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RESEARCH ARTICLE

Clinical utility of histology in non-erosive gastro-esophageal reflux disease.

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Abstract

Background: in the absence of gross oesophageal erosions longterm ph monitoring is the present gold standard for diagnosing gastro-esophageal reflux disease**.**this method, however, is invasive, time consuming and not generally available.

Aim of the study: this study was specifically designed to determine the diagnostic value of histological findings in patients with non-erosive gastro-esophageal reflux disease.

Study design: an observational, cross-sectional study.

Patients and methods:68 patients (36 males & 32 females) complaining of typical gerd symptoms for at least 12 weeks, not necessarily consecutive, during the previous 12 months were enrolled in this study. They underwent upper gi endoscopy and los angeles classification was used for grading cases with mucosal breaks and biopsies were taken from esophago-gastric junction. 24 hour ph monitoring and esophageal manometry were also performed for each patient.correlation/regression analysis was done on clinical, endoscopic and histological findings. Fifteen apparently healthy subjects (9 males and 6 females) not complaining of any gerd symptoms were recruited as controls.

Results:four groups were identified on the basis of symptoms, endoscopy, and ph monitoring: (1) erosive esophagitis (n=24), (2) non-erosive esophagitis with abnormal ph (n=33), (3) non-erosive esophagitis with normal ph (n=11), and (4) controls (n=15).the presence of erosive esophagitis was directly correlated to male gender (p=0.0225) , current smoking (p= 0.0227) and hiatus hernia (p=0.0086).biopsies were assessed for basal cell hyperplasia, papillae elongation, intercellular space dilatation, intraepithelial eosinophils infiltration, intraepithelial neutrophil infiltration and erosions. A global severity score was calculated on the basis of the above parameters and allowed the distinction of patients from controls with 82.4% sensitivity and 86.7% specificity.there global severity score was directly correlated to the frequency of reflux episodes. It was also directly related to the time elapsed below ph 4.0 and the composite ph score.

Conclusions:in contrast with previous reports on the marginal role of histology in patients with gerd, our study shows that this technique can be a useful diagnostic tool, particularly in patients With nerd where it may contribute to diagnosis and play a role in the comparative evaluation of different therapies.

Introduction:-

Gastroesophageal reflux disease (GERD) is defined as an abnormal reflux of the gastric contents into the esophagus at least once a week, leading to symptoms, such as heartburn and/or acid regurgitation, and/or esophageal mucosal damage, which may also provoke long-term complications, such as Barrett's esophagus ***(1).***

GERD is a multifactorial disease in which anatomical and functional factors both play a pathogenetic role. The main pathogenetic mechanism of GERD is considered to be transient lower esophageal sphincter relaxation (TLESR) which may account for the majority of reflux episodes, in patients with esophagitis and in those with non-erosive reflux disease (NERD). An increased number of TLESR episodes, combined or not with an impaired LES basal tone or with gastric or esophageal motor dysfunction, may lead to GERD, but the underlying causes of these functional disorders are still partially unknown ***(2).***

The diagnosis of GERD poses a challenge for even the most skilled clinician. Without a test that is 100% sensitive and specific for disease, the physician must assimilate the patient's presentation and adopt an individualized strategy that will best arrive at a diagnosis. This demands a fundamental understanding of the many tests available for detecting GERD, the strengths and weaknesses of each test, and the indications for their use. By employing this approach, the physician optimizes the opportunity to correctly identify GERD and its relationship to the patient's complaints ***(3).***

Moreover, GERD encompasses a wide spectrum of clinical manifestations, ranging from symptoms without anatomical gross lesions (nonerosive reflux disease = NERD) to erosive esophagitis (erosive reflux disease = ERD) and complications such as ulcers, strictures, hemorrhage, and Barrett's esophagus ***(4)***. Because of this heterogeneity, it has been always difficult to define the gold standard diagnostic test.

Symptom collection by structured questionnaires is frequently deceiving because it is mainly based on subjective variables***(5).*** Endoscopy can fail to detect lesions in up to two-thirds of cases with symptomatic reflux ***(6),*** and 24-h esophageal pH monitoring can be normal in about one-third of patients with NERD ***(7)***. Hence, it is highly required to study the multi-investigational approach for diagnosis trying to define an objective diagnostic tool with high sensitivity and specificity.

The present study is based on the systematic investigation of a series of GERD patients compared with an appropriate control group. All patients and controls underwent clinical investigation for symptoms, endoscopy, and 24-h pH monitoring coupled with a careful, systematic histological analysis aimed to define whether GERD is associated with microscopic esophagitis, what lesions are mostly expressed, and if the is a correlation between histopathology and other investigations.

Aim of the study:-

This study was specifically designed to determine the diagnostic value of histological findings in patients with non-erosive gastro-oesophageal reflux disease.

Study design:-

An observational, cross-sectional study.

Patients and methods:-

This study was conducted on 68 patients referred to Specialized Medical Hospital (SMH) outpatient clinic complaining of classic gastroesophageal reflux symptoms were enrolled in this study. The researcher completed a detailed symptom assessment for each patient using a validated gastrointestinal symptom questionnaire that has previously been shown to be reproducible ***(8)***.This questionnaire recorded the frequency and severity of clinically relevant gastrointestinal symptoms during the previous 12 months, using the Rome II cut-off which defines clinically relevant symptoms as those occurring for ''at least 12 weeks, not necessarily consecutive'' ***(9)***.

For the purposes of this study, the typical reflux symptoms of heartburn (described as ''a burning pain or discomfort behind the breastbone in the chest'') and acid regurgitation (''a bitter or sour fluid coming into the throat or mouth'') were recorded as frequent (symptoms on at least 2 days per week) or infrequent (symptoms on fewer than 2 days per week). Data were also collected on age, sex, weight, height, smoking, consumption of alcohol and coffee, and use of aspirin, non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids.

Fifteen subjects (9 males and 6 females, mean age 31.4 yr, range: 23–49 years) without esophageal symptoms were used as controls. They underwent upper GI endoscopy and 24-h esophageal pH monitoring with normal results of both examinations.

