Sudan University of Science and Technology

College of Graduate Studies

Immunohistochemically Detection of Carcino Embryonic Antigen in Gastric Lesions among Sudanese Patients

الكشف المناعي الكيميائي النسيجي عن المستضد السرطاني الجنيني في آفات المعدة لدى المرضى السودانيين

A dissertation submitted in the fulfillment for requirements of master degree in medical laboratory science (Histopathology and Cytology)

By

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الآيــة

بِسْمِ اللهِ الرَّحمـٰنِ الرَّحِيمِ

قَالَ تَعَالَى:

الرَّحْمَٰنُ (١) عَلَّمَ الْقُرْءانَ (٢) خَلَقَ الْإِنسَانَ (٣) عَلَّمَهُ الْبَيَانَ (٤)

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سورة الرحمٰن من الآية (١-٤)

Dedication

To everyone who was beside me at the first time when I learned how to write my name.

To my parents for their long life love and encouragement.

To my friends who with their big hearts and loyalty give me love and life.

To all Muslims everywhere.

To all and every person that I know.

Acknowledgements

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Abstract

This is descriptive cross sectional hospital-based study was conducted at Omdurman teaching hospital in Khartoum state, during the period from January 2019 to February 2020. The study was aimed to detect CEA expression in gastric lesions using immunohistochemistry.

Fifty paraffin blocks were collected from patient samples previously diagnosed as gastric tumors, 30 (60%) were gastric adenocarcinoma and 20 (40%) were benign. Each paraffin blocks were cut at (3µ) by rotary microtome, then stained by immunohistochemical method (new indirect technique). The data obtained was analyzed using Statistical Package for Social Science (SPSS) program version 20.

The age of patients ranged between 12 and 95 years with mean age of 57.7 ± 17.9 years. Most of patients were more than 50 years representing 33 (66%) and the remaining 17 (34%) were equal or less than 50 years.

Most of patients were male representing 32 (64%) and the remaining 18 (36%) were female, with a male to female ratio of 1.8:1.0,

Gastric adenocarcinoma revealed positive CEA expression in 17 (56.7%) samples and negative expression in 13 (43.3%) samples, while gastric lesions showed positive expression in 3 (15%) samples and negative expression in 17 (85%) samples, with significant statistical association (P.value 0.003).

CEA positive expression was found in 8 (26.7%) samples of grade I, 5 (16.67%) samples of grade II, and 4 (13.3%) samples of grade III, with no significant association between CEA expression and tumor grade (P.value = 0.171).

The study concluded that CEA is biomarker for gastric adenocarcinoma with no statistical association between CEA expression and tumor grade.

المستخلص

أُجريت هذه الدراسة الوصفية المقطعية في مستشفى أم درمان بولاية الخرطوم خلال الفترة من يناير ٢٠١٩ وحتى فبراير ٢٠٢٠، هدفت هذه الدراسة للكشف عن ظهور المستضد السرطاني الجنيني في أورام المعدة بإستخدام كيمياء الأنسجة المناعية.

جمع ٥٠ قالب نسيجي مطمور في شمع البارفين من عينات مرضى كانوا مشخصين مسبقاً على أنهم مصابين بأمراض في المعدة، منها ٣٠ (٦٠%) كانت أورام معدة خبيثة و ٢٠ (٤٠%) كانت أمراض معدة. قطع من كل قالب مقطع (٣ مايكروميتر) بإستخدام المشراح الدوار وصبغت بواسطة طريقة كيمياء الأنسجة المناعية (الطريقة الجديدة غير المباشرة)، وتم تحليل البيانات بإستخدام النسخة ٢٠ من برنامج الحزم الإحصائية للعلوم الإجتماعية.

تراوحت أعمار المرضى بين ١٢ و ٩٥عاما بمتوسط عمر ١٧،٥٧±٩،٧ عاما، أظهرت الدراسة أن معظم المرضى كانت أعمارهم أكثر من ٥٠ سنة وكان عددهم ٣٣ مريضاً بنسبة ٦٦%، بينما ١٧ مريضاً بنسبة ٣٤% كانت أعمارهم أقل من أو تساوي ٥٠ سنة.

غالبية المرضى من الذكور وكان عددهم ٣٢ بنسبة ٦٤% أما الإناث فكان عددهن ١٨ بنسبة ٣٦%. وكان معدل الذكور إلى الإناث هو ١،٨:١،٠.

