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Histopathological Changes and DNA Pattern in Esophageal and Gastric Mucosa Before and after Treatment of Esophageal and Gastric Varices

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Abstract: Variceal bleeding is the most health emergencies in Egypt and is a frequent and life-threatening complication of portal hypertension and endoscopic sclerotherapy has emerged as an effective treatment for bleeding esophageal and gastric varices Recent reports claim that there might be a relationship between sclerotherapy for esophageal varices and cancer of the esophagus. Our aim is to study the histopathological changes, the DNA pattern of the mucosal lining of both esophagus and stomach (peri-variceal area) under the use of 5% ethanolamine oleate histoacryl and 50% hypertonic glucose water during eradication of esophageal and gastric varices respectively and to detect any early premalignant or malignant changes in the mucosal lining of both esophagus and stomach and the proliferating index (PI) of the proliferating cell nuclear antigen (PCNA) in correlation with S phase fraction of cell cycle. *Materials and methods:* This study was carried out on 75 patients, they were grouped into: Group I: Esophageal varices: Subgroup IA: 20 patients treated with injection of glucose 50%. Subgroup IB: 20 patients treated with injection of 5% ethanolamine oleate. Group II: Fundal varices: Subgroup IIA: 10 patients treated with injection of glucose 50 %.Subgroup IIB: 10 patients treated with injection of histoacryle. Group III: 15 patients served as control. Endoscopic biopsies were taken. And paraffin blocks were made; sections were stained by haematoxyline and eosin for histopathological examination, feulgen stain for DNA evaluation using Cell Image Analyzer and using indirect imuonohistocheimstry technique (IHC) for detection of PCNA antibody. *Results:* Esophageal varices cases, most of subgroup IA patients treated by injection with glucose 50% showed chronic mild esophagitis in (65%) of cases, while in subgroup IB patients treated by injection ethanolamine oleate 5% showed moderate and sever chronic esophagitis in (50%). Patients in subgroup IA showed 19/20 of patients elaborated diploid histograms with increase in S phase (95%) and aneuploid histograms in 1/20 of patients (5%), while patients in subgroup IB elaborated aneuploid histogram in 6/20 of patients (30%) and diploid histograms in 14/20 of patients (70%), there was significant difference between 2 subgroups (IA & IB) $p<0.01$ as regard the type of histogram. The percentage of cells occupying the S phase fraction showed significant increase in both subgroup IA and IB compared to control group ($p<0.01$) on the expense of cells at 2C. PCNA (PI) increased in subgroup IB compared to control cases $p<0.05$ and to subgroup IA ($p<0.05$). In fundal varices cases treated by injection of glucose 50% showed chronic atrophic gastritis (moderate and sever) in (60%) of patient while patients treated by injection of histoacryle showed chronic atrophic gastritis (mild, moderate and sever) in (50%) of patients. 5/10 of patients in subgroup IIA showed aneuploid histograms (50%), while 7/10 of patients in subgroup IIB elaborated aneuploid histogram in (70%). The percentage of cells occupying the S phase fraction showed significant increase in both subgroup IA and IB compared to control group ($p<0.01$) on the expense of cells at 2C. The cells at 4C showed significant increase in subgroup IIB compared to control group ($p<0.05$). PCNA (PI) increased in both subgroups IIA & IIB compared to control cases $p<0.01$ for each. *Conclusions:* It can be concluded that ethanol amine 5% and histoacryl, the popular sclerosants used in Egypt, are effective in eradication of esophageal and gastric varices respectively. Their use may lead to histopathological changes in the form of esophagitis (mild to sever) and chronic atrophic gastritis (mild to sever), in the surrounding peri-variceal tissues. DNA study by image analysis is a highly sensitive method for detecting the effect of different sclerosants on both esophageal and gastric mucosa and DNA content and pattern of esophageal and gastric mucosa did not reach pre-malignant or malignant changes. These data ensure the safety of use of both sclerosants (5% ethanolamine oleate and histoacryl) in clinical practice.

Key words: DNA, 5% ethanolamine oleate, histoacryl, hyper tonic glucose water50%, PCNA, esophageal and gastric varices.

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INTRODUCTION

Gastroesophageal variceal bleeding is a potentially deadly complication in patients with liver cirrhosis and portal hypertension (Zakaria *et al.,* 1987; Bhasin *et al.,* 2002; Brandenburger *et al.,* 2002; Garcia-Tsao, 2007) Liver diseases constitute the most prevented disease among Egyptian, Schistosomiasis and hepatitis viruses cause hepatic fibrosis and cirrhosis leading to portal hypertension (D'Amico *et al*., 1995; Abed Wahab, 1997). Schistosomiasis is a widespread, endemic parasitic disease, in regions of Africa, South America and Asia (Ezzat *et al.,* 1989) that may set some 600 million people at risk of infection-induced morbidity (Mahmoud and Wahab, 1990) Hepatitis C Virus (HCV) is recognized as a major threat to global public health. An estimated 170 million people worldwide are infected, most of them chronically infected and at risk for liver cirrhosis and hepatocellular carcinoma (Doumenge *et al.,* 1997) Egypt has possibly the highest HCV prevalence in the world; 10%–20% of the general populations are infected and HCV is the leading cause of HCC and chronic liver disease in the country (El-Zayadi *et al.,* 2001; Habib *et al.,* 2001). In patients with cirrhosis, the incidence of esophageal varices ranges from 35% to 80% and approximately a third of patients with esophageal varices experience variceal bleeding and up to 70% of the survivors have one or more additional episodes of bleeding (Hassan *et al.,* 2001)

Oesophageal varices are the commonest cause of acute upper gastrointestinal bleeding in Egypt, due to the prevalence not only of schistosomiasis but also chronic hepatitis (Yassin *et al.,* 2005). It was reported by El Zayadi, 1989) that oesophagitis as assessed by fibrosis and ulceration or polymorphs infiltration may play a role in the pathogenesis of bleeding varices and one of theses factors may be the integrity of the mucosal cell lining which may be detected by DNA study of these cells.

Treatment of variceal bleeding at esophagus, is the endoscopic injection sclerotherapy by a sclerosant, which may be 5 % ethanolamine oleate for intavariceal injection or poidecanole for paravariceal use (Sherlok and Dooley, 1997).