Upper GI endoscopy:
Upper GI endoscopy (esophago-gastro-dudenoscopy) was done for all patients in the study at admission.
All the following endoscopic findings were recorded for all patients:
Presence or absence of gross endoscopic signs of gastroesophageal reflux.

- Grading of the degree of gastroesophageal reflux according to the Los Angeles Classification - Other esophageal findings (e.g. ulcer, stricture…etc) - Presence or absence and the size of hiatal hernia. A hiatal hernia was diagnosed when gastric mucosa was fixed or prolapsed 2 cm or more above the diaphragmatic hiatus during quiet respiration without excessive air insufflation.

Esophageal histopathology:
Multiple esophageal biopsies were obtained from each patient using esophageal biopsy forceps (ACMI Martin biopsy forceps), 2-3 cm above the squamocolumnar junction (the Z line).
All biopsy specimens were fixed in 10% neutral buffered formaldehyde and then embedded in paraffin. Sections were cut from the paraffin blocks and stained with haematoxylin and eosin and Giemsa. Histological assessment was performed by our pathologist independently and in a blind manner.
The histopathological report included : basal cell hyperplasia (BH), papillae elongation (PE), and dilatation of intercellular spaces (DIS). Each one of those parameters were semiquantitatively scored as 0 (absent), 1 (mild), and 2 (marked) on hematoxylin–eosin stained slides obtained from each biopsy site. Basal cell thickness (normal values= <15% at 2 and 4 cm and <20% at the Z-line) and length of papillae (normal values=<50% at 2 and 4 cm and <66% at the Z line) were recorded as a percentage of the total epithelial thickness. The cytological features of basal and intermediate layer nuclei helped in defining basal cell hyperplasia in poorly oriented samples. DIS were scored on the basis of their size. Before starting blind assessment, some reference pictures of the different grades of basal cell hyperplasia, papillary elongation, and DIS were selected and formed a sort of visual analogic scale ranging from 0 to 2 ***(10).***
In addition, the presence of intraepithelial infiltration of eosinophils (IE) (score 0=absent, 1=1 eosinophil, 2=>1 eosinophil per HPF 40×), neutrophils (0=absent, 2=present), and necrosis/erosions (0=absent, 2=present) were recorded.
When lesions were not homogeneously distributed in a given sample, the most severe change was considered.
Global severity score
The overall severity of all the lesions was described by means of a global score (GS). Calculation of the score was obtained by summing up all the scores for BH, PE, ISD, IE and intraepithelial neutrophils (IN) (range 0–2) and dividing by the number of assessable lesions. The GS spanned from 0 to 2. In the presence of intraepithelial neutrophils or necrosis/ erosion (found almost exclusively in erosive disease), the assigned score.
was automatically 2 because such lesions represent the most severe end of the spectrum ***(11)***.

Esophageal Manometry:
Esophageal Manometry was performed to locate the LES using water perfused eight lumen pressure catheter with an outside diameter of 4.5mm, side holes at 5,5,5,2,1,1 and 1 cm apart from each other, from the proximal and they are radially oriented by 360° and were constantly perfused with distilled water from "Mui-scintific" perfusion pump at a rate of 0.5ml/min. The catheter assembly was passed through the nose and advanced into the stomach ***(12).***

Ambulatory twenty-four hour pH monitoring:
Ambulatory twenty-four hour pH monitoring was performed using a disposable antimony or glass in gold pH electrodes was passed and secured to the nose with an electrode placed 5cm above the LES as (located by manometric examination) and the electrode was connected to a portable Digtrapper MKIII (Synectics medical Sweden).
Esophageal acid exposure was then scored according to DeMeester and Johnson scoring system **(*13*)**.

Results:-

The study included 68 patients, 36 males (53%) and 32 females (47%),with age ranging between 20 and 50 years with the mean age 38.2.

The control group included 15 healthy subjects: 9 males and 6 females with age range 23–49 with the mean age 35.4 years.

Table (1): Baseline demographic and clinical data

Variable	Patients Males		Control	
	Males	**Females**	**Males**	**Females**
Total number of patients	36(53%)	32(47%)	9 (60%)	6 (40%)
Age (years): -range - Mean ± SD	(23 – 49) 38.2 ±13.3	(20- 50) 31.7 ±12.8	(23-47) 34.1±7.9	(24-49) 37.5±9.6
Body mass index (kg. /m2) - < 20 - 20-24.9 - 25-29.9 - ≥ 30	 0 (0%) 13(36.1%) 17(47.2%) 6(16.6%)	 1(3.1%) 11 (34.3%) 10 (31.2%) 10(31.2%)	 1(11.1%) 6 (66.6%) 2(22.2%) 0 (0%)	 0 (0%) 1(16.6%) 4(66.6%) 1(16.6%)
Smoking status - Current - Former - Never	 16(44.4%) 7(19.4%) 13(36.1%)	 1(3%) 1 (3%) 30 (94%)	 3 (33.3%) 0(0%) 12(66.6%)	 0(0%) 0(0%) 0(0%)
Coffee consumption - Yes - No	 8(22.2%) 28(77.8%)	 10(31.2%) 22(68.7%)	 1(11.1%) 8(88.9%)	 1(16.6%) 5(83.4%)
Medication use - Yes - No	 7(19.4%) 31(80.6%)	 9 (28.1%) 23 (71.8%)	 1(11.1%) 8(88.9%)	 1(16.6%) 5(83.4%)

- Hiatus hernia was significantly associated only with frequent symptoms.
- A positive association was observed between female sex and frequent reflux symptoms.
- Factors that did not increase the risk of frequent reflux symptoms included (as a whole or after stratifying by sex): H. pylori infection, BMI, current or former smoking, coffee consumption, and use of drugs.

