD- HRQL score between premenopausal and postmenopausal GERD women

Pearson's correlation coefficient between Overweight and GERD - HRQL score yielded (r = - 0.077, p = 0.777) and (r = - 0.010, p = 0.970) in

premenopausal women and postmenopausal GERD women suggested very weak negative correlation. The calculated p value in both the groups was not statistically significant.

Table 17 : Correlation between Obesity and GERD- HRQL score between premenopausal and postmenopausal GERD women.

Pearson's correlation coefficient between Obesity and GERD - HRQL score yielded a weak positive correlation (r = 0.473, p = 0.237) in premenopausal GERD women and (r = 0.938, p = 0.062) in postmenopausal GERD women yielded strong positive correlation. The calculated p value in both the groups was not statistically significant.

Table 18 : Correlation between BMI and estrogen level between premenopausal and postmenopausal GERD women.

Pearson's correlation coefficient between BMI and estrogen level yielded a weak positive correlation (r = 0.131, p = 0.490) in premenopausal GERD women and (r = - 0.113, p = 0.553) in postmenopausal GERD women yielded weak negative correlation. The calculated p value in both the groups was not statistically significant.

Table 19 : Correlation between GERD-HRQL score and estrogen level between premenopausal and postmenopausal GERD women

In this study, there was weak negative correlation (r = - 0.189, p = 0.316) in premenopausal GERD women and there was very weak positive correlation (r = 0.004, p = 0.983) in postmenopausal women. The calculated p value in both the groups was not statistically significant.

TABLES

TABLE:1

Comparison between premenopausal and postmenopausal GERD women Normal Basal Lower Esophageal Sphincter Pressure (LES) with estrogen level

Groups	No of patients	Mean ± SD (LES)	Mean ± SD (estrogen)	p value
Premenopausal	18	20.48 ± 5.50	151.57 ± 39.88	0.000**
Postmenopausal	12	13.77 ± 2.56	16.15 ± 5.28	

TABLE: 2

Comparison between premenopausal and postmenopausal GERD women Reduced Basal Lower Esophageal Sphincter Pressure (LES) with estrogen level

Groups	No of patients	Mean ± SD (LES)	Mean ± SD (estrogen)	p value
Premenopausal	12	8.48 ± 1.02	73.37 ± 17.90	0.000**
Postmenopausal	18	6.81 ± 1.31	6.38 ± 3.58	

TABLE: 3

Comparison between premenopausal and postmenopausal GERD women Normal EGJ – Cl with estrogen level

Groups	No of patients	Mean ± SD (EGJ–CI)	Mean ± SD (estrogen)	p value
Premenopausal	16	45.31 ± 16.30	152.14 ± 43.35	0.000**
Postmenopausal	12	26.86 ± 4.17	15.66 ± 6.31	

TABLE: 4

Comparison between premenopausal and postmenopausal GERD women

Reduced EGJ – Cl with estrogen level

Groups	No of patients	Mean ± SD (EGJ–CI)	Mean ± SD (estrogen)	p value
Premenopausal	14	10.74 ± 4.75	85.67 ± 25.56	0.000**
Postmenopausal	18	6.26 ± 1.39	6.11 ± 2.82	

TABLE: 5

Comparison between premenopausal and postmenopausal GERD women

Normal Motility pattern with estrogen level

Groups	No of patients	Mean ± SD (estrogen)	p value
Premenopausal	18	128.34 ± 43.59	0.000**
Postmenopausal	10	17.30 ± 5.43	

TABLE: 6

Comparison between premenopausal and postmenopausal GERD women

Motility disorders with estrogen level

Groups	No of patients	Mean ± SD (estrogen)	p value
Premenopausal	12	110.30 ± 56.43	0.000**
Postmenopausal	20	6.25 ± 2.82	

TABLE: 7

Comparison between premenopausal and postmenopausal GERD women

Normal Endoscopy findings with estrogen level

Groups	No of patients	Mean ± SD (estrogen)	p value
Premenopausal	17	150.43 ± 40.80	0.000**
Postmenopausal	9	17.22 ± 5.76	

TABLE: 8

Comparison between premenopausal and postmenopausal GERD women

Abnormal Endoscopy findings with estrogen level

Groups	No of patients	Mean ± SD (estrogen)	p value
Premenopausal	13	82.80 ± 27.91	0.000**
Postmenopausal	21	6.80 ± 3.76	

TABLE: 9

Comparison between premenopausal and postmenopausal GERD women

Normal LES finding in Endoscopy with estrogen level

Groups	No of patients	Mean ± SD (estrogen)	p value
Premenopausal	17	147.54 ± 43.48	0.000**
Postmenopausal	11	16.63 ± 5.60	

TABLE: 10

Comparison between premenopausal and postmenopausal GERD women

Lax LES finding in Endoscopy with estrogen level

Groups	No of patients	Mean ± SD (estrogen)	p value
Premenopausal	13	86.56 ± 31.46	0.000**
Postmenopausal	19	6.05 2.75	

TABLE: 11

Comparison between premenopausal and postmenopausal GERD women

Non- erosive reflux findings in Endoscopy with estrogen level

Groups	No of patients	Mean ± SD (estrogen)	p value
Premenopausal	21	136.79 ± 46.53	0.000**
Postmenopausal	10	17.30 ± 5.43	

TABLE: 12

Comparison between premenopausal and postmenopausal GERD women

Reflux esophagitis finding in Endoscopy with estrogen level

Groups	No of patients	Mean ± SD (estrogen)	p value
Premenopausal	9	84.55 ± 33.87	0.000**
Postmenopausal	20	6.25 ± 2.82	

TABLE: 13

Comparison between premenopausal and postmenopausal GERD women

Normal Endoscopy findings with GERD - HRQL score

Groups	No of patients	Mean ±SD	p value
Premenopausal	17	19.41 ± 7.24	0.655
Postmenopausal	9	20.77 ± 7.44	

TABLE: 14

Comparison between premenopausal and postmenopausal GERD women

Abnormal Endoscopy findings with GERD - HRQL score

Groups	No of patients	Mean ±SD	p value
Premenopausal	13	23.92 ± 6.48	0.045
Postmenopausal	21	19.47 ± 5.74	

TABLE: 15

Correlation between Normal BMI and GERD- HRQL Score GERD

between premenopausal and postmenopausal women

Women	Pearson Correlation coefficient	p value
Premenopausal	0.378	0.460
Postmenopausal	0.219	0.603

TABLE: 16

Correlation between Overweight and GERD- HRQL score GERD

between premenopausal and postmenopausal women

Women	Pearson Correlation coefficient	p value
Premenopausal	- 0.077	0.777
Postmenopausal	-0.010	0.970

TABLE: 17

Correlation between Obesity and GERD- HRQL score between premenopausal and postmenopausal GERD women

Women	Pearson Correlation coefficient	p value
Premenopausal	0.473	0.237
Postmenopausal	0.938	0.062

TABLE: 18

Correlation between BMI and estrogen level between premenopausal and postmenopausal GERD women

Women	Pearson Correlation coefficient	p value
Premenopausal	0.131	0.490
Postmenopausal	- 0.113	0.553

TABLE: 19

Correlation between GERD - HRQL score and estrogen level between premenopausal and postmenopausal GERD women

Women	Pearson Correlation coefficient	p value
Premenopausal	- 0.189	0. 316
Postmenopausal	0.004	0. 983