كشفت الدراسة أن المستضد السرطاني الجنيني كان موجب الظهور في ١٧ عينة بنسبة ٥٦,٧% في أورام المعدة الخبيثة وسالب الظهور في ١٣عينة بنسبة ٤٣,٣%، بينما في أمراض المعدة الحميدة كان موجب الظهور في ٣ عينات بنسبة ١٥% وسالب الظهور في ١٧عينة بنسبة ٨٥%، مع وجود علاقة ذات دلالة إحصائية بين ظهور المستضد السرطاني الجنيني والتشخيص النسيجي للمرض (القيمة الإحتمالية ٠,٠٠٣).

وفيما يتعلق بظهور المستضد السرطاني المضغي ودرجة تمايز الورم، كان موجب الظهور في ٨ عينات بنسبة ٧،٢٦% من درجة التمايز الأولى، و ٥ عينات بنسبة ٦٧،١٦% من درجة التمايز الثانية، و ١٤ عينة بنسبة ٣،١٣% من درجة التمايز الثالثة. أظهرت الدراسة عدم وجود علاقة ذات دلالة إحصائية بين ظهور المستضد السرطاني المضغي ودرجة تمايز الورم (القيمة الإحتمالية ٠,١٧١).

خلصت الدراسة إلى وجود علاقة إحصائية بين ظهور المستضد السرطاني الجنيني والتشخيص النسيجي، مع عدم وجود علاقة ذات دلالة إحصائية بين ظهور المستضد السرطاني الجنيني و درجة تمايز الورم.

List of abbreviations

CEA	Carcinoembryonic Antigen
CT	Computed tomography
ECL	Enterochromaffin-like
EUS	Endoscopic ultrasonography
GC	Gastric cancer
GISTs	Gastrointestinal stromal tumors
MRI	Magnetic resonance imaging
PET	Positron emission tomography

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Chapter one

Introduction

Chapter one

Introduction

1.1 Introduction

Gastric cancer (GC) is an aggressive disease that represents a serious problem and has a daunting impact on global health, these cancers develop within the cells of the mucosa, the innermost lining of the stomach. Other GC histotypes are lymphoma, gastrointestinal stromal tumors (GISTs), carcinoid tumors, and other rare tumors (Canzonieri and Giordano, 2019).

Stomach cancer remains an important cancer worldwide and is responsible for over 1,000,000 new cases in 2018 and an estimated 783,000 deaths, making it the fifth most frequently diagnosed cancer and the third leading cause of cancer death, and the rates are 2-fold higher in men than in women (Bray, *et al*. 2018).

In Sudan GC is the 15th most commonly diagnosed cancer among adults (Saeed, *et al*. 2016).

The risk factors of GC include genetics, *H.pylori* infection, smoking, alcohol, diet, obesity, sex and ethnicity (Rawla and Barsouk, 2019).

The diagnosis of gastric cancer is done by barium meal, magnetic resonance imaging, computed tomography, positron emission tomography, fibreoptic endoscopy, endoscopic ultrasonography and laparoscopy (Neal and Hoskin, 2009; Hallinan, and Venkatesh, 2013).

The treatment options for gastric cancer include surgery, chemotherapy, radiation therapy, and targeted therapy (Strong, 2015).

CEA It is a set of highly related glycoproteins that are involved in cell adhesion. CEA is usually produced in the gastrointestinal tissue during the

development of the foetus and terminates before birth. In adult it is expressed only in cancer cells, primarily adenocarcinomas, and may be used for diagnostic purposes (Canzonieri, *et al*. 2019).

1.2 Objectives

1.2.1 General objective

To assess CEA expression in gastric lesions among Sudanese patients by immunohistochemical method.

1.2.2 Specific objectives

- To detect CEA in gastric lesions, using IHC.
- To correlate CEA expression and histopathological diagnosis.
- To correlate CEA expression and gastric cancer grade.

Chapter two

Literature review

Chapter Two

Literature Review

2.1 Scientific background:

Gastric cancers include a heterogeneous group of malignant epithelial lesions with a variety of predisposing conditions and etiological factors. Gastric cancer remains a significant health issue and accounts for the 4th leading cause of cancer, although the incidence of gastric cancer has steadily declined in past decades, it remains the second leading cause of death from cancer worldwide (Tang and Selby, 2015).

2.2 Anatomy of the Stomach:

The stomach is J-shaped. The stomach is divided into four regions: the cardiac region is the small area within about 3 cm of the cardiac orifice, the fundic region is the domelike roof superior to the esophageal attachment, the body which makes up most of the stomach distal to the cardiac orifice and the pyloric region which is a slightly narrower pouch at the distal end (Saladin, *et al*. 2017).