Table(2): Correlation between the frequency of reflux episodes and other factors

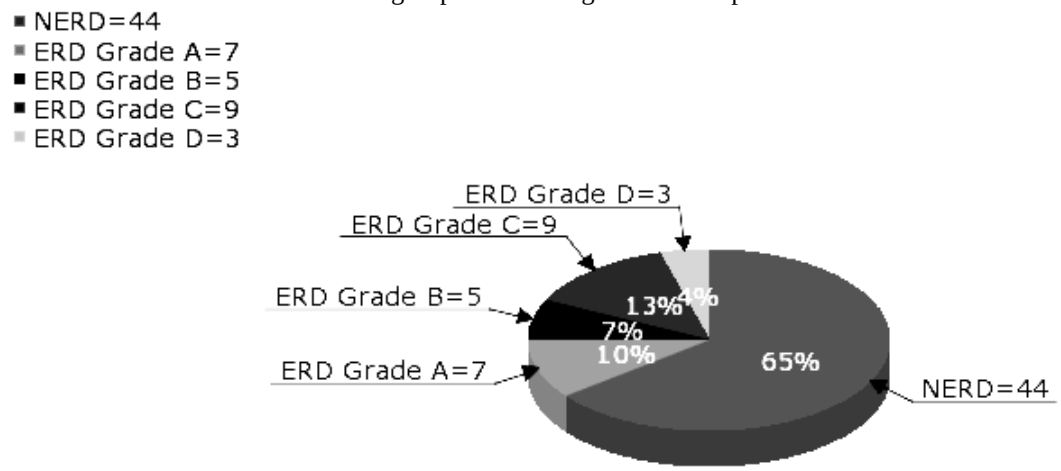
	Frequency of reflux episodes						
	None (control group) n=15	**Less than twice per week n=30(44.1%)**			**At least twice per week n=38 (55.9%)**		
		N (%)	RR (95% CI)	Sig. level	N (%)	RR (95% CI)	Sig. level
Age (years)							
- 20-29	2(13.3%)	4(13.3%)	1	1.0000	6(15.7%)	1.3421	0.6996
- 30-39	5 (33.3%)	8(26.6%)	0.8	0.6380	10(26.3%)	0.7895	0.6034
- 40-49	5 (33.3%)	7(23.3%)	0.7	0.4692	9(23.6%)	0.7105	0.4643
- ≥ 50	3 (20%)	9(36.6%)	1.50	0.4896	13(34.2%)	1.7105	0.3406
Sex							
- Male	8 (53.4%)	20(66.6%)	1.2500	0.4152	16(42.1%)	0.7895	0.4420
- Female	7 (46.6%)	10(33.3%)	0.7143	0.3733	22(57.9%)	1.2406	0.4850
Body mass index (kg. /m2)							
- < 20	1(6.6%)	0(0%)	0.1185	0.1844	1(2.6%)	0.6250	0.7326
- 20-24.9	7 (46.6%)	11(36.6%)	0.5357	0.1005	12(31.5%)	1.0714	0.8407
- 25-29.9	6(40%)	13(43.3%)	0.7386	0.4404	17(44.7%)	1.7708	0.0950
- ≥ 30	1(6.6%)	7(23.3%)	2.3864	0.3968	8(21%)	5.0000	0.1104
Smoking status:							
- Current	3 (20%)	8(26.6%)	1.3333	0.6308	9(23.6%)	1.1842	0.7755
- Former	0(0%)	3(10%)	3.6129	0.3855	5(13.1%)	4.5128	0.2977
- Never	12(80%)	19(63.3%)	0.7917	0.2180	24(63.1%)	0.7895	0.1865
Coffee consumption							
- Yes	2(13.3%)	8(26.6%)	2.0000	0.3388	10(26.3%)	2.6786	0.1625
- No	13(68.7%)	22(73.7%)	0.8462	0.2641	28(74.7%)	0.7023	0.0232
Medication use							
- Yes	2(13.3%)	6(20%)	1.5000	0.5901	10(26.3%)	2.5000	0.1950
- No	13(68.7%)	24(80%)	0.9231	0.5572	28(73.7%)	0.8502	0.2471
H. pylori infection	6(40%)	17(56.6%)	1.4167	0.3255	23(60.5%)	1.5132	0.2262
Hiatus hernia	0(0%)	12 (40%)	12.9032	0.0695	22(57.9%)	18.4615	0.0371

Figure (1) shows the morphological endoscopic results.

- Erosive reflux disease was detected in 24 patients (35.2%) whereas the remaining 44 patients (64.7%) were labeled as non erosive reflux disease.

Of the 24 patients with erosive reflux disease, 7 patients belonged to grade A, 5 patients belonged to grade B, 9 patients belonged to grade C and 3 patients belonged to grade D.

- Endoscopic hiatus hernia was detected in 34 patients out of 68 (50%).
- Individuals of the control group showed no gross endoscopic abnormalities.



The presence of erosive esophagitis was directly correlated to male gender, current smoking and hiatus hernia.
The presence of erosive esophagitis was directly correlated with marked higher frequency of reflux symptoms.
There was no significant correlation between an increased risk of esophagitis and H. pylori infection, drug intake, Coffee consumption or BMI.