CHARTS

Chart:1

Comparison between premenopausal and postmenopausal GERD women

Normal Basal Lower Esophageal Sphincter Pressure (LES) with estrogen level

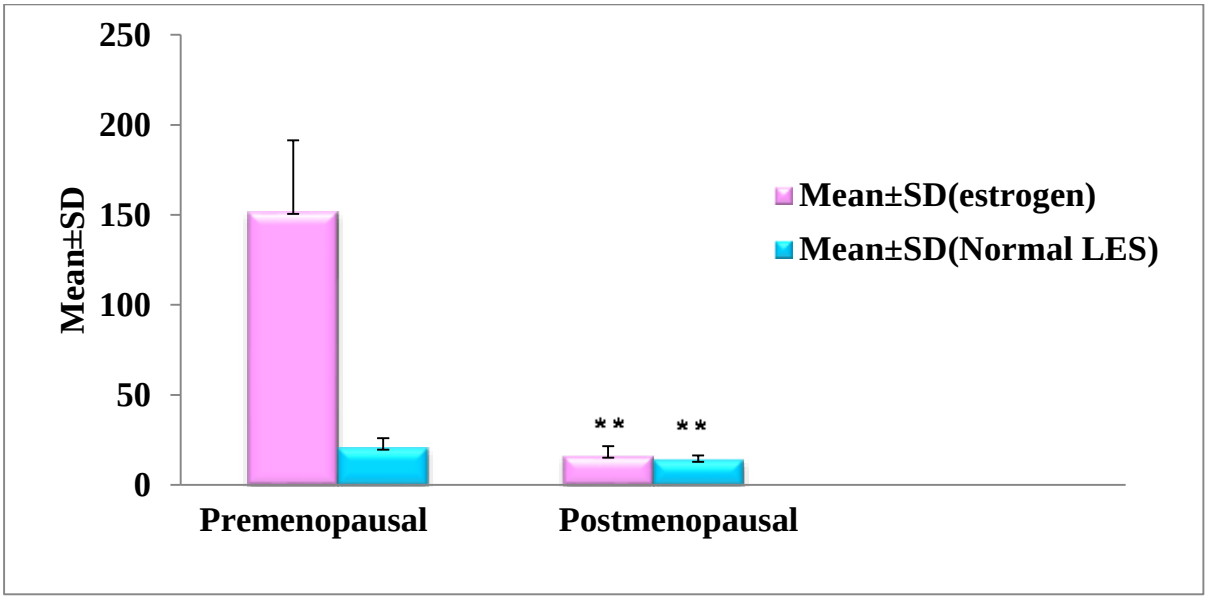
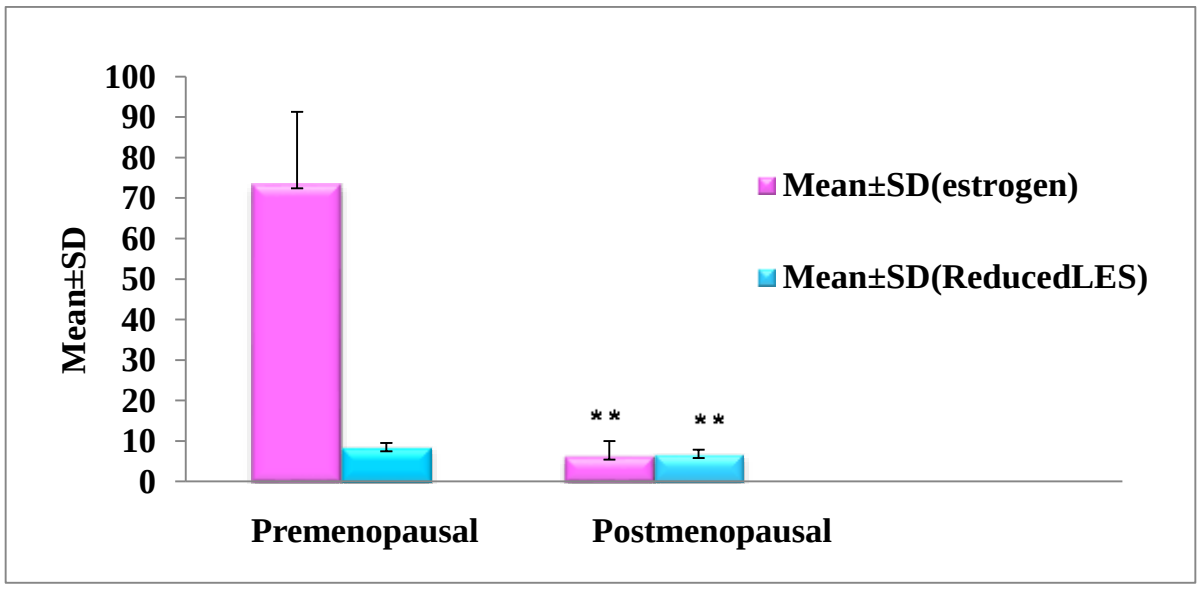


Chart: 2

Comparison between premenopausal and postmenopausal GERD women

Reduced Basal Lower Esophageal Sphincter Pressure (LES) with estrogen level



**** p value < 0.001 – statistically significant**

Chart: 3

Comparison between premenopausal and postmenopausal GERD women

Normal EGJ – Cl with estrogen level

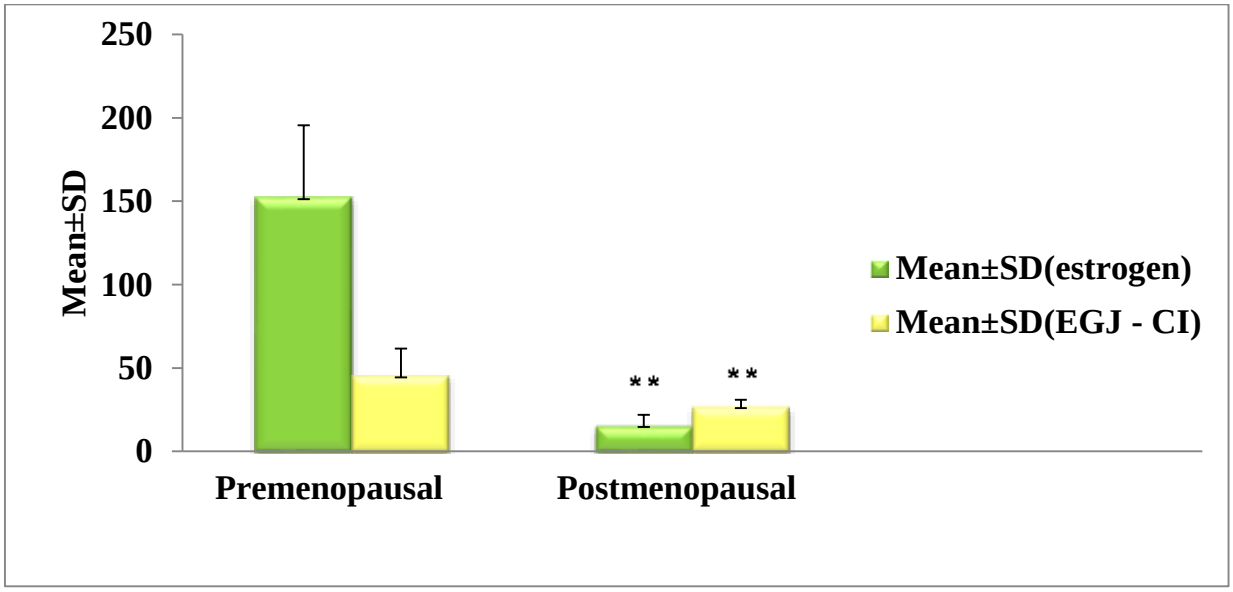
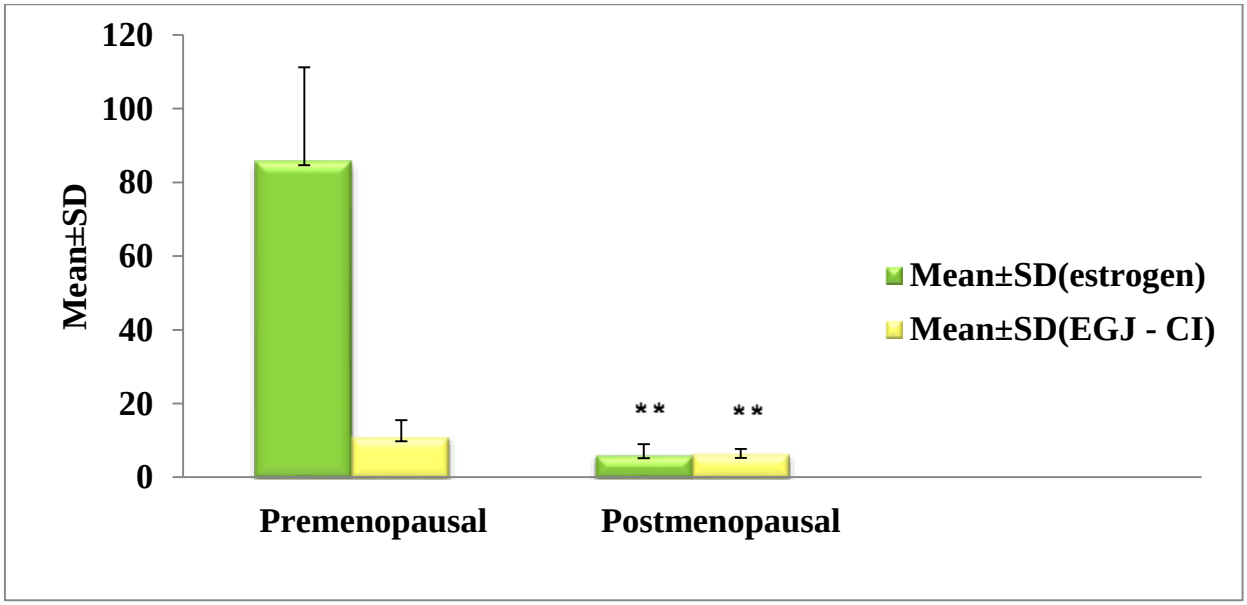