The mucosal surface contains millions of gastric pits that lead to mucosal glands. The mucosal surface is composed of columnar, mucin-secreting epithelium, while deeper in the gastric pits are mucus neck cells. The gastric glands vary depending on their anatomic region; Cardia (mucin-secreting cells), fundus/body (parietal cells (acid), chief cells (pepsin), and scattered endocrine cells), and antrum/pylorus (endocrine (mostly gastrin G cells) and mucin-secreting cells) (Allen and Cameron, 2017).

2.3 Diseases of the stomach:

2.3.1 Benign tumors:

2.3.1.1 Benign gastric epithelial polyps:

Gastric epithelial polyps are defined as lesions, which lay above the plain of the mucosal surface. The most common polyps are represented by fundic gland polyps that account for up to 77% of all gastric polyps followed by hyperplastic polyps (Rotondo, *et al*. 2019).

2.3.1.2 Gastric adenoma:

Gastric adenomas are characterized by lesions with raised polyps may be classified as tubular, tubulovillous or villous based on the architecture. Gastric adenomas may be also sub- typed, based on the epithelial phenotype, into intestinal and gastric types (Canzonieri and Giordano, 2019).

2.3.2 Malignant tumors of the stomach:

2.3.2.1 Adenocarcinoma:

Adenocarcinoma: forms the majority of gastric malignancy. Histological patterns are intestinal (50%), diffuse (20%), or mixed/solid (25%). Intestinal carcinomas arise from intestinal metaplasia/dysplasia, form ulcerated or polypoid lesions. Diffuse carcinomas (signet ring cells), or poorly cohesive carcinoma in the WHO 2010 classification, form diffusely infiltrating linitis plastica, undermining the mucosa with transmural spread to the peritoneum

(McManus, *et al*. 2017).

2.3.2.2 Carcinoid:

Carcinoid (well-differentiated neuroendocrine) tumor: of gastric endocrine or enterochromaffin-like (ECL) cell origin, either related to gastric atrophy (type 1), ZE syndrome (type 2), or sporadic (type 3) (Allen and Cameron, 2017).

2.3.2.3 Gastrointestinal Stromal Tumor (GIST):

GIST is the most common mesenchymal tumor of the abdomen, and more than half of these tumors occur in the stomach. for example, leiomyomas or leiomyosarcomas, schwannomas and glomus tumors (Kumar, *et al*. 2013).

2.4 Epidemiology of gastric cancer:

The incidence rate of gastric cancer in the entire Sudan has yet to be identified; however, in a hospital-based data set from the Radiation and Isotopes Center in Khartoum (RICK), collected between 2009 and 2013, Gasric cancer ranked the 11^{th} most common cancer among adult male and 19^{th} among adult female (Saeed, *et al*. 2016).

2.5 Risk factors of gastric tumors:

2.5.1 Genetics

Inherited mutations of certain genes, such as the GSTM1-null phenotype or CDH1 gene, have been found to increase the risk of stomach cancer. Loss of one of the copies of the CDH1 gene results in hereditary diffuse gastric cancer (Boland and Yurgelun, 2017).

2.5.2 *H.pylori* infection

H. pylori triggers a number of innate and adaptive immune responses entangled in tumor formation process. CagA+ strains present an increased risk of gastric cancer, and elevated levels of inflammatory cytokines have been observed in H. pylori-infected individuals. Through these mediators, several kinds of immune cells are stimulated to cooperate in the modulation of the oncogenic and anti-suppressive pathway activity. Methylation of tumor suppressor genes increases the risk of adenocarcinoma in the stomach (Zanussi, *et al*. 2019).

2.5.3 Diets

Ingestion of salt has been shown to increase gastritis and the carcinogenic effects of known gastric carcinogens such as N-methyl-N-nitro-N-nitrosoguanidine. Salt is known to erode the mucosal barrier of the stomach, thereby leading to inflammation. Preserved meats are rich in N-nitroso compounds, which can elicit a similar effect in the body. Grain-fed red meat is especially rich in saturated fats and low in protective fats such as omega-3, which contributes to its inflammatory processes and thus increases gastric cancer risk (Rawla and Barsouk, 2019).

2.5.4 Sex

Gastric cancer rates have been considerably lower in females than males. A possible explanation might be that the protective effect of estrogen that may lower the risk of gastric cancer in women (Camargo, *et al*. 2012).

2.5.5 Obesity:

Possible biological mechanisms linking obesity with GC risk and progression include insulin resistance and hyperinsulinaemia, increased levels of circulating growth factors, chronic inflammation, and altered levels of sex hormones (Jochem, *et al*. 2018).