In the last decade, several patients with esophageal varices treated with endoscopic injection sclerotherapy developed carcinoma of the esophagus and gastroesophageal junction have been reported in the literature. This may only be a coincidence, although the existence of an undemonstrated relationship cannot be discarded. Tung *et al., 2001*, reported more than 20 cases of esophageal carcinoma have been reported to develop after endoscopic injection sclerotherapy. One patient was diagnosed as adenocarcinoma of the esophagus who underwent several sessions of endoscopic sclerotherapy for esophageal varices with ethanolamine oleate, reported by Larrubia *et al.,* 1998, Nakamura *et al.,* 1988 reported early esophageal cancer in a male after treatment for esophageal varices by a series of endoscopic sclerotherapeutic injections using 5% ethanolamine oleate, Ng *et al.,* 2001 reported two cases of squamous cell carcinoma of the esophagus following endoscopic injection sclerotherapy for esophageal varices. Guillemot *et al.,* 1988 reported 4 male patients, treated by sclerotherapy for bleeding esophageal varices. Ohta *et al.,* 1995, report three cases of squamous-cell carcinoma of the esophagus following endoscopic injection sclerotherapy (5% ethanolamine oleate) for esophageal varices. The interval between sclerotherapy and the development of carcinoma was 9, 10 and 33 months. Macías Rodríguez *et al.,* 1992 report one case of a male, who developed a squamous cell carcinoma of the esophagus after undergoing endoscopic injection sclerotherapy for bleeding esophageal varices. Kokuodo *et al.,* 1990 report two cases of squamous cell carcinoma of the esophagus following endoscopic injection sclerotherapy for esophageal varices. Umekita *et al.,* 1985, reported one case of carcinoma of the esophagus developing two years after endoscopic injection sclerotherapy of esophageal varices. Tanoue *et al.,* 1995 reported one case, after endoscopic injection sclerotherapy on the lower esophagus using 5% ethanolamine oleate.

From all reported cases of esophageal carcinoma that developed after endoscopic injection sclerotherapy, whether the development of esophageal carcinoma in those patients is coincidental or consequential is controversial, we need a new methods to detect early premalignant and malignant changes in the mucosa (peri-variceal area) of both esophagus and gastric in patient with bleeding gastroesophageal varices treated by endoscopic injection sclerotherapy.

Image analysis is a new histopathological technique that combines computer software and microscopic image scanning techniques to allow quantitative analysis of the DNA content within individual cell in tissue sections that contains only slices of the nuclei, in a specific cell types that can be defined by their morphologic characters and histologic location and to detect early premalignant and malignant changes (Barclay *et al.,* 1993).

Bleeding from gastric varices is not as common as esophageal varices, it is always a serious and difficult problem. First- line therapy for active bleeding from isolated gastric varices is endoscopic obliteration through sclerotherapy (Ryan *et al.,* 2004). Sarin *et al*., 2002, 2004 demonstrated that N- butyl -cyanoacrylate promotes primary hemostasis better than conventional sclerotherapy. Endoscopic injection with butyl cyanoacrylate (BC), the mainstay of the therapy for gastric varices (GV), has been reported to be effective for hemostasis of bleeding varices, but its efficacy in the obliteration of GV and impact on the survival of patients still needs clarification (Cheng *et al.,* 2007).

Proliferation markers have been used to evaluate cell replication in various tissue lesions including inflammation and neoplasia. An intranuclear polypeptide whose synthesis reaches its maximum during the S phase of the cycle has recently been identified as a DNA damage-inducible protein that performs an essential function in DNA replication and repair. The molecular mass of accessory protein is 36 kd; it was originally described as the proliferating cell nuclear antigen (PCNA) Taksaki, *et al.,* 1991.

Our aim is to study the histopathological changes, the DNA pattern of the mucosal lining of both esophagus and stomach (peri-variceal area) under the use of 5% ethanolamine oleate, histoacryl and 50% hypertonic glucose water during eradication of esophageal and gastric varices respectively and to detect any early premalignant or malignant changes in the mucosal lining of both esophagus and stomach and the proliferating index of the proliferating cell nuclear antigen (PCNA) in correlation with S phase fraction of cell cycle.

MATERIAL AND METHODS

Subjects and Plain of Work:

This study was carried out on 60 patients, with liver cirrhosis and portal hypertension; all patients presented with symptoms of gastrointestinal bleeding in the form of hematemesis or melena. In addition fifteen patients served as control. The study protocol was approved by the Ethics Committee of TBRI and Monifya University according to the Institutional Committee for the Protection of Human Subjects and adopted by the 18th World Medical Assembly, Helsinki, Finland. Informed consent from all patients and controls underwent endoscopy and biopsies were taken. Patients were grouped into:

Group I:	Esophageal varices group classified into:
Subgroup IA:	20 patients treated with injection of glucose 50%.
Subgroup IB:	20 patients treated with injection of 5% ethanolamine oleate.
Group II:	Fundal varices group classified into:
Subgroup IIA:	10 patients treated with injection of glucose 50%.
Subgroup IIB:	10 patients treated with injection of histoacryle.
Group III:	15 patients are control. Who are presenting with other symptoms rather than hematemesis or melena. Biopsies were taken from the lower esophagus (seven patients) and from the fundus of the stomach (eight patients) and are proved histopathologically free with normal mucosa.

All patients were subjected to full history, thorough clinical examination, routine investigations including, urine and stool analysis, liver and kidney functions tests and complete blood picture.Upper endoscopic examination using Olympus GIF XQ30 fibroptic endoscope (Tokyo- Japan) had been performed to all patients and injection sclerotherapy had been done using needle injection Welson Cock MH21G as follows:

- Patients of subgroup IA with esophageal varices had undergone injection with hyper tonic glucose water 50% injected intravariceal or paravariceal which is a dehydrating sclerosant (Change *et al.,* 1996) and subgroup IB with esophageal varices had undergone injection with 5% ethanolamme oleate strictly intravariceally, till complete eradication and until they achieved full sclerosis.
- Patients of subgroup IIA with gastric fundal varices had undergone injection using hyper tonic glucose water 50% intravariceal or para variceal and patients in subgroup IIB with gastric fundal varices had undergone injection of histoacryl (N-butyl-cyanoacrylate, Braun, Melsungen, Gemany). Histoacryle injected strictly intravariceally, it is a tissue adhesive molecule that has the ability to rapidly polymerize on exposure to weak bases such as water and blood. This polymerization results in the immediate occlusion of varices and arrested the bleeding resulting in better hemostasis than conventional therapy (Sarin *et al.,* 2002).