Chart: 4

Comparison between premenopausal and postmenopausal GERD women

Reduced EGJ – Cl with estrogen level



**** p value < 0.001 – statistically significant**

Chart: 5

Comparison between premenopausal and postmenopausal GERD women

Normal Motility pattern with estrogen level

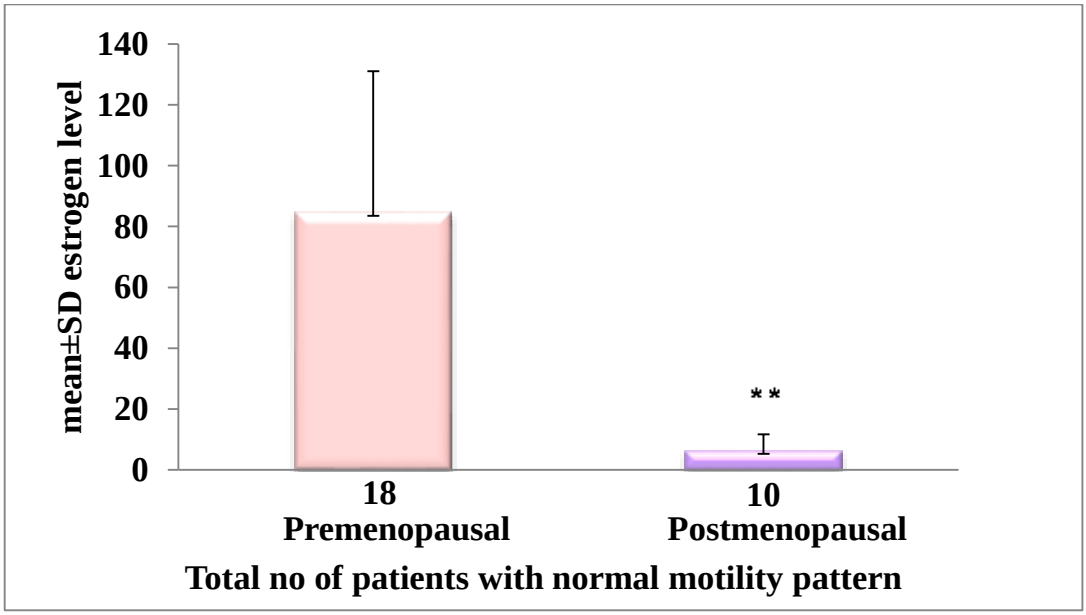
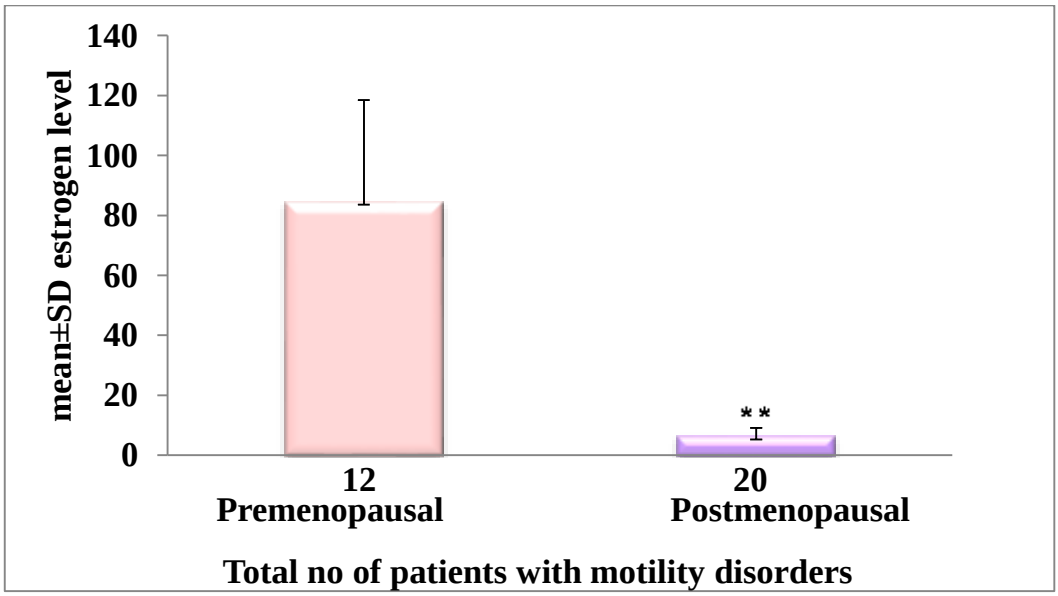