Table (3): Correlation between erosive esophagitis and other factors

Characteristics	NERD n=44 (64.7%)	ERD n=24 (35.2%)	RR (95% CI)	Significance level
Age (years)				
- 20-29	12(27.2%)	6(25%)	0.9167	0.8399
- 30-39	17(38.6%)	10(41.6%)	1.0784	0.8059
- 40-49	13(29.5%)	7(29%)	0.9872	0.9739
- ≥ 50	2(4.5%)	1(4.1%)	0.9583	0.9717
Sex				
- Male	19(43.2%)	17(70.8%)	1.6404	0.0225
- Female	25(56.8%)	7(29.2%)	0.5133	0.0527
Body mass index (kg. /m2)				
- < 20	2(4.5%)	1(4.1%)	0.9583	0.9717
- 20-24.9	16(36.3%)	8(33.3%)	0.8333	0.5999
- 25-29.9	18(40.9%)	11(45.8%)	1.1204	0.6916
- ≥ 30	8(18.1%)	4(16.6%)	0.5000	0.1993
Smoking status				
- Current	7(6.8%)	10(58.3%)	2.6190	0.0227
- Former	3(6.8%)	5(20.8%)	3.0556	0.1029
- Never	34(77.2%)	9(37.5%)	0.4853	0.0088
Coffee consumption				
- Yes	11(25%)	7(29.1%)	1.1667	0.7080
- No	33(75%)	17(70.9%)	0.9444	0.7163
Medication use				
- Yes	9(20.5%)	7(29.2%)	1.1039	0.8246
- No	35(79.5%)	24(70.8%)	0.9733	0.8264
Frequency of reflux symptoms				
- At least twice per week	20(45.5%)	18 (75%)	1.6500	0.0136
- Less than twice per week	24(54.5%)	6 (25%)	0.5000	0.0735
H. pylori infection				
- Yes	26 (59%)	14 (58.3%)	0.9872	0.9518
- No	18(41%)	10(41.7%)	1.0185	0.9515
Hiatus hernia				
- Yes	17 (38.6%)	17 (70.8%)	1.8333	0.0086
- No	27(61.4%)	7(29.2%)	0.4753	0.0286

Table (4): Overall assessment by using the global severity score

Group	Number of cases	Score ≥0.3	%
ERD	**24**	**23**	**95.8%**
NERD pH +ve	**33**	**24**	**72.7%**
NERD pH -ve	**11**	**6**	**54.5%**
All patients	**68**	**53**	**77.9%**
Control group	**15**	**2**	**13.3%**

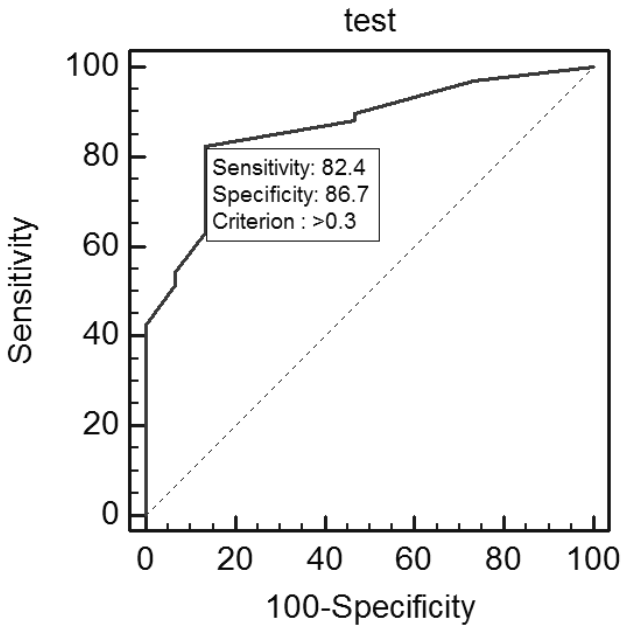
Four groups were identified on the basis of symptoms, endoscopy, and pH monitoring: (1) erosive esophagitis (n=24), (2) non-erosive esophagitis with abnormal pH (n=33), (3) non-erosive esophagitis with normal pH (n=11), and (4) controls (n=15).

Biopsies were assessed for basal cell hyperplasia, papillae elongation, intercellular space dilatation, intraepithelial eosinophils infiltration, intraepithelial neutrophil infiltration and erosions.

A global severity score was calculated on the basis of the above parameters. Calculation of the score was obtained by summing up all the scores for BH, PE, ISD, IE and intraepithelial neutrophils (IN) (range 0–2) and dividing by the number of assessable lesions.

The global severity score allowed the distinction of patients from controls with 82.4% sensitivity and 86.7% specificity with cut off value of 0.3.

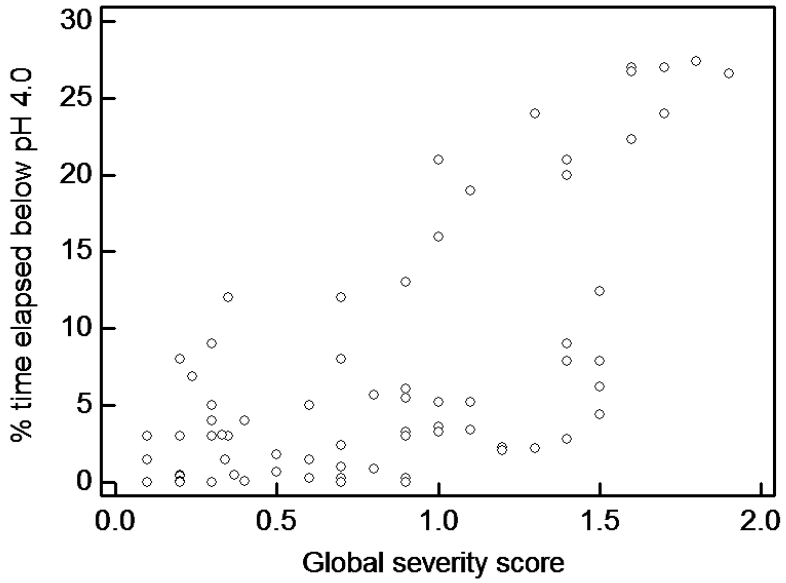
Figure (2): ROC curve analysis to the Global Severity Score for distinction of patients from controls