Follow up by upper endoscopy was done after 6 months to 24 months and biopsies were taken, using biopsy forceps from the mucosa of the lower esophagus for patients with esophageal varices in group I and from the fundus of the stomach for patients of group II with gastric varices from the perivariceall areas for histopathologic evaluation by:

Histopathological Study:

Endoscopic biopsies taken were fixed in 10% 10% buffered formalin for 24 hours and then preceded in ascending grades of ethyl alcohol, xylene, wax and paraffin blocks. Five µm thick tissue sections were cut on albuminized glass slides and stained with haematoxylin and eosin stain for histopathological diagnosis of esophageal and gastric lesions according to (Rosai *et al.,* 2007; Whitenead *et al.,* 1972) respectively, we stress on mucosal changes as; the presence of intestinal metaplasia, gland atrophy or dysplasia or malignancy and recorded it if present.

Evaluation of DNA Content Using Image Analysis System

Principle of the Procedure:

The Feulgen stain specifically and quantitatively stains the nuclear DNA blue while the cytoplasm appears transparent. The quantity of this blue colored compound formed is directly proportional to the DNA content within the nucleus of the cell. In a population of normal and abnormal cells there is an obvious difference between the nuclei in the staining intensity Cells in the S phase appear darker than their normal counterparts because of their increased amount of DNA content (Schutte *et al.,* 1989).

DNA Image Analysis:

Automated image analysis assessment of nuclear DNA of esophageal and gastric epithelial cells was performed using the computer controlled analysis system (Kontron Image Analysis System, Germany), This essentially consists of a computer controlled microscope (Zeiss Axioscope microscope), video camera, two monitors and a computer unit.Image analysis technique was performed using the soft ware program CIRES, which allowed the colored compound that develops in the stained nuclei by feulgen to be directly proporitional to DNA content within the nucleus and can be measured as quantifiable integrated optical density (IOD) (Paque *et al.,* 1991) A number of nuclei ranging from 150-200 cells were submitted for DNA analysis in each case at 400x lens magnification. Only single monolayered nuclei without overlapping were analyzed. Reference cells are necessary for DNA scaling of densometric measurements. Mucosal biopsies from esophagus and stomach of control group III were used as standard control. Reference cells were stained exactly as the cells under analysis in the same staining bath with slides of the sample.On analyzing their DNA content, reference histograms were elaborated and considered reference control histograms for sections under study (Schutte *et al.,* 1989).

The elaborated DNA histograms were classified into: Diploid or aneuploid histograms based on DNA index (DI) of the main peak.

The Diploid Histograms:

Exhibiting a significant peak in the diploid range (DI=1±10%) were further subclassified according to the percentage of proliferating cells in the S phase fraction into:

- Diploid histogram.
- Diploid histogram with increase in the synthesis of S phase according to the increase in the percentage of proliferating cells, which classified into:
- Diploid histogram with mild increase in S phase, when the percentage of proliferating cells at S phase equal 30-40% of total number of analyzed cells).
- Diploid histogram with moderate increase in S phase, when the percentage of proliferating cells at S phase equal 41-50% of total number of analyzed cells).
- Diploid histogram marked increase in S phase, when the percentage of proliferating cells at S phases equal 51-60% of total number of analyzed cells).
- Aneuploid histogram: displaying a mass peak or multiple peaks outside the diplod or tetrapolid range or when the percentage of proliferating cells at S phase is more than 60% (Yosef *et al.,* 1996; Eskelino *et al.,* 1995)

Terms used in DNA analysis:

Diploid: DNA content of the normal cell or 2C.
Ploidy: Analysis of DNA content
Aneupioid: Abnormal DNA content.
S Phase: The synthesis phase during which, there is rapid replication of the DNA content of the cell, which varied from 2C to 4C.
DNA Index (DI): DI= Modal aneuploid Go/Gl DNA content /Modal diploid GO/Gl DNA content

The DI is commonly used to compare the DNA content of abnormal with that of normal cells
G0: Resting phase of the cell cycle.
G l: pre-synthetic phase.
M phase: period of mitosis (Borgmama *et al.,* 1991, Aziz *et al.,* 1991).

Immunohistochemistry Procedure for PCNA Detection:

Immunohistochemical reaction was performed using the using an avidin biotin complex (ABC) immunoperoxidase technique according to Akyol *et al.,* 1999 using anti human PCNA on paraffin 4µm thick sections which were mounted on poly-L-Lysine coated slides, dewaxed in xylene and rehydrated to water through graded alcohols. Sections were incubated with absolute methanol containing 0.3% hydrogen peroxide for 20 minutes. Antigen retrieval was performed by microwaving the sections in citrate buffer (pH 6.0) for 15 min at 700 W. After antigen activation, sections were cooled to room temp, washed in phosphate buffered saline (PBS buffer, PH 7.2) and incubated with 10% normal swine serum for 30 mintes to decrease the non specific back ground staining. Subsequently sections were allowed to stand over night at 4°C in a 50 fold dilution of the anti human PCNA antibody in PBS (DAKO, Denmark). Next day, after wash in PBS buffer incubated with biotinylated, anti rabbit IgG at room tepmerature for 30 minutes. Sections were washed in PBS buffer and incubated with streptavidine perioxidase complex for 20 minutes(DAKO, Denmark).The reagent covering the peroxidase reaction was 3, 3 diaminobenzidine tetrahydrochloride. Mayer's haematoxyline was used for counter staining before mounting.

Immunohistochemical reaction for PCNA were considered positive when the nuclei stained brown. We have assessed the number of positive nuclei in 1000 epithelial cells either esophageal or gastric mucosal cells in 10-15 high power optical fields (at power of magnification X 400) per section. Value of PCNA was expressed as percentage of positive epithelial nuclei reported to total epithelial nuclei, representing PCNA proliferation index (Akyol *et al.,* 1999).