Chart: 6

Comparison between premenopausal and postmenopausal GERD women

Motility disorders with estrogen level



**** p value < 0.001 – statistically significant**

Chart: 7

Comparison between premenopausal and postmenopausal GERD women

Normal Endoscopy findings with estrogen level

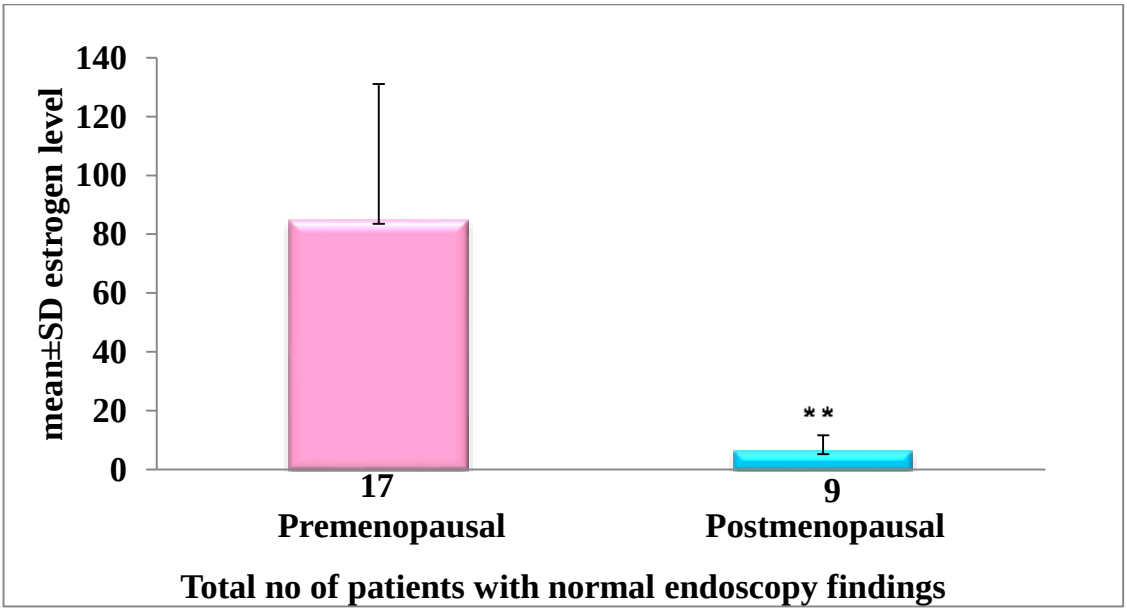
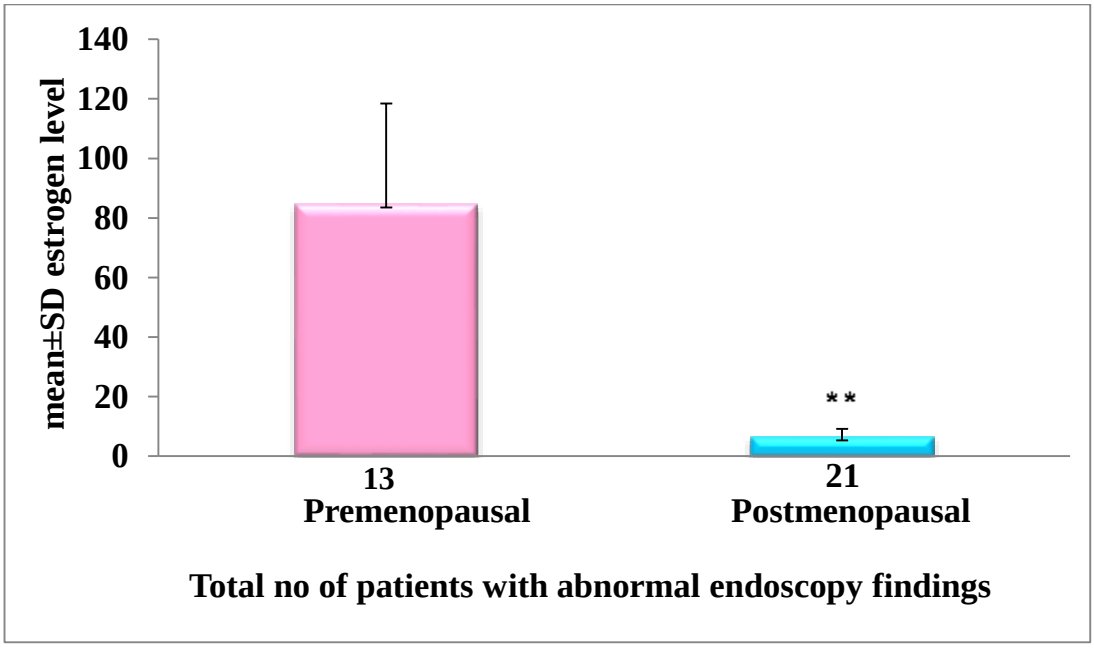


Chart: 8

Comparison between premenopausal and postmenopausal GERD women

Abnormal Endoscopy findings with estrogen level

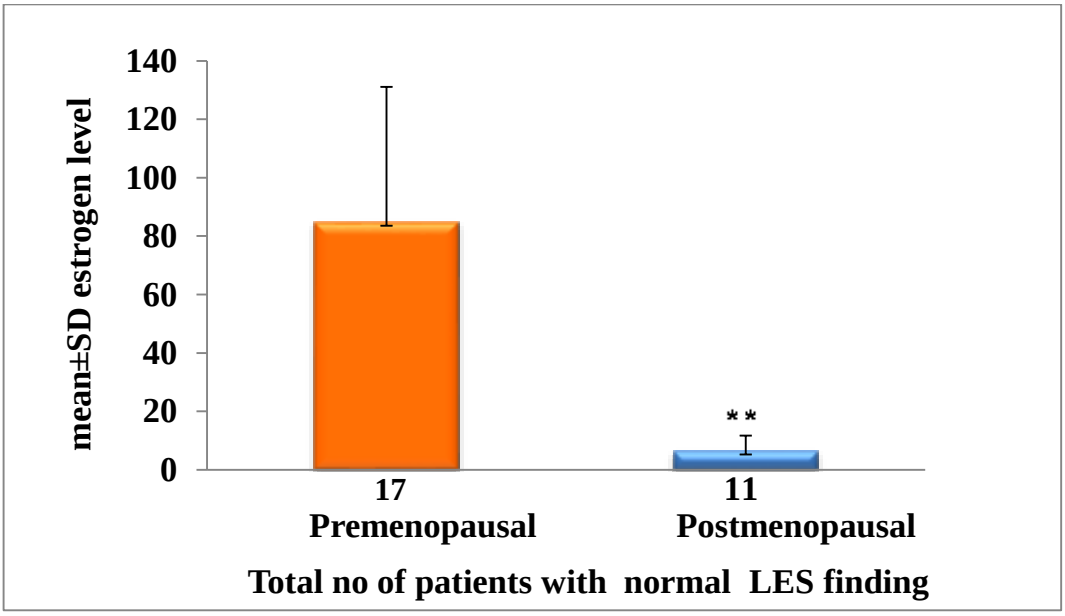


** p value < 0.001 – statistically significant

Chart: 9

Comparison between premenopausal and postmenopausal GERD women

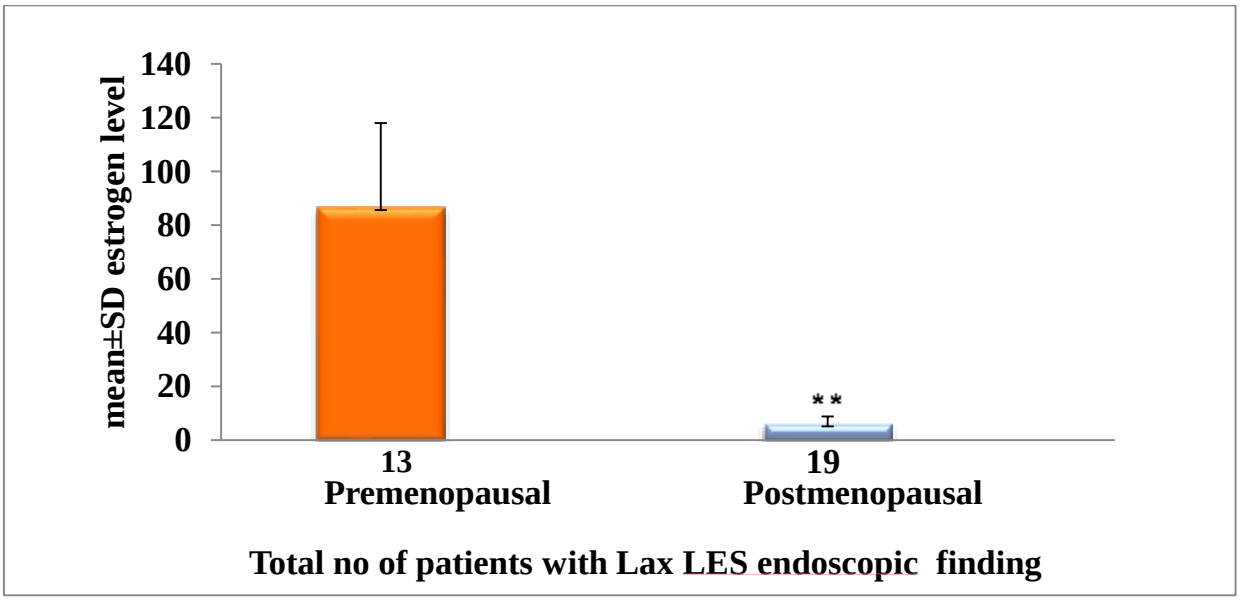
Normal LES finding in Endoscopy with estrogen level