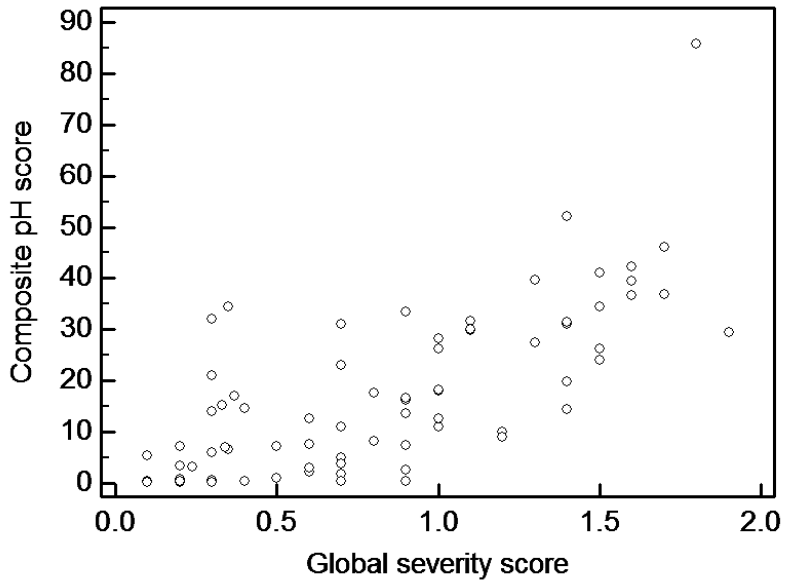
There global severity score was directly correlated to the frequency of reflux episodes. It was also directly related to the time elapsed below pH 4.0 and the composite pH score.

Figure (3): Correlation between esophageal histopathological changes and 24-hour pH monitoring

1- Correlation between the global severity score and the percent time elapsed below pH 4:



2- Correlation between the composite acid score (DeMeester Score) and the percent time elapsed below pH 4 :



DISCUSSION:-

Gastroesophageal reflux disease (GERD) is caused by the reflux of gastric contents into the esophagus. GERD affects 3% to 4% of the population ***(14).***

The diagnosis of GERD is based on the combination of clinical symptoms, endoscopic findings, pH monitoring, and histologic changes. However, GERD is a heterogeneous disease, and the findings in these tests do not always

correspond. For example, some patients report typical symptoms but lack other positive tests, whereas other patients are asymptomatic yet exhibit typical endoscopic and histologic features of GERD ***(15).***

The present study was specifically designed to assess the esophageal histopathological, endoscopic, radiologic, manometric and pH changes in GERD patients in comparison to a group of healthy volunteers.

In our study, male gender (P=0.0225) and current smoking (P=0.0527) appeared to be risk factors for erosive esophagitis. This is similar to results reported by Labenz et al (2004) ***(16).*** On the contrary, other studies believed that the association between lifestyle factors (eg smoking - coffee) and GERD is still unclear and lacking strong association ***(17)(18).***

We found that the frequency of reflux symptoms was not affected by cigarette smoking, coffee consumption or drug intake (NSAIDs or corticosteroids). This result is similar to that adopted by Dent et al, 2005 and Moayyedi &Talley, 2006. ***(17)(18).***

On the other hand, a study performed by Zheng and his colleagues (2007) reported an association between frequent smoking and an increased risk of symptoms of GERD. Different mechanisms have been suggested to justify the association between smoking and GERD. Cigarette smoking can reduce the LES pressure and decrease salivary bicarbonate secretion, thus reducing the physiological neutralizing effect of saliva on intraesophageal acid and prolonging acid clearance. Furthermore, abrupt increases in intra-abdominal pressure, as occur during coughing or deep inspiration, have been associated with reflux symptoms in smokers **(*19)***.

Even though the role of these life style factors appears to be controversial, lifestyle changes are still recommended for all patients with or at high risk for GERD (for example, meal size and timing, not lying down after a meal or lying down where the head is elevated, not smoking, not consuming alcohol, not eating heavily spiced or fatty food and having a physically active life) ***(20).***

In the current study, H. pylori infection did not appear to increase the frequency of reflux episodes or increase the incidence of erosive disease. The role of H. pylori infection in the natural history of GERD is an area of ongoing debate.

Similar to our study results, some authors reported that the incidence H. pylori in GERD patients is similar to that in normal population ***(21)***, another study by Bingbing et al (2011) ***(22)*** proved that H. pylori eradication does not affect the clinical outcomes in terms of short-term and long-term posteradication occurrence of GERD & there is no association between *H. pylori* eradication and the development of GERD**.**

On the opposite side, other studies concluded that H. pylori infection is considered as a definite risk factor for development of erosive esophagitis ***(23)*** and furthermore, documented eradication of H. pylori appears to significantly improve GERD symptoms ***(24)***.

Moreover, Chung and his colleagues (2011) ***(25)*** studied a large sample of Korean population and reported that H. pylori seropositivity had strong inverse relationships with the risk and severity of reflux esophagitis and concluded that H. pylori is a protective factor against GERD. Even more, Pandya and his co-workers (2011) ***(26)*** claimed that H. pylori infection is protective against development of severe forms and complications of GERD. Labenz et al. (1997) ***(27)*** reported an observation that reflux esophagitis newly developed in up to 26% of patients with duodenal ulcer after the clearance of H. pylori, whereas it was present only in 13% of those with persistent infection. Barrett's esophagus and adenocarcinoma related to it have been recognized as a complication of GERD, so their report raised a special concern that H. pylori eradication therapy may be a potential risk factor for developing esophageal adenocarcinoma ***(28)***.

In our study, H. pylori infection was found in 40% of the individuals of the control group. This figure is similar to that reported in a population based study by Jackson et al. (2009) ***(29).***

This study found no association between BMI and reflux symptoms or esophagitis, either in the population as a whole or after stratifying by sex. This data comes in accordance with two population based studies performed by Lagergren et al (2000) ***(30)*** and Zagari et al (2008) ***(21)*** .

On the other hand, other studies conclude that increasing BMI is a well recognized risk factor for GERD ***(31)(32).***They explained the association between obesity and GERD by the fact that increased abdominal waist circumference, could be associated with increased intra abdominal pressure which would, in turn, promote GERD by increasing intragastric pressure

An explanation of this discrepancy may be found in a meta-analysis that, evaluating US and European data separately ***(33)*** showed that the association between elevated BMI and the presence of GERD reported within the European studies was less clear than that reported in the US studies. A potential explanation may be that the relationship between BMI and percentage body fat differs between different populations and different ethnic groups. It has been reported, for example, that the percentage body fat of individuals with the same BMI differs between Hispanic and European American women ***(34)***.