Statistical Analysis:

Statistical analysis was performed using the SPSS computer program (version 9.0 windows). Data were recorded as mean ± standard deviation (mean ± SD) or number or percentages. Comparison between means of two groups was compared using unpaired t-test. For comparing more than two groups, ANOVA test was used. Comparison between percent of positive cases was calculated by Chi-square test. "P" value of less than 0.05 was considered statistically significant

RESULTS AND DISCUSSION

Results:

Patients are 51 males and 9 females, while the 15 control cases were 10 males and 5 females with the mean age of patients was 45.2±3.5 years, while that of controls was 39.2±4.5. Glucose 50% and 5% ethanolamine oleate were comparable to each other as regard the cessation of bleeding from esophageal varices and eradication of varices, but 5%ethanolamine oleate was better than glucose 50% as it needs less amount of injected sclerosant and less number of sessions needed for eradication of varices. On the other hand glucose 50% and histoacryle had different results on bleeding fundal varices, glucose 50% failed to stop bleeding of fundal varices in 5 patients out of 10 included in this study (50% failure rate) that necessitate immediate conversion to histoacryle for controlling of the bleeding varices. Histoacryle had dramatic effect on bleeding either when used as first choice of controlling bleeding fundal varices or secondary to failure of glucose 50% to stop bleeding, with no record cases of rebleeding or recurrence of varices on follow up.

Table 1: Comparative study of endoscopic data of studied groups.

	Group I (40 patients)		Group II (20 patients)	
Characteristics	No.	%	No.	%
Esophagus				
Reflux	16	40%	12	60%
Hiatus hernia	6	30%	0	0%
Varices	40	100%	0	0%
Cherry red spots	13	32.5%	12	60%
Stomach				
Congestive gastropathy	15	37.5%	12	60%
Fundal varices	0	0%	20	100%
Biliary reflux	8	20%	10	50%
Duodenum				
Congestion	8	(20%)	2	10%
Ulcers	4	(10%)	1	5%

Table 2: Histopathological results of the esophageal varices patients (subgroup IA and subgroup IB).

	Subgroup IA 20 patients		Subgroup IB 20 patients		
Histopathological results	No.	%	No.	%	Chi
Acute esophagitis:	5	25%	8	40%	1.89
* Acute mild esophagitis	2	10%	5	5%	P>0.05
* Acute moderate esophagitis	3	15%	2	10%	
* Acute sever esophagitis	0	0%	1	5%	
Chronic esophagitis:	15	75%	12	60%	14.19
* Chronic mild esophagitis	13	65%	2	10%	P<0.01
* Chronic moderate esophagitis	2	10%	5	25%	
* Chronic sever esophagitis	0	0%	5	25%	

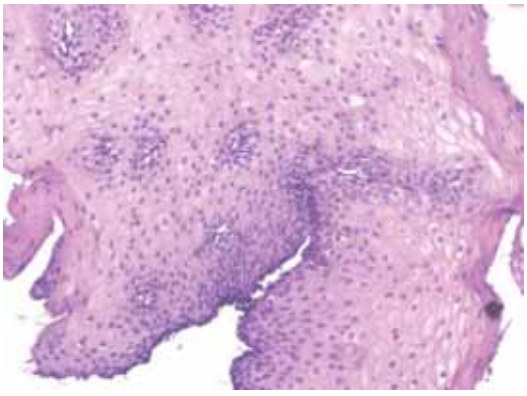

Fig. 1: A case of chronic esophagitis with moderate activity, showing epithelial hyperplasia and infiltration by neutrophils, lymphocytes, and scattered esinophils (H&E, x200).

Endoscopic data of the patients in the study are in Table 1.

In this study the histopathological results of esophageal varices patients' subgroup IA and IB. As regard acute esophagitis mild, moderate or sever activity, there was no statistical significant difference between both subgroups. As regard chronic esophagitis mild, moderate or sever activity, there was statistical significant difference between the 2 subgroups. Chronic mild esophagitis we seen in 13/20 (65%) vs. 2/20 (10%) in cases injected with glucose 50% and 5% ethanolamine oleate (P<0.01) respectively. While 5% ethanolamine oleate, caused moderate esophagitis in 5/20 (25%) of cases compared to 2/20(10%) caused by the glucose 50%. 0% patients of subgroup IA showed chronic sever esophagitis compared to 5 patients (25%) of subgroup IB, there was statistical significance difference between subgroup IA and IB as regards chronic sever esophagitis (P<0.01), (Fig.1 & Table 2).

Histopathological results of the fundal varices patient's subgroup IIA and IIB. As regard chronic gastritis mild, moderate or sever activity, there was no statistical significant difference between both subgroups. Glucose 50% caused chronic sever atrophic gastritis in 2/10 (20%) patients versus 1/10 (10%) in subgroup IIB patients. Glucose 50% caused chronic moderate atrophic gastritis in 4/10 (40%) patients versus 3/10 (30%) subgroup IIB patients. As regards chronic atrophic lesions there was no statistical significant difference between both subgroups (P>0.05), (Fig.2 & Table 3).

Table 3: Histopathological results of the gastric fundal varices patients (subgroup IIA and subgroup IIB).

	Subgroup IIA 10 patients		Subgroup IIB 10 patients		
Histopathological results	No.	%	No.	%	Chi
Chronic gastritis:	4	40%	5	50%	1.5
* Chronic mild gastritis	0	0%	0	0%	P>0.05
* Chronic moderate gastritis	3	30%	1	10%	
* Chronic sever gastritis	1	10%	4	40%	
Chronic atrophic gastritis:	6	60%	5	50%	1.14
Chronic mild atrophic gastritis	0	0%	1	10%	P>0.05
Chronic moderate atrophic gastritis	4	40%	3	30%	
Chronic sever atrophic gastritis	2	20%	1	10%	

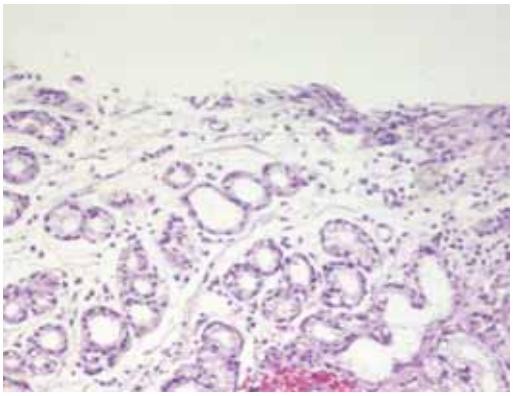

Fig. 2: A case of chronic atrophic gastritis with moderate activity, showing glandular atrophy, condensation of fibrous tissue in the lamina propria and infiltration by lymphocytes, plasma cells and scattered esinophils (H&E X 200).