Charts: 10

Comparison between premenopausal and postmenopausal GERD women

Lax LES finding in Endoscopy with estrogen level



** p value < 0.001 – statistically significant

Charts: 11

Comparison between premenopausal and postmenopausal GERD women

Non- erosive reflux finding in Endoscopy with estrogen level

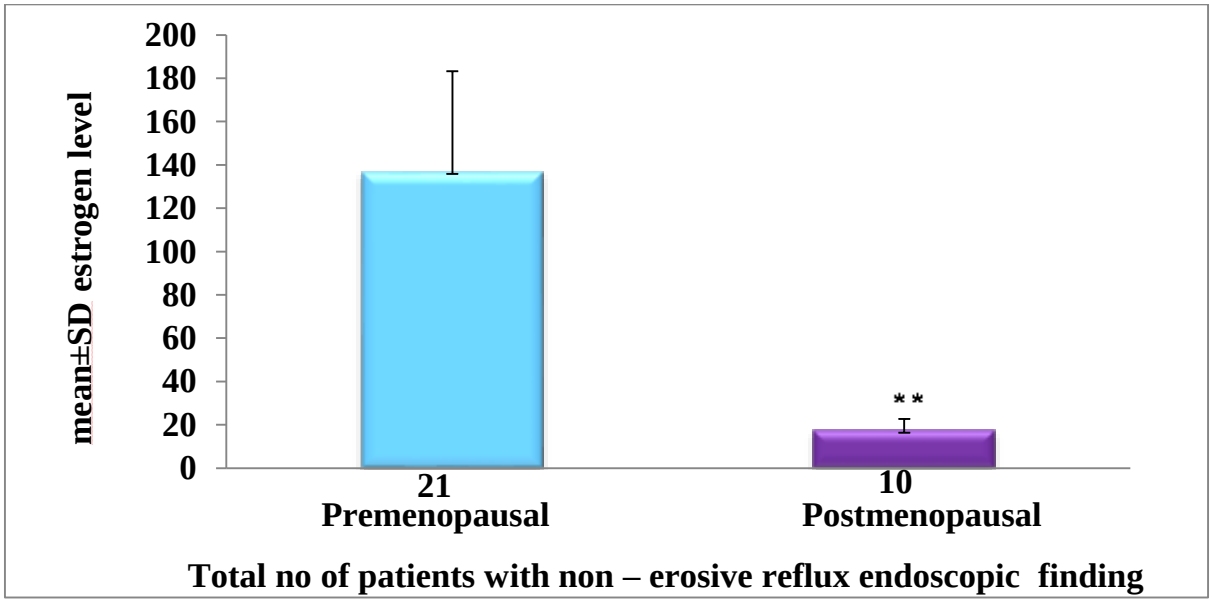
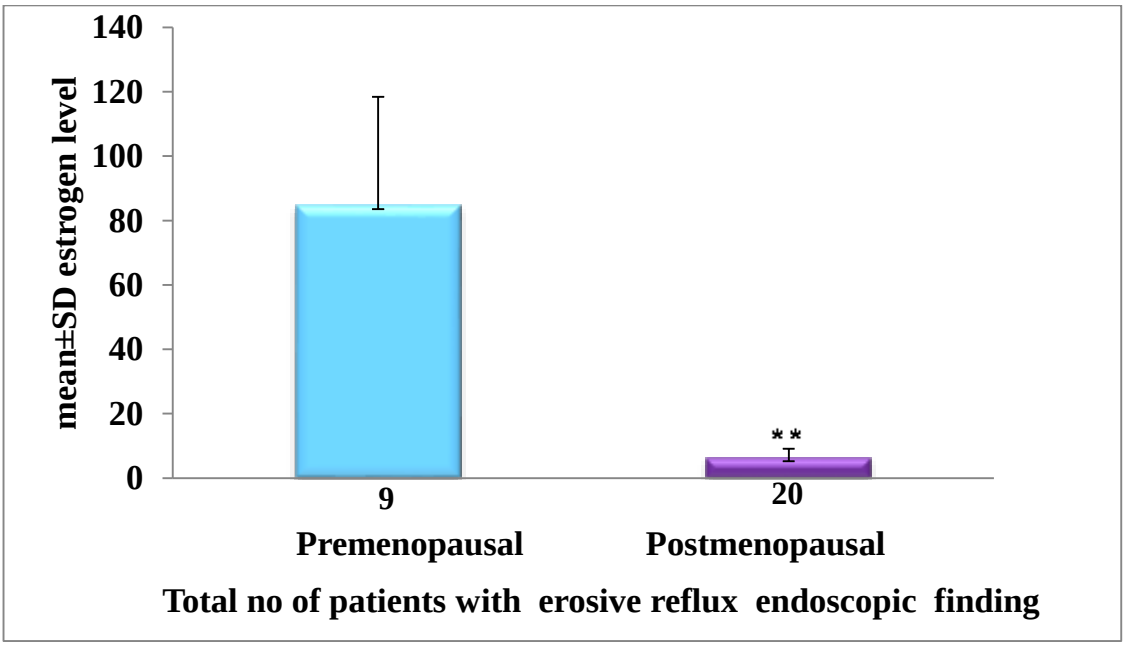


Chart: 12

Comparison between premenopausal and postmenopausal GERD women

Reflux esophagitis finding in Endoscopy with estrogen level



** p value < 0.001 – statistically significant

DISCUSSION

DISCUSSION

Gastroesophageal disease is one of the most common disorder of the upper gastrointestinal tract. GERD is characterized by heart burn and regurgitation symptoms which accounts from 2.5% to more than 25% in the community.[90] The prevalence of GERD is closely related to the generative phase of women which denotes that estrogen might evoke a significant role in premenopausal women. The severity of GERD rapidly increases in postmenopausal women because the disease spectrum is closely related to the estrogen levels of women. Estrogen has anti inflammatory action and decreases activities like migration, adhesion and production of chemical mediators of inflammation associated with the epithelial damage induced by acid reflux.

The most important risk factor for GERD is considered to be obesity, and the severity of the disease runs parallel to it. The sex steroid hormone, estrogen modulates the adipose tissue metabolism. In postmenopausal women, the estrogen levels in the circulation are sufficiently low which might lead to the accumulation of visceral fat.[8] On compiling the above said information, the spectrum of GERD in women has a possible association with sex steroid hormone estrogen. In this study, the aim was to compare the association between premenopausal and postmenopausal women presenting with GERD and serum estrogen level.

Comparison between premenopausal and postmenopausal GERD women Lower Esophageal Sphincter Pressure (LES) with estrogen level.

In this study, the normal basal LES pressure was found to be observed 60% in premenopausal and 40% in postmenopausal GERD women with mean value of normal LES pressure and estrogen which were found to be higher in premenopausal than postmenopausal GERD women. The reduced basal LES pressure was found to be observed 40% in premenopausal and 60% in postmenopausal GERD women with mean value of reduced LES pressure and estrogen was found to be lower in postmenopausal women than premenopausal GERD women. This finding is supported by the study done by Shyam et al [91] showed that there was a significant correlation between basal LES pressure and estrogen in the postmenopausal group and quoted it as distinct finding. Since the female sex hormone estrogen increases synthesis of nitric oxide causing LES relaxation and results in reflux of acid in animal models.

In contrast to this finding, Masaka et al [57] revealed that exposure to exogenous nitric oxide did not produce any damage in the esophagus of the female rat, due to the resistance offered by the sex hormone estrogen. The damage to the esophageal tissue can be avoided by decreasing the secretion of tumour necrosis factor alpha. The secretion of tumour necrosis factor alpha (TNF-α) is limited by estrogen mediated mast cell inactivation.

Kim and Lee et al [92] in his animal model study revealed that estrogen plays a vital role in modulating the muscle contraction and mucin secretion of

esophagus. The serum E2 levels increases the intracellular calcium by regulating the calcium related genes expression which contributes to reduced muscle contraction and increased mucus secretion in the esophagus. With the decreasing level of hormone, there is modification in mucin content leading to impaired protective barrier function of lower esophagus.

Zia and Heitkemper et al [93] found that reduced LES pressure observed in the luteal phase of menstrual cycle in healthy women and highlighted that no studies explained about the changes in menstrual cycle in GERD women. This study also revealed that estrogen and progesterone could be the cause for GERD, the disease spectrum can be increased in postmenopausal women.

Infantino et al [94] in his study found that menopausal symptoms are related to low estrogen and progesterone levels, symptoms related to GERD would increase to higher percentages in menopausal women due to the modulation of contractile function of gastrointestinal tract.

Thus the female sex hormone estrogen might influence the LES pressure, which is higher in postmenopausal compared to premenopausal GERD women in this study due to fluctuations in hormone levels seen in menopause.