In this study, the presence of hiatus hernia (proved by endoscopy) was found to increase both the frequency of reflux episodes and the incidence of endoscopic erosive disease (ERD). This comes in accordance with the results of previous studies by Durk et al (2008) ***(35).***This may be explained by the physiological dysfunction of the LES and the anatomic distortion of the gastroesophageal junction that occur with hiatus hernia ***(36).***

The role of hiatus hernia may be attributed to the anatomic distortion of the gastroesophageal junction. Moreover, during abrupt increases in intra-abdominal pressure, the crural diaphragm normally serves as a "second sphincter," and this mechanism is substantially impaired in individuals with a gaping hiatus. Large, non-reducing hernias also impair the process of esophageal emptying, thereby prolonging acid clearance time following a reflux event (especially while in the supine posture) ***(37)***.

The histopathological results of this study provide consistent data showing that histological alterations characterize most patients with documented abnormal reflux. The good sensitivity of histology (85%) in all GERD patients coupled with a high specificity in controls (86%) suggests that histopathology is a reliable diagnostic tool in patients with GERD. The sensitivity in this study is comparable to that found by Mastracci et al. (2009) ***(11).***

On the contrary, other studies conducted by Seefeld U et al. (1977)***(38),*** Collins BJ, et al. (1985) ***(39)*** have reported that the sensitivity of histology is lower, ranging from 17% to 62%.

This discrepancy in results may be attributed to the fact that - in our study - we have added a sensitive variable, which is dilatation of intracellular spaces (DIS), to the histological criteria proposed by Ismail et al. (1970) ***(40)*** and adopted by other studies ***(39)***.

This histological marker of microscopic esophagitis was first proposed by Solcia et al. (2000) ***(41)*** and validated by Villanacci et al. (2001) ***(42).*** A later study also utilized this diagnostic parameter successfully but using an electron microscopy approach ***(43)***. However, this latter technique is time consuming, expensive, and not generally available and therefore cannot be proposed for routine diagnostic purposes.

The assessment of DIS by optical microscopy is certainly easier, simpler, and cheaper than electron microscopy. DIS was associated with good sensitivity and was as reliable as the elongation of papillae at the same biopsy sites. Its frequent occurrence in patients with NERD can also be of help in explaining the mechanism by which acid stimulates the appearance of heartburn, because DIS may favor the diffusion of hydrogen ions and their contact with nerve endings deep in the esophageal mucosa ***(44).***

Since the expected contribution of histology to the diagnosis of reflux disease is mainly limited to NERD patients, the lesions that provided useful information in this context were BH, DIS, PE, and .

Regarding the sensitivity of histology in relation to the presence or absence of endoscopic esophagitis, it was 96% in patients with ERD and 76% in patients with NERD. The former number confirms that nearly all patients with gross endoscopic esophagitis have histological lesions. The few cases with normal histology may be explained by sampling error due to the patchy nature of microscopic changes ***(40)***. However, this finding does not add useful information to endoscopy, and biopsies are not recommended in patients with erosive esophagitis, except for excluding metaplastic or neoplastic changes ***(11)*** .

On the other hand, histology provided additional valuable diagnostic information in 76% of NERD patients where endoscopy showed no lesions. Taking multiple biopsies at the Z line and 2 cm above it can improve the diagnostic yield in patients without gross mucosal breaks. The value of pathology seems even more important, when remembering that 24-h pH monitoring, which is known as the most reliable procedure for detecting GERD ***(45)***, can give false negative results in about one-third of cases with NERD ***(46)***. The presence of histological alterations in the majority of patients with NERD is also important because it permits objective follow up of the effectiveness of antireflux therapies ***(47)***, instead of depending on the non reliable subjective resolution of symptoms.

In this study, there was a direct correlation between global severity score calculated for all biopsies and the time elapsed below pH 4.0 for the total monitored period. So we can conclude that the histological lesions detected were mainly related to acid reflux. However, we found significant histological changes in 6 of 11 patients with NERD pH -ve (normal endoscopy and normal pH testing), but with GERD symptoms assessed by means of a validated questionnaire. This means that histology has the advantage of objective diagnosis in about half of patients with functional heartburn. It is also possible that nonacid reflux may play a role in determining histological lesions in patients with normal pH testing ***(48).***

There are three other studies with a larger population than ours, but important differences exist between them and the present study. Funch et al. (1986)***(49)*** evaluated histopathological changes in esophageal biopsies in 200 patients with reflux symptoms and 74 controls, but esophageal pH monitoring was not done in any of those two groups, moreover, dilatation of intracellular spaces (DIS) was not evaluated. Another study by Vieth et al. (2004) ***(50)*** was performed in 1,475 patients but, once again, DIS was not evaluated and, more importantly, a control group was lacking. Despite the limitations of the above mentioned two studies, their authors recommended histology as a reliable tool in the diagnosis of GERD. Finally, Steiner et al. (2004) ***(51)*** carried out a retrospective study in 305 children, but they focused only on the presence of eosinophils in biopsy samples and excluded any correlation between them and gastroesophageal reflux.

The control group in our study was composed of asymptomatic volunteers who underwent upper GI endoscopy and 24-h esophageal pH monitoring with normal results of both examinations. Most of the previous similar studies (***(49)(50)*** recruited as controls asymptomatic individuals or subjects with symptoms other than GERD with normal endoscopy and no pH metry was done. It is now established that patients with GERD can be entirely asymptomatic and endoscopy has a low sensitivity because only the minority of patients with GERD have gross mucosal breaks ***(6)***. Thus, a normal 24-h pH testing must be added to the absence of both symptoms and endoscopic lesions in defining a proper control group, since pH-metry remains the most reliable method to exclude the presence of GERD. Whereas only few previous studies have followed this methodology, all our control subjects underwent pH monitoring in order to be sure that their esophageal acid exposure was normal. However, evident histopathological lesions were found in two of them. This unexpected result may be explained by the fact that these two subjects- not suffering from any reflux symptom- could have been GERD patients, in whom pH test failed to detect abnormal acid exposure ***(52)***.