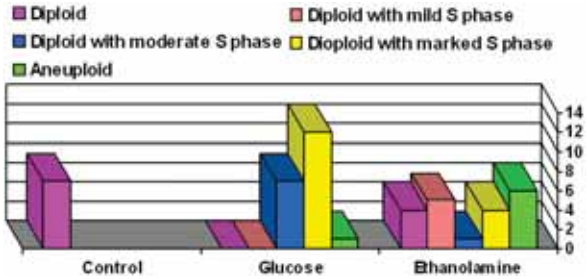

Fig. 3: Histogram showed comparative study of the DNA pattern in cases of patients with esophageal varices injected by glucose 50% and 5% ethanolamine oleate versus control group.

The image analysis of the DNA content of the esophageal mucosa of the control group showed all patients (100%) elaborated normal diploid histogram pattern, while esophageal varices patients injected by 50% glucose showed 19/20 patients (95%) elaborated diploid histograms with increase in S phase population (7/20 patients (35%) elaborated diploid histogram with moderate increase in S phase, 12/20 patients (60%) elaborated diploid histogram with marked increase in S phase) and 1/20 patients (5%) elaborated aneuploid histogram (the percentage of proliferating cells at S more than 60% of the analyzed cells). On the other hand, patients treated by injection of 5% ethanolamine oleate, showed 14/20% patients (70%) elaborated diploid histograms with increase in S phase population (4/20 of patients (20%) elaborated diploid histogram, 5/20 of patients (25%) elaborated diploid histogram with mild increase in S phase and 1/20 of patients (5%) elaborated diploid histogram with moderate increase in S phase, 4/20 of patients (20%) elaborated diploid histogram with marked increase in S phase) and 6/20 of patients (30%) elaborated aneuploid histogram(the percentage of proliferating cells at S more than 60% of the analyzed cells).There was no statistical significant difference between the control group and subgroup IA ($p>0.01$), while there was statistical significant difference between the control group and subgroup IB ($p<0.05$) and there was highly significant difference between the 2 subgroups (IA and IB) $p<0.01$, as regard the type of histogram as detected by Chi square test (Figure 3-6).

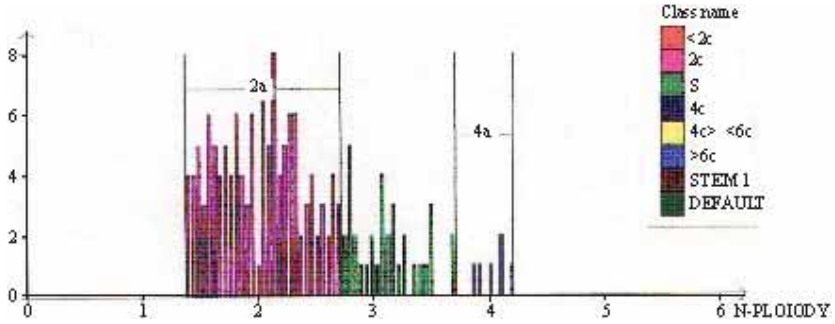

Fig. 4: A histogram of normal esophageal mucosa (control case), the majority of the nuclei (75.61%) are diploid at 2C with S phase (20.73%) and DNA index peak 1.

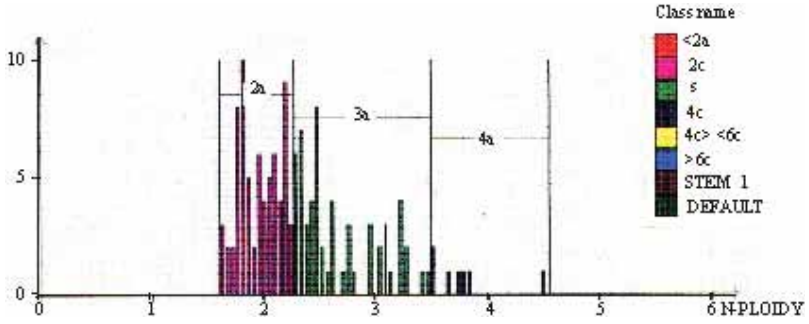

Fig. 5: Chronic mild esophagitis case, injected by glucose 50% elaborated a diploid histogram, with moderate increase in S phase (42.86%), DNA index peak 0.90.

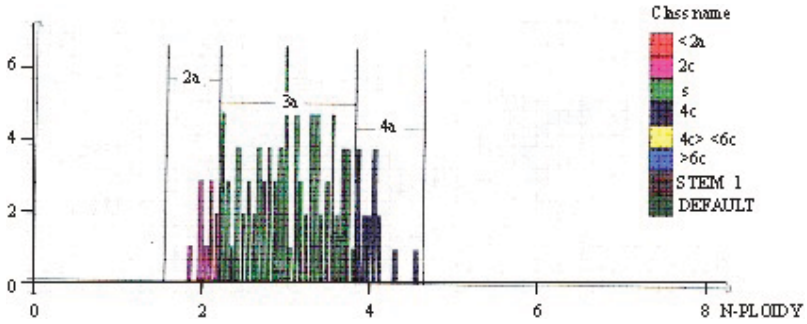

Fig. 6: Chronic sever esophagitis case, injected by 5% ehanolamine oleate, elaborated an aneuploid histogram, the proliferating cells at S phase (76.99%), DNA index peak 1.48.