2.5.6 Ethnicity

High rates of stomach cancer were observed in Indigenous Siberians, Mapuche in South America, Inuit in Arctic regions of Greenland, Canada and Alaska and Maoris in New Zealand (Arnold, *et al*. 2014).

2.5.7 Cigarette smoking:

A cohort study of a more than 300000 women reported that there are association between smoking duration, pack-years, and number of cigarettes smoked per day, and both invasive and borderline mucinous GC, but not associated with serous or endometrioid GC (Licaj, *et al*. 2016).

2.5.8 Alcohol consumption:

Alcohol is known to irritate and erode the stomach lining, resulting in gastritis, a precursor for stomach cancer (Ma, 2017).

2.6 Diagnosis of gastric cancer:

2.6.1 Barium meal:

Double contrast barium meal will outline the gastric mucosa and is a sensitive investigation for detecting mucosal abnormalities. Although carcinomas often have a characteristic appearance, they can be confused with benign peptic ulcers and therefore further investigation to obtain a tissue diagnosis is mandatory (Neal and Hoskin, 2009).

2.6.2 Magnetic resonance imaging (MRI):

The role of MRI in gastric cancer is as an imaging tool to further refine preoperative staging and treatment response evaluation, and provide more valuable information for diagnosis and treatment, particularly for patients who cannot receive iodine contrast agents and those with peritoneal implants and small hepatic metastatic lesions (Zhang and Yu, 2020).

2.6.3 Computed tomography (CT):

CT is most useful for detection of distant metastases and recurrent postoperative GC. CT are also useful modalities for staging and treatment response assessment (Hallinan, and Venkatesh, 2013).

2.6.4 Positron emission tomography (PET):

PET with 2-[^{18}F] fluoro-2-deoxy-D-glucose (FDG) has been recognized as a useful diagnostic technique in clinical oncology. PET has low sensitivity for the primary tumor and lymph node metastases. The major advantage is in the detection of distant metastases to the liver, lungs, and skeleton. In contrast, FDG-PET/CT has limited accuracy in the detection of peritoneal disease (Smyth and Shah, 2011).

2.6.5 Fibreoptic endoscopy:

This allows direct visualization of the gastric mucosa with a more accurate assessment of the macroscopic appearances of an abnormality. It also allows biopsy and brushings for cytology to give a tissue diagnosis (Neal and Hoskin, 2009).

2.6.6 Endoscopic ultrasonography (EUS):

EUS is a combined technique of high-frequency ultrasound (5-12Hz) and endoscopy that allows evaluation of the digestive tract wall and immediate adjacent structures. EUS can distinguish T1-2 tumors from T2-4 tumors with a sensitivity of 0.86 and specificity of 0.91 (Mocellin, *et al*. 2011).

2.6.7 Laparoscopy:

This facilitates direct visualization of the stomach, regional lymph nodes, liver and peritoneal surfaces. It is complementary to high quality cross-sectional imaging and is important in a condition with a high rate of lymphatic involvement and transcoelomic spread (Neal and Hoskin, 2009).

2.7 Treatment of gastric cancer:

2.7.1 Surgery:

Surgical resection is the cornerstone of curative treatment but only two-thirds of patients are deemed operable. A partial or total gastrectomy is performed in operable cases depending on the size and site of the tumor. Total gastrectomy in smaller tumors will have the advantage in surgically clearing occult mucosal spread and synchronous second primary cancers (Neal and Hoskin, 2009).

2.7.2 Radiation therapy:

Radiation treatment for gastric cancer can be technically challenging and associated with significant toxicities. maintenance of adequate nutrition during therapy and supportive care are critical. Treatment interruptions or dose reductions for manageable acute toxicities should be avoided. Patients

should undergo a CT scan for radiation-treatment planning and are positioned supine with arms up in an immobilization device for reproducibility of daily set-up (Hajj and Goodman, 2015).

2.7.3 Chemotherapy:

Preoperative/perioperative chemotherapy using cisplatin and 5FU with or without epirubicin increases the rate of subsequent curative surgery and yields a 5-year improvement in overall survival of 10–15% (Neal and Hoskin, 2009).

2.7.5 Targeted therapy:

The majority of patients with gastric cancer present with advanced disease, which is incurable. Molecularly targeted therapies, such as those targeting HER2, are anticipated to improve the current status of systemic treatment beyond conventional cytotoxic therapy. Trastuzumab is a monoclonal antibody which binds to the extracellular domain of the HER2. It mediates antibody dependent cellular cytotoxicity by inhibiting proliferation of cells that overexpress HER2 protein, resulting in the blockade of receptor dimerization (Gomez-Martin, *et al*. 2013).