Although the diagnosis of GERD is done nowadays merely on a clinical basis and symptoms mainly make the therapeutic decisions, the results of this study indicate that histopathological examination of esophageal samples combined with pH metry can be an accurate and reliable method that can improve the approach to patients with reflux disease and can help in finding a correlation between microscopic esophagitis and acid or nonacid reflux, particularly in patients with NERD ***(5)***.

The finding that most NERD patients do have histopathological changes contributes to the general knowledge of disease because it demonstrates that the majority of these patients belong to a condition with an organic rather than functional origin. The recognition of microscopic changes in NERD has also practical implications: histopathology might contribute to the assessment of some subgroups of patients (i.e., those with atypical symptoms) and play a role in the comparative evaluation of different therapies or drug regimens for NERD treatment.

References:-

1- Davide Festi, Eleonora Scaioli, Fabio Baldi, Amanda Vestito, Francesca Pasqui, Anna Rita Di Biase, and Antonio Colecchia. Body weight, lifestyle, dietary habits and gastroesophageal reflux disease. World J Gastroenterol. 2009 April 14; 15(14): 1690–1701.

2- El-Serag H. Role of obesity in GORD-related disorders. Gut 2008; 57: 281-284

3- Nimish V and Peter JK. GERD Diagnosis: Pretest Probability and the "Gold" Standard Alter Outcome. Am J Gastroenterol 2012; 107:322–323; doi:10.1038/ajg.2011.428

4- Stanghellini V, Cogliandro R, Cogliandro L, et al. Unsolved problems in the management of patients with gastroesophageal reflux disease. Dig Liver Dis 2003;35:843–8.

5- Stanghellini V, Armstrong D, Monnikes H, et al. Systematic review: Do we need a new gastro-esophageal reflux disease questionnaire? Aliment Pharmacol Ther 2004;19:463–79.

6- Schindlbeck NE, Klauser AG, Berghammer G, et al. Three year follow-up of patients with gastresophageal reflux disease. Gut 1992;33:1016–9.

7- Quigley EM. 24-h pH monitoring for gastresophageal reflux disease: Already standard but not yet gold? Am J Gastroenterol 1992;87:1071–5.

8- De Luca L, Zagari RM, Pozzato P, et al. Measuring dyspepsia: a new severity index validated in Bologna. Dig Liver Dis 2004;36:806–10.

9- Drossman DA. The functional gastrointestinal disorders and the Rome II process. Gut 1999;45(Suppl 2):1–5

10- Jarvis LR, Dent J, Whitehead R (1985) Morphometric assessment of reflux oesophagitis in fibreoptic biopsy specimens. J Clin Pathol 38:44–48

11- Mastracci L, Spaggiari P, Grillo F et al. Microscopic esophagitis in gastro-esophageal reflux disease: individual lesions, biopsy sampling, and clinical correlations. Virchows Arch (2009) 454:31–39

12- Bodger K, Trudgill N: Guidelines for oesophageal manometry and pH monitoring London (UK): British Society of Gastroenterology (BSG);2006.

13- DeMeester TR, Johnson LF: Development of the 24 hour intraesophageal pH monitoring composite scoring system. In: DeMeester TR, Skinner DB, eds. Esophageal disorders: Pathophysiology and therapy. New York, NY: Raven Press, 1985:561–570

14- Pluta RM, Gabriela D. Perazza, Robert M. Golub. Gastroesophageal Reflux Disease. JAMA. 2011;305(19):2024.

15- Gersonet LB, Peter J. Kahrilas, Ronnie Fass. Insights into Gastroesophageal Reflux Disease–Associated Dyspeptic Symptoms. Clinical Gastroenterology and Hepatology October 2011.Volume 9, Issue 10 , Pages 824-833.

16- Labenz J, Blum AL, Bayerd◌rffer E, Meining A, Stolte M, B◌rsch G: Curing Helicobacter pylori infection in patients with duodenal ulcer may provoke reflux esophagitis. Gastroenterology 1997;112:1442–1447.
17- Dent J, El-Serag HB, Wallander MA, et al. Epidemiology of gastro–oesophageal reflux disease: a systematic review. Gut 2005;54:710–7.

18- Moayyedi P, Talley NJ. Gastro-oesophageal reflux disease. Lancet 2006;367:2086–100.

19- Zheng Z, Nordenstedt H, Pedersen NL, et al. Lifestyle factors and risk for symptomatic gastroesophageal reflux in monozygotic twins. Gastroenterology 2007;132:87–95.

20- Festi D, Scaioli E, Baldi F et al. Body weight, lifestyle, dietary habits and gastroesophageal reflux disease. World J Gastroenterol 2009 April 14; 15(14): 1690-1701

21- Zagari RM, Fuccio L, Wallander MA et al. Gastro-oesophageal reflux symptoms, oesophagitis and Barrett's oesophagus in the general population: the Loiano–Monghidoro study. Gut 2008;57;1354-1359

22- Bingbing Q, Shijie M, Li S et al. Effects of Helicobacter pylori Eradication on Gastroesophageal Reflux Disease. Helicobacter 2011Volume 16, Issue 4, pages 255–265.

23- Wang PC, Ching-Sheng Hsu, Tai-Chung Tseng, Tsung-Cheng Hsieh et al. Male Gender, Hiatus Hernia and Helicobacter Pylori Infection Associated with Asymptomatic Erosive Esophagitis. J Gastroenterol Hepatol. 2011 Aug 22. doi: 10.1111/j.1440-1746.

24- Saad AM., Choudhary A & Bechtold ML.. Effect of Helicobacter pylori treatment on gastroesophageal reflux disease (GERD): meta-analysis of randomized controlled trials. 2012, Vol. 47, No.2,129-135

25- Chung SJ, Lim SH, Choi J et al. Helicobacter pylori Serology Inversely Correlated With the Risk and Severity of Reflux Esophagitis in Helicobacter pylori Endemic Area: A Matched Case-Control Study of 5,616 Health Check-Up Koreans. J Neurogastroenterol Motil. 2011 July; 17(3): 267–273.