The image analysis of the DNA content of the gastric fundal mucosa of the control group showed all patients (100%) elaborated normal diploid histogram, while fundal varices patients treated by injection of 50% glucose showed showed 50/10 patients (50%) elaborated diploid histograms with increase in S phase population(4/10 of patients (40%) elaborated diploid histogram with moderate increase in S phase, 1/10 of patients (10%) elaborated diploid histogram with marked increase in S phase) and showed 5/10 patients (50%) aneuploid histogram (the percentage of proliferating cells at S more than 60% of the analyzed cells). On the other hand, patients treated by injection of histoacryle showed 3/10 patients (30%) elaborated diploid histograms with increase in S phase population (1/10 of patients (10%) elaborated diploid histogram and 1/10 of patients (10%) elaborated diploid histogram with moderate increase in S phase, 1/10 of patients (10%) elaborated diploid histogram with marked increase in S phase) and 7/10 of patients (70%) elaborated aneuploid histogram (the percentage of proliferating cells at S more than 60% of the analyzed cells). There was no statistical significant difference between the control group and both subgroup IIA and IIB as detected by Chi square test ($p>0.05$), there was no statistical difference between both sub groups IIA and IIB as regard the type of histogram as detected by Chi square test $p>0.05$ (Figures 7-10).

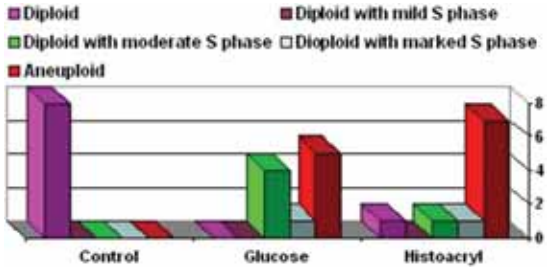

Fig. 7: Histogram showed comparative study of the DNA pattern in cases of patients with gastric fundal varices injected by glucose 50% and histoacryle versus control group.

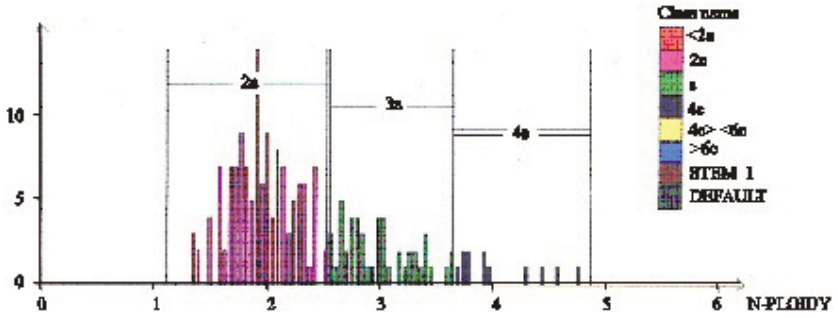

Fig. 8: A histogram of gastric control case, the majority of the nuclei (68.59%) are diploid at 2C with S phase (25.13%) and DNA index peak 0.95.

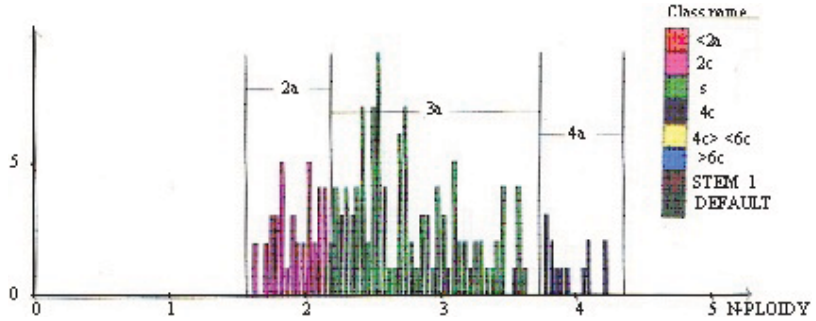

Fig. 9: Chronic sever atrophic gastritis case, patients injected by glucose 50% elaborated aneuploid histogram, with proliferating cells at S phase (68.32%), DNA index peak 1.26,cells at 4C 8.07%.

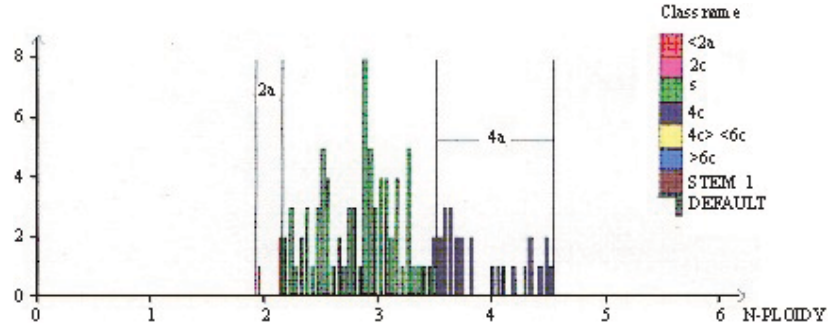

Fig. 10: Chronic moderate atrophic gastritis case, patients injected by histoacryl, elaborated an aneuploid histogram, the proliferating cells at S phase (72.38%) of the nuclei, DNA index peak 1.44.

As regard the different items of the image analysis of the DNA study, DNA ploidy, 2C, 4C, S phase and 2C deviation rate in the esophageal varices patients. Subgroup IA and subgroup IB showed increase in mean polidy (2.45 ± 0.21 & 2.63 ± 0.33 respectively) compared to control group (2.0 ± 0.02) ($P<0.05$ for each). Both subgroup

Table 4: DNA ploidy, 2C, 4C, S phase, 2C deviation rate and PCNA (PI) in the esophageal varices patients (subgroup IA and subgroup IB).

Group	DNA ploidy (Mean ±S.D)	2C (Mean ±S.D)	4C (Mean ±S.D)	S phase % (Mean ±S.D)	2C deviation rate (Mean ±S.D)	PCNA (PI) (Mean ±S.D)
Control (7)	2.0 ± 0.02	69.72±7.99	4.5±0.90	26.78±7.9	0.39±0.09	20.5±4.7
- Subgroup IA	2.45±0.21‡	39.70±7.67#	8.0±1.4#	52.3±15.65#	0.69±0.04‡	21.01±6.9
- Subgroup IB	2.63 ±0.33‡	41.36±6.3#	10.19 ±1.69#	47.83±11.98#	0.92±0.08‡	36.1±6.9‡,,§

‡Significant difference from control group (P<0.05).

Highly significant difference from control group (P<0.01).