Comparison between premenopausal and postmenopausal GERD women EGJ – Cl with estrogen level.

In this study, the EGJ – CI was maintained in 53.33% in premenopausal and 40% in postmenopausal GERD women with increased mean value of EGJ – CI and estrogen in premenopausal women. The EGJ – CI was reduced in

46.67% of premenopausal and 60% in postmenopausal women with lower mean value of EGJ – CI and estrogen in postmenopausal women than premenopausal GERD women. The reduced estrogen level, which was not able to maintain the EGJ – Cl function, was found to be higher in postmenopausal compared to premenopausal GERD group.

The above findings supported by the study conducted by Honda et al [20] using rat model, explained the effects of estrogen on esophageal barrier function on exposure of acidified nitrite and luminal acid reflux in esophagus of rabbit. This study explained that treatment with estrogen reduced the esophageal barrier permeability, dilatation of intercellular spaces and transmembrane resistance induced by hydrochloric acid and nitrite exposure. This study also revealed that administration of estrogen increased the expression of occludin which enhanced the adhesion between the adjacent epithelial cells of esophagus and potentiate the tight junction. This study signifies the importance of estrogen in maintaining the esophageal barrier function.

The epithelium barrier is the intercellular junctional complex made up of tight junction for the transport of ions and molecules in gastrointestinal tract. The gastric acid refluxate destroys the tight junction causing increased paracellular permeability, decreased transmembrane resistance and intercellular space dilatation indicating a relation between gut permeability and estrogen. The nitric oxide causes distal esophageal damage in GERD patients after intake of meal with high nitrate level. This nitrate is converted into reactive nitrogen oxide species and damages the esophageal epithelium in the lower esophagus [8].

The EGJ – CI denotes the esophageal barrier function in manometry. The barrier function of esophagus plays a vital role in preventing the reflux of acid in GERD. The findings in this study also signify that the sex hormone estrogen helps in preserving the function of esophageal gastric junction.

Comparison between premenopausal and postmenopausal women Motility pattern with estrogen level.

In this study, 60% of premenopausal women and 33.33% of postmenopausal women were presented with normal esophageal motility with mean estrogen value higher in premenopausal GERD women. The motility disorder pattern was found in 40% and 66.67% in premenopausal women and postmenopausal GERD women. The mean estrogen value and normal motility is reduced in postmenopausal than premenopausal GERD women.

The abnormal motility of esophagus namely TLESR, hypotensive LES, ineffective esophageal motility are considered to be the important factor in GERD [95]. The study conducted by Zia and Heitkemper et al [93] revealed that receptors for both estrogen and progesterone are found throughout the gastrointestinal tract, might have an effect on motility. These hormones influence the effects on motility by the changes in neurons containing nitric oxide in the myenteric plexus and by modulating the mast cell number and function in the mucosa of gastrointestinal tract.

The findings in this study suggest that estrogen influences in maintaining the motility of the esophagus.

Comparison between premenopausal and postmenopausal GERD women with Endoscopy findings and estrogen.

In this study, 56.67% of premenopausal women and 30% of postmenopausal women were presented with normal endoscopy finding with mean estrogen value higher in premenopausal GERD women. The abnormal endoscopy finding was found in 43.33% and 70% in premenopausal women and postmenopausal GERD women. The mean estrogen value was reduced in postmenopausal women than premenopausal GERD women. Similarly normal LES and Lax LES findings were observed in 56.67% and 43.33% in premenopausal women and in postmenopausal GERD women 36.67% and 63.33% found to have normal LES and Lax LES findings.70% of premenopausal women and 30% of postmenopausal GERD women were observed to have non erosive reflux disease.

The reflux esophagitis was found in 30% and 66.67% in premenopausal women and postmenopausal women respectively. The mean estrogen value was reduced in postmenopausal women than premenopausal GERD women.

The gastroesophageal reflux disease is classified into non erosive reflux disease and reflux esophagitis based on upper endoscopy. The exposure of refluxed contents constantly into the epithelium of esophagus results in erosive reflux esophagitis.

Non erosive reflux diseases generally affect women more than men suggesting that gastric inflammation caused by bacterial infection and chemicals are low in females. In animal experimental study done by Masaka et

al explained the role of exogenous administration of estrogen causing mild damage to the mucosa of esophagus in the ovariectomized rats and male rats. The female sex hormone estrogen exerts anti inflammatory action by decreasing the esophageal macrophage inhibitory factor levels. Thus estrogen binds with E2 receptor and reduces mast cell mediated cytotoxicity and production of TNF-α that mediates inflammation [57].

The low estrogen level in postmenopausal period could be the reason for severity of reflux esophagitis in this study.

Comparison between premenopausal and postmenopausal GERD women

Endoscopy findings with GERD - HRQL score.

In this study, 56.67% and 30% of premenopausal and postmenopausal GERD women presented with normal endoscopy finding with mean score value of lower than postmenopausal GERD women. 43.33% of premenopausal and 70% of postmenopausal GERD women presented with abnormal endoscopy finding with mean score value of lower in postmenopausal GERD women. The endoscopy finding of reflux esophagitis confirms the diagnosis of gastroesophageal disease. However, normal endoscopy does not exclude the diagnosis of GERD. There will be no endoscopic evidence of gastroesophageal disease in most of the patients presenting with heartburn and regurgitation [96].

The cause for visceral hypersensitivity is due to the presence of transient receptor potential vanilloid subfamily member-1 receptors (TRPV1) in esophageal mucosa considered as a major factor involved in pathogenesis of non erosive reflux disease. The activation of the transient receptor potential

vanilloid subfamily member-1 receptors (TRPV1) releases calcitonin-gene-related peptide and substance P from primary afferent neurons and causes inflammatory reaction. The cause for visceral hypersensitivity is due to the presence of TRPV1 in esophageal mucosa considered as major factor involved in pathogenesis of non erosive reflux disease. The activation of TRPV1 releases calcitonin-gene-related peptide and substance P calcitonin gene -related peptide from primary afferent neurons and causes inflammatory reaction [97].

Thus, severity of symptoms of GERD does not coincide with the risk of damage to the esophagus in this study.

Correlation between BMI and GERD- HRQL score between premenopausal and postmenopausal GERD women.

In this study, Pearson's correlation coefficient between Normal BMI and GERD- HRQL score yielded a weak positive correlation in both groups. There was very weak negative correlation observed between Overweight and GERD-HRQL score in premenopausal women and postmenopausal GERD women. Pearson's correlation coefficient between Obesity and GERD - HRQL score yielded a weak positive correlation in premenopausal women and strong positive correlation in postmenopausal women with GERD.

The study conducted by Jacobson et al [98] found that an increased BMI and normal BMI was associated with an increase risk of reflux symptoms in women by using supplemental questionnaire. The study showed that 40% of normal BMI presented with increased reflux symptoms, while women with overweight and obese presented two to three times with frequent symptoms.

This study also states that BMI as a marker to assess the severity of GERD symptoms than abdominal distribution of fat.

Asanuma et al [8] quoted that the complications of GERD like Barrett's esophagus negatively correlated to the serum leptin level in women. Obesity is the major risk factor for gastroesophageal reflux disease, Barrett's esophagus and adenocarcinoma of esophagus. The spectrum of this disease is increased due to obesity. The central obesity increases the abdominal pressure causing acid reflux to be exposed into the distal esophagus by the relaxation of lower esophageal sphincter. The cause for obesity associated with interleukin 6, insulin-like growth factor, tumour necrosis factor α and leptin. The adipocytes secrete leptin which plays avital role in controlling intake of food and consumption of energy. The obese subjects were considered leptin resistant due to increased serum leptin level. Leptin predisposes to esophageal carcinoma due to its angiogenesis and mitosis action on the cell lines of esophagus [99].

In this study, there was a strong positive correlation between obesity and GERD- HRQL score in postmenopausal GERD women. Thus obesity plays an important role in pathogenesis of GERD in postmenopausal women.

Correlation between BMI and estrogen level between premenopausal and postmenopausal GERD women.

In this study, there was weak positive correlation in premenopausal women and postmenopausal GERD women yielding weak negative correlation between BMI and estrogen level.