2.8 Carcinoembryonic Antigen (CEA)

CEA belongs to the immuno- globulin gene "superfamily". CEA is a glycoprotein with a molecular weight of 150 to 300 kDa. It is a single polypeptide chain consisting of 641 amino acids and containing 45–55% carbohydrate. It displays a cell adhesion activity and signal regulatory properties. CEA localized mainly to epithelial cell membranes (Steffan, *et al*. 2019).

CEA is usually produced in the gastrointestinal tissue during the development of the foetus and terminates before birth (Canzonieri, *et al*. 2019). Their biological role in malignancy is the suppression of the host immune system, while in pregnancy they affect the

maternal immune response, generating maternal tolerance toward the embryo (Angeliki Sarandakou, *et al*. 2007).

Some study show that CEA is significantly more in malignant cells than benign cells (Shafaghi, *et al*. 2017).Other study found that tissue CEA expression was not correlated with degree of differentiation (Park, *et al*. 2008).

Chapter three

Materials and Methods

Chapter three

Materials and Methods

3.1 Materials:

Archived tissue blocks of gastric tumor were selected for this study.

3.2 Methods:

3.2.1 Study design:

This is descriptive cross-sectional hospital-based study aimed to study CEA expression in gastric lesions.

3.2.2 Study sample:

Fifty paraffin block samples were collected from patients previously diagnosed as gastric tumor, 30 (60%) of them were malignant and the remaining 20 (40%) were benign. Patient's identification information (age, gender, histopathological diagnosis, malignant tumor grade) were obtained from patient's records.

3.2.3 Study area:

This study conducted at Omdurman teaching hospital during the period from 2019-2020.

3.2.4 Sample processing:

Section of 3 µm in thickness was obtained from each formalin fixed paraffin wax embedded tissue block using rotary microtome, mounted into charged slides then dewaxed in oven.

3.2.5 Immunohistochemical staining:

Immunohistochemical staining was carried out using new indirect-dextran polymer immune peroxidase technique. Tissue sections were deparaffinized in xylene and rehydrated through graded alcohol (100%,

90%, 70%, and 50%) to DW. The antigens were retrieved using water bath with tris EDTA buffer (pH 9) for 20 minutes and then cooled down to room temperature for 5 min, then washed in phosphate buffer saline (pH 7.4), Endogenous peroxidase activity was blocked by 3% peroxidase blocker for 10 minutes, then washed in phosphate buffer saline for 3 minutes. Each slide then treated with anti-CEA primary antibody (45µl) for 25 min at room temperature in a humid chamber, then washed in phosphate buffer saline for 3 minutes. Then sections were incubated in dextran polymer-HRB (horseradish peroxidase) secondary antibody (45µl) for 20 minutes then washed in three changes of phosphate buffer saline, after that incubated in 3, 3 diaminobenzidine tetrahydrochloride substrate solution for 5 minutes, then washed in running tap water. Then counter stained in Mayer's haematoxylin stain for one minute, then washed and blued in running tap water. After that dehydrated through ascending concentration of ethanol, cleared and mounted in DPX mounting media (Bancroft, *et al*. 2013).

3.2.6 Result interpretation:

All quality control measures were adopted. A negative control slide was completed by omission of the primary antibody. A known positive CEA section obtained from colon carcinoma blocks used as positive control during immunohistochemical staining. Positive staining for CEA appeared as brown particles at the plasma membrane using X40 lens. Under microscopy, detection of more than 5 cells per one field considered as positive result.

3.2.7 Data analysis:

Data was analyzed using SPSS version 20 computer program. Frequency, mean, standard deviation, minimum, maximum and chi-square test values were calculated.

3.2.8 Ethical consideration:

Samples were collected after taking ethical approval from Omdurman teaching hospital to use the tissue blocks for research purposes.

Chapter Four

Results

Chapter Four

Results

The study includes fifty samples, 30 (60%) samples were gastric adenocarcinoma and 20 (40%) samples were gastric lesions, of which 12 (60%) samples were gastritis, 4 (20%) samples were peptic ulcer, 3 (15%) samples were dysplasia and 1 (5%) sample was fundic polyp, as indicated in table (4.1).

The age of study population ranged between 12 and 95 years with mean age of 57.7 ± 17.9 years. Most of patients were more than 50 years representing 33 (66%) and the remaining 17 (34%) were equal or less than 50 years. as indicated in table (4.2).