§ Significant difference from glucose group (P<0.05).

Table 5: DNA ploidy, 2C, 4C, S phase, 2C deviation rate and PCNA (PI) in the gastric fundal varieces patients (subgroup IIA and subgroup IIB).

Group	DNA ploidy (Mean ±S.D)	2C (Mean ±S.D)	4C (Mean ±S.D)	S phase % (Mean ±S.D)	2C deviation rate (Mean ±S.D)	PCNA (PI) (Mean ±S.D)
Control (8)	2.10 ± 0.08	67.45±9.83	5.34±1.88	27.21±4.6	0.77±0.04	23.78±7.14
- Subgroup IIA	2.72±0.6‡	26.8±16.31#	7.36±1.6	65.64±10.91#	1.02±0.08‡	58.55±10.98#
- Subgroup IIB	2.92 ±0.06‡	33.04±26.3#	11.26±2.3‡	55.7±12.11#	1.32±0.05‡	59.37±14.1#

‡ Significant difference from control group (P<0.05).

High Significant difference from control group (P<0.01).

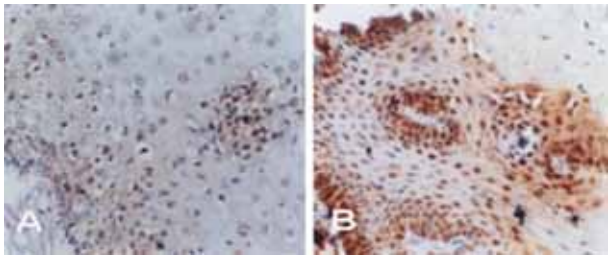

Fig. 11: A case of chronic mild esophagitis with esophageal varices injected by glucose 50% showed PI= 20, the positivity appear as brownish colored nuclei of the stratified squamous epithelium of the esophagus (IHC, PCNA, DAB X400) (A). A case of chronic moderate esophagitis with esophageal varices injected by 5% ethanolamine oleate showed PI= 40, (IHC, PCNA, DAB X 400)(B)

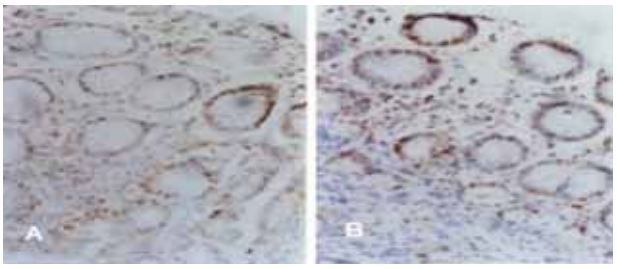

Fig. 12: A case of chronic moderate atrophic gastritis with fundal varices injected by glucose 50% showed PI= 30 the positivity appear as brownish colored nuclei of the gastric mucosa (IHC, PCNA, DAB X200)(A). A case chronic moderate gastritis with fundal varices injected by histoacryle (PI= 50) (IHC, PCNA, DAB X 200)(B).

IA and IB showed highly significant increase in the percentage of cells occupying the S phase fraction compared to control group (P<0.01) on the expense of cells at 2C. Subgroup IIB showed 4C equal to 10.19 ± 1.69 versus 8.0 ±1.4 in subgroup IIA, both groups showed significant difference from control group (P<0.01). The mean value of 2C deviation rate showed stepwise increase in cases of group IA and IB (0.69 ± 0.04 &0.92 ± 0.08 respectively) compared to control cases (0.39 ± 0.09) (P<0.05 for each), (Table 4).

The proliferating cells with positive nuclei stained with PCNA antibody, showed significant increase in proliferation index (PI) in subgroup IB compared to control (P<0.01) and compared to subgroup IA p<0.05 (Table 4, Figure 11).

The DNA study, DNA ploidy, 2C, 4C, S phase and 2C deviation rate in the gastric fundal varieces patients. Subgroup IA and subgroup IB showed increase in mean polidy (2.72 ± 0.6& 2.92 ± 0.06 respectively) compared to control group (2.10 ± 0.08) (P<0.05 for each). The S fraction showed high significant increase in the number of cells both in subgroup IIA and IIB compared to control group (p<0.01 for each) on the expense of cells at 2C.

Subgroup IIB showed 4C equal to 11.26 ± 2.3 versus 7.36 ±1.4 in subgroup IIA and subgroups IIB showed significant difference from control group (P<0.05). The mean value of 2C deviation rate showed significant increase in cases of group IIA and IIB (1.02 ± 0.08 &1.32 ± 0.05) compared to control cases (0.77± 0.04) (P<0.05 for each), (Table 5). The proliferating cells with positive nuclei stained with PCNA antibody, showed increased (PI) in both subgroups IIA and IIB compared to control cases p<0.01 (Table 5, Figure 12).

Discussion:

In this study, the histopathological results of esophageal varices patients subgroup IA revealed, no patients reported with sever esophagitis either in the acute or chronic forms and this may be related to the osmotic dehydrating effect of glucose 50% which does not affect the mucosa markedly and not cause sever inflammation either acute or chronic (Change *et al.,* 1996; Jensen 1993) On the contrast of the mode of action, 5% ethanolamine oleate, cause necrosis of the mucosa, sometimes ulceration which heel by fibrosis and obliteration of the varices (Jutabha and, Jensen, 1994; Paquet *et al.,*1991) and this matches with the 50% of patients reported with chronic moderate and sever esophagitis in our cases of patients with esophageal varices injected by 5% ethanolamine oleate.

In this study, histopathological results of the fundal varices subgroup IIA caused chronic atrophic gastritis in 6/10 (60%) of patients (40% moderate and 20% sever) compared to 5/10 (50%) of subgroup IIB (10% mild, 30% moderate and 10% sever), this may be explained by the fact that glucose 50% had more hazardous effect on the gastric mucosa than histoacryle which is clinically explained by the role that histoacryle showed by strictly injected intravariceally as any injection of it in the gastric rouge would result in heroic bleeding and ulceration which are difficult to be controlled (Change *et al.,* 1996). Glucose 50% could be injected intravariceally and injected paravariceally which increased the chance of mucosa to be affected, also due to the effect of glucose 50% on the gastric epithelium (simple columnar) that could be a cause of the marked effect of glucose 50% on gastric mucosa and may explain the lesser effect of the same sclerosant on esophageal mucosa which is stratified squamous epithelium. Sever atrophic gastritis is percancerous lesions that could turn into dysplasia and secondary gastric carcinoma which could be diagnosed by the early study of the DNA content of gastric mucosa (Marrero *et al.,* 1996).