Heine et al [100] in his animal experiment study concluded that decreased synthesis of estrogen can lead to obesity due to lack of estrogen receptor alpha (ER) in hypothalamus thereby decreases leptin sensitivity. This study also explained that there was inverse relation between visceral fat distribution and steroid hormone estrogen.

Morita et al [101] concluded that deficiency of sex steroid hormone estrogen induced obesity in postmenopausal women. The epithelium of human esophagus expresses ER alpha receptors. The lack of ER alpha would causes leptin induced development of Barrett's esophagus in postmenopausal GERD women with low level of estrogen.

Thus, the sex steroid hormone estrogen might possess a role in controlling metabolism in adipose tissue which is more pronounced in postmenopausal GERD women. In this study, obesity acts as a major independent risk factor to GERD.

Correlation between GERD-HRQL score and estrogen level between premenopausal and postmenopausal GERD women

In this study, there was weak negative correlation in premenopausal GERD women and postmenopausal GERD women yielded very weak positive correlation GERD –HRQL score and estrogen level.

Yaseri et al [102] in his study concluded that severity and frequency of GERD symptoms of observed more in older females than in males due to female sex hormones. Similarly Infantino et al [94] concluded in his study that there is hormonal association between menopause and GERD symptoms by using

gastrointestinal symptom rating scale. This study also revealed that 80% of the menopausal women had never been diagnosed to have GERD. The mucin barrier function is altered which is closely related to variation in estrogen levels.

In this study there was very weak positive relation between GERD – HRQL score and estrogen level with no statistical significance in postmenopausal GERD women. So estrogen might have a role in delivering GERD symptoms.

CONCLUSION

CONCLUSION

In this study it has been concluded that female sex steroid hormone estrogen provides a protective effect on the epithelium of esophagus against acid refluxate. The high resolution manometry parameters like basal lower esophageal sphincter pressure, EGJ – CI, motility pattern when compared between premenopausal and postmenopausal GERD women in relation to estrogen levels showed statistical significance.

The basal LES pressure was found to be reduced in postmenopausal GERD women with low estrogen level when compared with premenopausal GERD women.

The esophageal gastric junction contractile integrity was highly impaired in postmenopausal GERD women with low estrogen level when compared with premenopausal GERD women.

The normal motility of esophagus found to be decreased in postmenopausal GERD women with low estrogen level when compared with premenopausal GERD women.

Similarly, the upper GI endoscopy findings also revealed that there was association between GERD and estrogen between premenopausal and postmenopausal women with statistical significance.

The presence of positive correlation between obesity and GERD symptoms in postmenopausal women has been concluded in this study. This study also revealed that there is a weak negative correlation, between BMI and estrogen and not statistically significant, in postmenopausal GERD women.

Further, this study also showed that there is very weak positive correlation between GERD-HRQL score and estrogen level in postmenopausal GERD women.

Since, this study showed certain consistencies and inconsistencies with previous studies. To validate the role of estrogen more potentially in both premenopausal and postmenopausal GERD women, the study with larger sample size is recommended to reach a balance between benefits and risks of sex steroid female hormone estrogen in the management of females with GERD.

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ANNEXURES

Study Volunteer ID:
Study Volunteer Name:

PSG Institute of Medical Science and Research, Coimbatore
Institutional Human Ethics Committee
INFORMED CONSENT FORMAT FOR RESEARCH PROJECTS

(strike off items that are not applicable)

I , Dr. M. Praveena, am carrying out a study on the topic: **A COMPARATIVE STUDY OF PREMENOPAUSAL AND POSTMENOPAUSAL WOMEN WITH GERD IN RELATION TO SERUM ESTROGEN LEVELS**
as part of my research project being carried out under the aegis of the Department of: Physiology

(*Applicable to students only*): My research guide is: Dr. R. Nagashree M.D.

The **justification for this study** is: To evaluate the relationship between symptoms of GERD among premenopausal and postmenopausal age in relation to serum estrogen levels.

The objectives of this study are:
Primary Objective: To compare the association between serum estrogen levels and GERD symptoms and severity in premenopausal and postmenopausal women.

Secondary Objective: To determine the pattern of symptoms and severity of GERD among premenopausal and postmenopausal women in relation to estrogen levels and endoscopy findings.

To determine the relation between body mass index and gastroesophageal reflux symptoms.

Sample size: 60

Study volunteers / participants are (specify population group & age group: Females (35 to 70 years).
Location: Gastroenterology department, PSGIMSR, Coimbatore

We request you to kindly cooperate with us in this study. We propose collect background information and other relevant details related to this study. We will be carrying out:

Initial interview (specify approximate duration):_____20____ minutes.

Data collected will be stored for a period of __3_ years. We will / will not use the data as part of another study.

Health education sessions: Number of sessions: _____. Approximate **duration** of each session:

_____ minutes.

Clinical examination (Specify details and purpose):

Blood sample collection: Specify quantity of blood being drawn: _____3_____ml.

No. of times it will be collected: ____1_____.

Whether blood sample collection is part of routine procedure or for research (study) purpose:

1. Routine procedure 2. Research purpose

Study Volunteer ID:
Study Volunteer Name:

Specify **purpose**, discomfort likely to be felt and side effects, if any: No

Whether blood sample collected will be stored after study period: Yes / No, it will be destroyed

Whether blood sample collected will be sold: Yes / No

Whether blood sample collected will be shared with persons from another institution: Yes / No

Medication given, if any, duration, side effects, purpose, benefits:

Whether medication given is part of routine procedure: Yes / No (If not, state reasons for giving this medication)

Whether alternatives are available for medication given: Yes / No (If not, state reasons for giving this particular medication)

Final interview (specify approximate duration):_____ mts. If **photograph** is taken, purpose:

Benefits from this study: To aid in the management of GERD in premenopausal and post menopausal women.

Risks involved by participating in this study: No

How the **results** will be used:

If you are uncomfortable in answering any of our questions during the course of the interview / biological sample collection, **you have the right to withdraw from the interview / study at anytime.** You have the freedom to withdraw from the study at any point of time. Kindly be assured that your refusal to participate or withdrawal at any stage, if you so decide, will not result in any form of compromise or discrimination in the services offered nor would it attract any penalty. You will continue to have access to the regular services offered to a patient. You will **NOT** be paid any remuneration for the time you spend with us for this interview / study. The information provided by you will be kept in strict confidence. Under no circumstances shall we reveal the identity of the respondent or their families to anyone. The information that we collect shall be used for approved research purposes only. You will be informed about any significant new findings - including adverse events, if any, – whether directly related to you or to other participants of this study, developed during the course of this research which may relate to your willingness to continue participation.

Consent: The above information regarding the study, has been read by me/ read to me, and has been explained to me by the investigator/s. Having understood the same, I hereby give my consent to them to interview me. I am affixing my signature / left thumb impression to indicate my consent and willingness to participate in this study (i.e., willingly abide by the project requirements).