The distribution of gender showed that most of patients were male representing 32 (64%) and the remaining 18 (36%) were female, with a male to female ratio of 1.8:1.0, as shown in graph (4.1).

The tumor grade of study samples revealed 10 (33.3%) grade I, 10 (33.3%) grade II and 10 (33.3%) grade III, as showed in table (4.3).

Gastric lesions revealed positive CEA expression in 20 (40%) samples and negative expression in 30 (60%) samples, as indicated in graph (4.2).

Gastric adenocarcinoma revealed positive CEA expression in 17 (56.7%) samples and negative expression in 13 (43.3%) samples, while gastric lesions showed positive expression in 3 (15%) samples and negative expression in 17 (85%) samples. This result showed significant statistical association (P.value 0.003), as indicated in table (4.4).

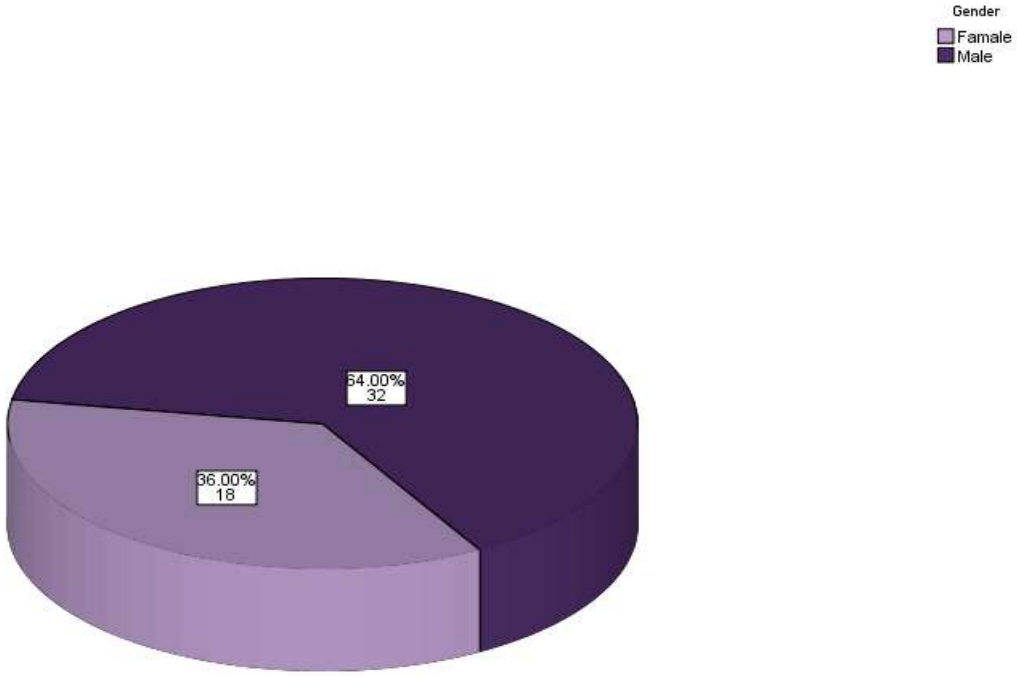
CEA positive expression was found in 8 (26.7%) samples of grade I, 5 (16.67%) samples of grade II, and 4 (13.3%) samples of grade III, while negative CEA expression founded in 2 (6.6%) samples of grade I, 5 (16.67%) samples of grade II and 6 (20%) samples of grade III. This result showed no significant association between CEA expression and tumor grade (P.value = 0.171), as showed in table (4.5).

Table (4.1): Distribution of histopathological diagnosis among the study Sample

Histopathological diagnosis		Frequency	Percentage
Gastric adenocarcinoma		30	60%
Gastric lesions	Gastritis	12	60%
	Peptic ulcer	4	20%
	Dysplasia	3	15%
	Fundic polyp	1	5%
Total		50	100%

Table (4.2): Distribution of age groups among the study population:

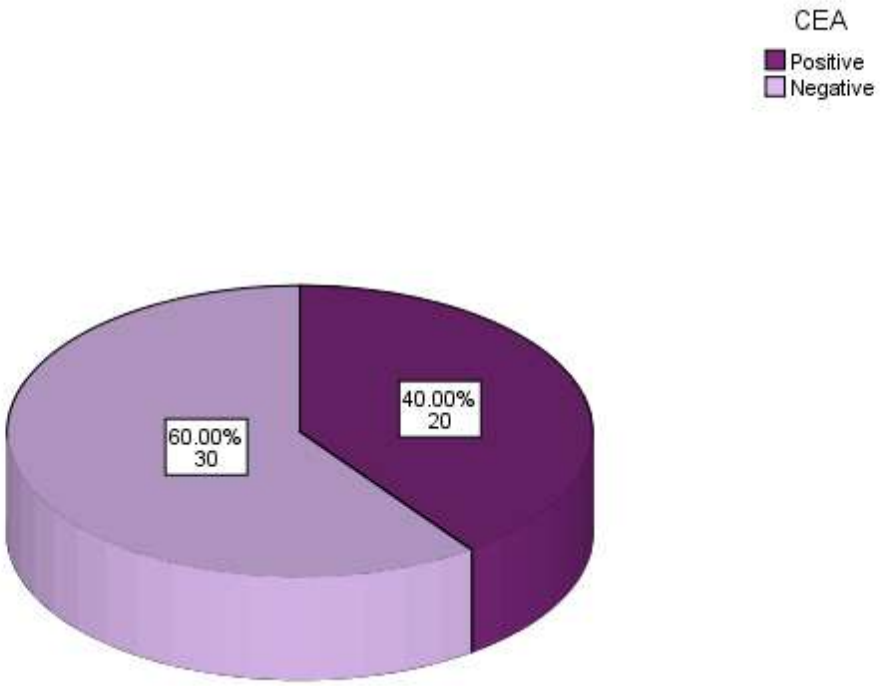
Age groups	Frequency	Percentage
Equal or less than 50 years	17	34%
More than 50 years	33	66%
Total	50	100%



Graph (4.1): Distribution of sex among study samples

Table (4.3): Distribution of tumor grades among malignant gastric tumors:

Tumor grades	Frequency	percent
Grade I	10	33.3
Grade II	10	33.3
Grade III	10	33.3
Total	30	100



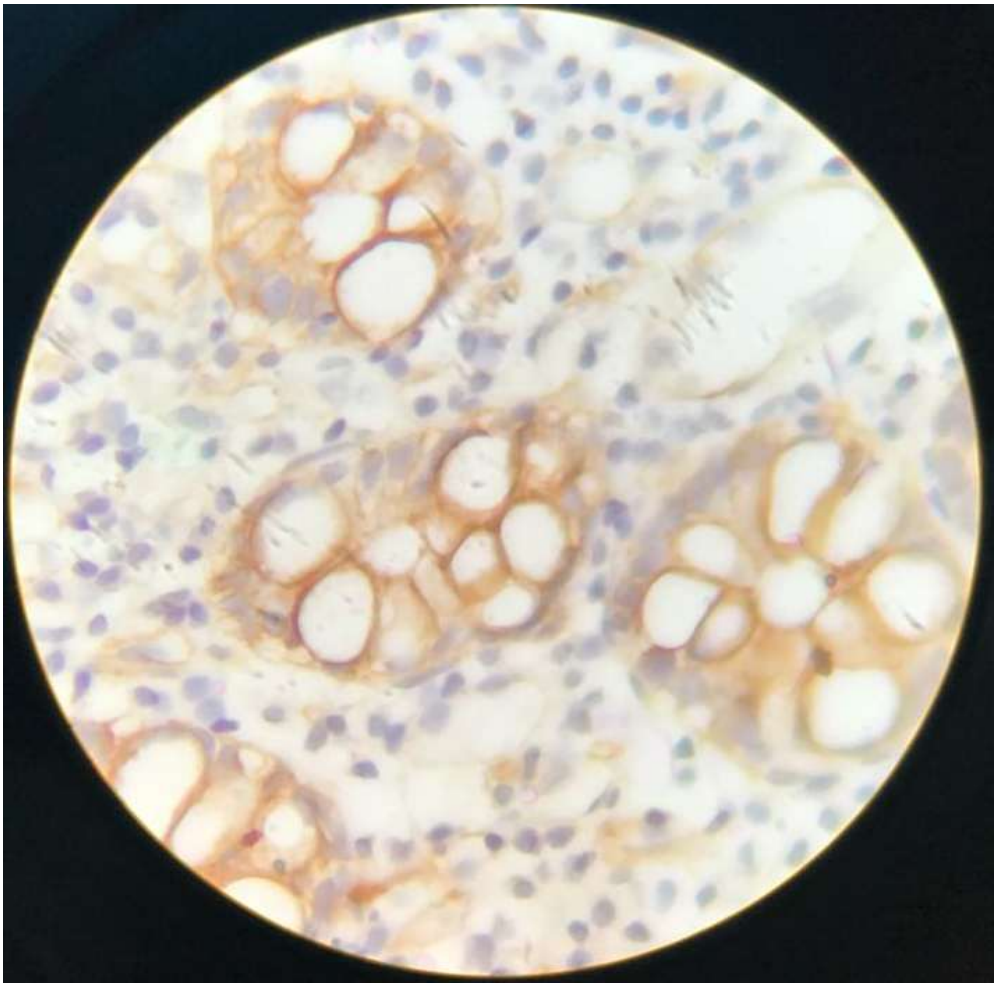
Graph (4.2): Frequency of CEA expression among study samples