Different grades of atrophic gastritis that were not present in the pretreatment gastritis which was detected by histopathological study of the mucosa of the stomach may play a role in the pathogenesis of rebleeding from distended varices. The integrity of the mucosal cell lining may play a role in the pathogenesis of bleeding fundal varices (eL- Zayadi *et al.,* 1989).

Automated image analysis of nuclear DNA of esophageal and gastric mucosal cells was performed using kontrom Image Analysis System (soft ware program, CIRES). It allows the colored compound that develops, in the stained nuclei to be directly proportional to the DNA content within the nucleus and can thus be measured as a quantifiable integrated optical density (IOD) (EL-Wakil *et al.,* 1994).

In the current study, DNA histogram pattern in esophageal control samples revealed all patients (l00%) elaborated normal diploid histograms, this goes in agreement with common findings in similar studies that demonstrated that normal cells elaborate normal diploidy (Yosef *et al.,* 1996;Nishida *et al.,* 1995; Sakusabe *et al.,* 1996).Patients with esophageal varices injected by glucose 50%(subgroup IA) elaborated (5%) of aneuploid histograms, increase in mean polidy(2.45±0.21), increase in the percentage of cells occupying the S phase fraction(52.3±15.65), increase in 2C deviation rate (0.69±0.04) compared to control cases, but to a lesser degree than patients treated by injection of 5% ethanolamine oleate (subgroup IB) whom showed increase in the duplication of the DNA content (4C), elaborated abnormal aneuploid histogram in 30% of cases, caused significant increase of the mean nuclear ploidy (2.63 ±0.33), increase in the percentage of cells occupying the S phase fraction (47.83±11.98), increase in 2C deviation rate (0.92±0.08), DNA histogram pattern in patients treated by injection of 5% ethanolamine oleate (subgroup IB) elaborated abnormal aneuploid histogram in 30% of cases, that can be explained by the chemical nature of 5% ethanolamine oleate and its ulcerogenic effect on the mucosa. This agreed with other studies that reported that, although the ploidy values showed no significant differences between the different sclerosants used in treatment of esophageal varices among different age groups however, they all cause significant mucosal changes through their actions (Mostafa *et al.,* 1997). Our opinion in this study that 5% ethanolamine oleate caused a change of the DNA content more than the effect of chronic inflammation but gave a picture similar to that of dysplasia effect on mucosa which elaborated 30% of cases with abnormal aneuploid histogram through its wide S phase.

In this study, all gastric control samples elaborated normal diploid histogram this in agreement of Yosef *et al.,* 1996, While 50% of the patients in subgroup IB and 70% of the patients in subgroup IIB elaborated abnormal aneuploid histogram (the percentage of proliferating cells at S more than 60% of the studding cells). This study revealed that histoacryle is an irritant material to the gastric mucosa and causes changes in DNA pattern (abnormal

aneuploid histogram), the mean value of 2C deviation rate showed stepwise increase in cases of group IIB (1.32 ± 0.05) than in control cases (0.77± 0.04). The 2C deviation index (which is a measure of cell number that is based on being unequal to ploidy value) achieved the lower most value in control cases. This fact goes in agreement with Marrero *et al.,* 1996, found that 2C deviation rate increased as gastric dysplasia progressed in severity. Cell image had the advantage of detection of aneuploid nuclei because it was more sensitive than flow cytometry for detection of aneuploidy and analysis of the DNA content of an endoscopic gastric biopsy specimen provides an adequate measure of the DNA content of the corresponding resected specimen (Li *et al.,* 1991) DNA aneuploidy could by a marker of malignant change of gastric precancerous lesions and mean DNA ploidy, S phase % and 2C deviation index are very useful parameters in diagnosing different lesions(Yosef *et al.,* 1996; Li *et al.,* 1991) and also can detect the early effects of sclerosants on esophageal and gastric mucosa. This outcome agreed with Masuda *et aI.,* 1994 Who described histoacryle as a highly irritant material acting as a glue to occupy the variceal lumen and causes marked irritation to the surrounded mucosa and significant change of DNA pattern, which may be similar to but not as typical dysplasia of the mucosa. Sugimoto *et al.,* 2007.

The percentage of cells at S phase fraction showed significant rising values from control group compared to group II (IIA & IIB) and also showed significant increase from control group compared to group I (IA & IB) It is clamed that, increased percentage of proliferating cells at S phase usually carrying real change of DNA ploidy and may be dysplastic changes and it was high in chronic gastritis with dysplasia than in those without dysplasia (Weiss *et al.,* 1989).

Synthesis of cyclic or proliferating cell nuclear antigen (PCNA) starts in the nucleus in the late G1 phase but attain peak only in S phase of cell cycle (Nakane *et al.,* 1989). Therefore detection of significant amount of this protein marker is a reliable indicator of cell replication. In the current study PCNA (PI) showed significantly increase in subgroup IB in patients injected with ethanolamine 5% compared to control cases. While in group II (IIA & IIB), PCNA (PI) showed significantly increase in both subgroups compared to control cases, which mean that these different sclerotheraputic agents may be an irritating materials and could lead to the development of mucosal alterations of esophageal and gastric mucosa and cause changes in the mucosal lining mimic dysplastic changes.

CONCLUSION

It can be concluded that ethanol amine 5% and histoacryl, the popular sclerosants used in Egypt, are effective in eradication of esophageal and gastric varices respectively. Their use may lead to histopathological changes in the form of esophagitis (mild to sever) and chronic atrophic gastritis (mild to sever), in the surrounding peri-variceal tissues. DNA study by image analysis is a highly sensitive method for detecting the effect of different sclerosants on both esophageal and gastric mucosa and DNA content and pattern of esophageal and gastric mucosa did not reach pre-malignant or malignant changes. These data ensure the safety of use of both sclerosants (5% ethanolamine oleate and histoacryl) in clinical practice.

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