Signature / Left thumb impression of the Study Volunteer / Legal Representative:

Signature of the Interviewer with date: Witness:

Contact number of PI: 9566371859

Contact number of Ethics Committee Office: 0422 4345818

DATA COLLECTION TOOL

Name :

Age :

Residential Address :

Occupation :

Menstrual status : Premenopausal / Postmenopausal

Marital History : Married / Unmarried

Dietary status : Vegetarian / Non- Vegetarian

Height :

Weight :

Body Mass Index :

Symptoms of GERD :

Endoscopy : Grade A / B / C / D

LES Manometry :

Serum Estrogen Levels :

QUESTIONNAIRE	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐	**5** ☐
1. How bad is the heartburn?	☐	☐	☐	☐	☐	☐
2. Heart burn when lying down?	☐	☐	☐	☐	☐	☐
3. Heartburn when standing up	☐	☐	☐	☐	☐	☐
4. Heart burn after meals?	☐	☐	☐	☐	☐	☐
5. Does heartburn change your diet?	☐	☐	☐	☐	☐	☐
6. Does heartburn wake you from sleep?	☐	☐	☐	☐	☐	☐
7. Do you have difficulty in swallowing?	☐	☐	☐	☐	☐	☐
8. Do you have pain with swallowing?	☐	☐	☐	☐	☐	☐
9. Does taking medications affect your daily life?	☐	☐	☐	☐	☐	☐
10. How bad is the regurgitation?	☐	☐	☐	☐	☐	☐
11. Regurgitation when lying down?	☐	☐	☐	☐	☐	☐
12. Regurgitation whenstanding up?	☐	☐	☐	☐	☐	☐
13. Regurgitation after meals?	☐	☐	☐	☐	☐	☐
14.Does regurgitation change your diet?	☐	☐	☐	☐	☐	☐
15.Does regurgitation wake you from sleep?	☐	☐	☐	☐	☐	☐

TOTAL SCORE : ☐

PREMENOPAUSAL

S.NO	Age	Sex	Height	Weight	BMI	Score	Endoscopy	LES lax	Reflux	LES pressure	EGJ-CI	Motility	Estrogen
1	39	F	154	71	29.9	12	Normal	Absent	Absent	29.7	31.7	Normal	105.6
2	37	F	170	82	28.4	32	Normal	Absent	Absent	22.6	5.8	Normal	98.4
3	36	F	158	61	24.4	12	Abnormal	Present	Present	8.2	5.7	Abnormal	66.9
4	43	F	161	77	29.7	13	Normal	Absent	Absent	23.4	47.3	Normal	144.2
5	41	F	156	66	27.1	12	Normal	Absent	Absent	16.3	17.4	Normal	200
6	38	F	153	66	28.2	24	Normal	Absent	Absent	13	34.8	Normal	76.4
7	40	F	160	93	36.3	35	Normal	Absent	Absent	31.4	50.9	Normal	157
8	45	F	155	52	21.6	18	Abnormal	Present	Absent	9.2	7.5	Abnormal	74
9	39	F	153	73	31.2	32	Abnormal	Present	Present	8.4	17.1	Abnormal	66
10	42	F	161	83	32	13	Abnormal	Present	Absent	8.2	15.7	Abnormal	79
11	36	F	165	60	22	30	Abnormal	Present	Present	21	27.5	Normal	171
12	35	F	162	75	28.6	16	Normal	Absent	Absent	21.5	45.9	Normal	150.1
13	34	F	149	60	27	24	Normal	Absent	Absent	9.2	11.3	Abnormal	86.1
14	35	F	156	60	24.7	30	Abnormal	Present	Present	8.5	7.8	Normal	56.3
15	48	F	153	59	25.2	25	Abnormal	Present	Present	8.5	6.2	Abnormal	76
16	38	F	152	59	25.5	23	Abnormal	Present	Absent	12.7	7.9	Normal	135
17	35	F	158	63	25.2	12	Normal	Absent	Absent	19.1	30.8	Normal	134.7
18	41	F	157	62	25.2	21	Normal	Absent	Absent	24.8	16.1	Normal	146.7
19	51	F	151	75	32.9	12	Normal	Absent	Absent	22.1	77.4	Abnormal	118.5
20	43	F	161	61	23.5	21	Abnormal	Present	Present	8.5	16.8	Normal	77.7
21	35	F	163	76	28.6	15	Normal	Absent	Absent	14.5	36.9	Normal	206
22	45	F	153	68	29	14	Normal	Absent	Absent	13.6	30	Abnormal	174
23	35	F	147	49	22.7	28	Abnormal	Present	Present	5.5	58.3	Abnormal	74
24	46	F	152	60	26	24	Abnormal	Present	Absent	9.2	7.2	Normal	76.31
25	46	F	168	82	29.1	24	Normal	Absent	Absent	18.8	77.4	Normal	134.42
26	43	F	141	51	25.7	27	Normal	Absent	Absent	17.6	30.3	Abnormal	236
27	41	F	155	73	30.4	24	Normal	Absent	Absent	12.9	55.5	Abnormal	184
28	41	F	147	49	22.7	27	Abnormal	Present	Present	5.2	56.7	Normal	84
29	39	F	160	55	21.5	15	Normal	Absent	Absent	13.8	33.7	Normal	156.3
30	45	F	145	65	30.9	26	Abnormal	Present	Present	9.2	7.9	Abnormal	89.1

POSTMENOPAUSAL

S.NO	Age	Sex	Height	Weight	BMI	Score	Endoscopy	LES lax	Reflux	LES pressure	EGJ-CI	Motility	Estrogen
31	68	F	156	62	25.5	24	Abnormal	Present	Present	8.2	5.9	Abnormal	5
32	60	F	157	67	27.2	25	Abnormal	Present	Present	8.2	6.2	Abnormal	5
33	53	F	156	72	29.6	27	Abnormal	Present	Present	8.2	7.9	Abnormal	5
34	54	F	170	77	26.6	15	Abnormal	Present	Present	8.2	5	Abnormal	8
35	47	F	155	66	27.5	26	Abnormal	Present	Present	8.2	7.4	Abnormal	5
36	53	F	156	63	25.9	24	Abnormal	Present	Present	8.2	5.5	Abnormal	5
37	58	F	151	68	29.8	12	Abnormal	Present	Present	8.2	6.3	Abnormal	12
38	50	F	144	77.5	37.4	24	Abnormal	Absent	Present	12	29.5	Abnormal	10
39	45	F	154	52	21.9	12	Normal	Absent	Absent	11.7	25.5	Normal	13
40	50	F	150	45	20	12	Abnormal	Present	Present	5.2	7.8	Abnormal	5
41	57	F	159	72.6	28.7	30	Abnormal	Present	Present	7.1	5	Abnormal	5
42	62	F	156	53	21.8	28	Normal	Absent	Absent	13.7	24.4	Normal	10
43	67	F	159	49	19.4	24	Abnormal	Present	Present	7.5	7.4	Abnormal	5
44	63	F	168	71.6	25.4	12	Normal	Absent	Absent	12.6	28.8	Normal	10
45	54	F	167	60	21.5	24	Normal	Absent	Absent	12.6	21.6	Normal	18
46	53	F	162	55	21	16	Abnormal	Present	Present	19.4	25.1	Abnormal	5
47	56	F	154	68	28.7	16	Abnormal	Absent	Present	12.6	22.4	Normal	18
48	47	F	152	46	19.9	12	Abnormal	Present	Present	7.6	5.8	Abnormal	15
49	61	F	149	55	25	21	Abnormal	Present	Present	5.5	2.5	Abnormal	5
50	59	F	166	63	22.9	24	Abnormal	Present	Present	5	7.5	Abnormal	5
51	57	F	156	62	25.5	24	Normal	Absent	Absent	15.2	24.4	Normal	15
52	57	F	160	68	26.6	15	Abnormal	Present	Present	5	6.7	Abnormal	5
53	60	F	160	77	30.1	18	Abnormal	Present	Present	7	5.4	Abnormal	5
54	70	F	149	68	30.6	19	Normal	Absent	Absent	11.5	24.2	Normal	17
55	69	F	154	62	26.1	12	Abnormal	Present	Present	7	5.2	Abnormal	5
56	70	F	160	65	25.4	24	Normal	Absent	Absent	17.6	36	Normal	24
57	70	F	156	60	24.7	16	Abnormal	Present	Absent	7	7.9	Abnormal	5
58	54	F	149	47	21.2	12	Normal	Absent	Absent	11.4	29	Normal	24
59	63	F	152	70	30.3	16	Abnormal	Present	Present	6.2	7.3	Abnormal	5
60	45	F	147	53	25	32	Normal	Absent	Absent	15	31.5	Normal	24

ABBREVIATION

S.No	Serial number
M	Male
F	Female
BMI	Body Mass Index
GERD	Gastro-esophageal reflux disease
EGJ-CI	Esophagogastric junction- Contractile index
LES	Lower esophageal sphincter
HRM	High resolution manometry
FSH	Follicle stimulating hormone (FSH)
LH	Luteinizing hormone
E1	Estrone
E2	17β-Estradiol
E3	Estriol
ERD	Erosive reflux esophagitis
NERD	Non - erosive reflux esophagitis
ECLIA	Electrochemiluminescence Immunoassay