26- Pandya H.B., Singh N.R., Singh S.P., Salvi P.N., Patel J.S., Agravat H., Ruparelia J.: Helicobacter Pylori Infection Is Protective Against Development Of Complications Of Gastro Esophageal Reflux Disease (GERD): A Study Done In Central Gujarat.. The Internet Journal of Gastroenterology. 2011 Volume 10 Number 1

27- Labenz J, Blum AL, Bayerd◌rffer E, Meining A, Stolte M, B◌rsch G: Curing Helicobacter pylori infection in patients with duodenal ulcer may provoke reflux esophagitis. Gastroenterology 1997;112:1442–1447.

28- Pisegna J, Holtmann G, Howden CW, Katelaris PH, Sharma P, Spechler S, Triadafilopoulos G, Tytgat G: Review article: oesophageal complications and consequences of persistent gastro-oesophageal reflux disease. Aliment Pharmacol Ther 2004;20(suppl 9):47–56.

29- Jackson L, Britton J, Lewis SA, et al. A population-based epidemiologic study of Helicobacter pylori infection and its association with systemic inflammation. Helicobacter. Oct 2009;14(5):108-13.

30- Lagergren J, Bergstrom R, Nyren O. No relation between body mass and gastro– oesophageal reflux symptoms in a Swedish population based study. Gut 2000;47:26–9.

31- Ronkainen J, Aro P, Storskrubb T, et al. High prevalence of gastroesophageal reflux symptoms and esophagitis with or without symptoms in the general adult Swedish population: a Kalixanda study report. Scand J Gastroenterol 2005;40:275–85.

32- Hampel H, Abraham NS, El-Serag HB. Meta-analysis: obesity and the risk for gastroesophageal reflux disease and its complications.Ann InternMed 2005;143:199–211.

33- Corley DA, Kubo A. Body mass index and gastroesophageal reflux disease: a systematic review and meta-analysis. Am J Gastroenterol 2006;101:2619–28.

34- Fernandez JR, Heo M, Heymsfield SB, et al. Is percentage body fat differentially related to body mass index in Hispanic Americans, African Americans, and European Americans? Am J Clin Nutr 2003;77:71–5.

35- Durk R, Margot A, Smout JPM et al. Gastroesophageal Pressure Gradients in Gastroesophageal Reflux Disease: Relations with Hiatal Hernia, Body Mass Index, and Esophageal Acid Exposure. The American Journal of Gastroenterology (2008) 103, 1349–1354

36- Soper J. Literature Review on Gastroesophageal Reflux Disease and Hiatal Hernia. Journal of Gastrointestinal Surgery (2011) 15:1472-1476.

37- Kahrilas PJ. The role of hiatus hernia in GERD. Yale J Biol Med. 1999 Mar-Jun;72(2-3):101-11

38- Seefeld U, Krejs GJ, Siebenmann RE, et al. Esophageal histology in gastresophageal reflux. Morphometric findings in suction biopsies. Dig Dis Sci 1977;22:956–64.

39- Collins BJ, Elliott H, Sloan JM, et al. Esophageal histology in reflux esophagitis. J Clin Pathol 1985;38:1265–72.

40- Ismail BF, Horton PF, Pope CE. Histological consequences of gastresophageal reflux in man. Gastroenterology 1970;58:163–74.

41- Solcia E, Villani L, Luinetti O, et al. Altered intercellular glycoconjugates and dilated intercellular spaces of esophageal epithelium in reflux disease. Virchows Arch 2000;436:207–16.

42- Villanacci V, Grigolato PG, Cestari R, et al. Dilated intercellular spaces as marker of esophageal reflux: Histology, semiquantitative score and morphometry upon light microscopy. Digestion 2001;64:1–8.

43- Caviglia R, Ribolsi M, Maggiano N, et al. Dilated intercellular spaces of esophageal epithelium in nonerosive reflux disease patients with physiological esophageal acid exposure. Am J Gastroenterol 2005;100:543–8.

44- Tobey NA, Carson JL, Alkiek RA, et al. Dilated intercellular spaces: A morphological feature of acid reflux—Damaged human esophageal epithelium. Gastroenterology 1996;111:1200–5.

45- Kahrilas PJ, Quigley EM. Clinical esophageal pH recording: A technical review for practice guideline development. Gastroenterology 1996;110:1982–96.

46- Johnsson F, Joelsson B and Isberg PE. Ambulatory 24 hour intraesophageal pH-monitoring in the diagnosis of gastroesophageal reflux disease. Gut1987;28:1145-1150 doi:10.1136/gut.28.9.1145

47- Kiesslich R, Kauzler S, Vieth M, et al. Minimal change esophagitis: Prospective comparison of endoscopic and histological markers between patients with non-erosive reflux disease and normal controls using magnifying endoscopy. Dig Dis 2004;22:221–7.

48- Zentilin P, Dulbecco P, Savarino E, et al. Combined multichannel intraluminal impedance and pH-metry: A novel technique to improve detection of gastro-esophageal reflux. Literature review. Dig Liver Dis 2004;36:565–9.

49- Funch JP, Kock K, Christensen LA, et al. Microscopic appearance of the esophageal mucosa in a consecutive series of patients, submitted to upper endoscopy. Correlation with gastresophageal reflux symptoms and macroscopic findings. Scand J Gastroenterol 1986;21:65–9.

50- Vieth M, Peitz U, Labenz J, et al. What parameters are relevant for the histological diagnosis of gastresophageal reflux disease without Barrett's mucosa? Dig Dis 2004;22:196– 201.

51- Steiner SJ, Gupta SK, Croffie JM, et al. Correlation between number of eosinophils and reflux index on the same day esophageal biopsy and 24 hour esophageal pH monitoring. Am J Gastroenterol 2004;99:801–5.

52- Mela GS, Savarino V,Vigneri S. Optimizing the information obtained from continuous 24-hour gastric pH monitoring. Am J Gastroenterol 1992;87:961–6.