Table (4.4): Relation between the expression of CEA and histopathological diagnosis:

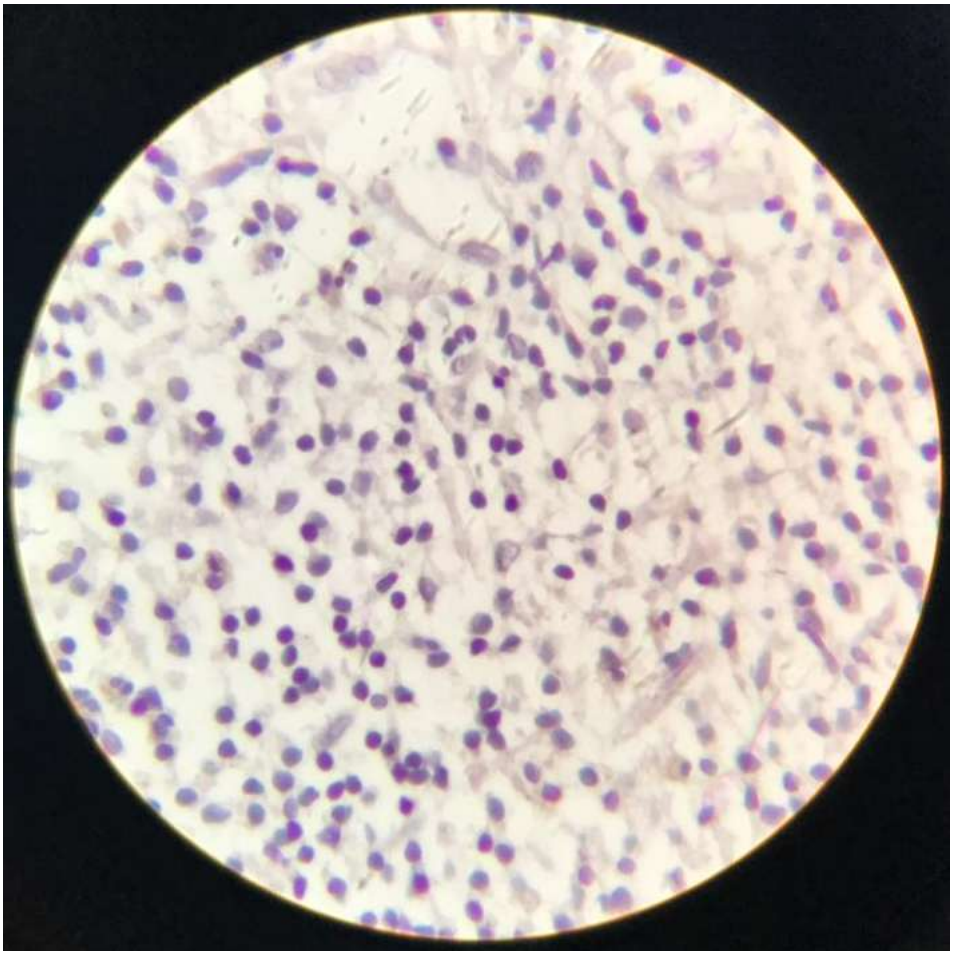
Histopathological diagnosis	CEA expression		P-value
	Positive	Negative	
	N (%)	N (%)	
Gastric adenocarcinoma	17 (56.7%)	13 (43.3%)	0.003
Gastric lesions	3 (15%)	17 (85%)	
Total	20 (40%)	30 (60%)	

Table (4.5): Relation between CEA expression and malignant tumor grade:

CEA expression	Tumor grade			P. value
	Grade I	Grade II	Grade III	
	N (%)	N (%)	N (%)	
Positive	8 (26.7%)	5 (16.67%)	4 (13.3%)	0.171
Negative	2 (6.6%)	5 (16.67%)	6 (20%)	
Total	10 (33.3%)	10 (33.3%)	10 (33.3%)	



Micrograph (4.1): Shows positive expression of CEA in gastric adenocarcinoma (X40).



Micrograph (4.2): Shows negative expression of CEA in gastric lesion (X40).

Chapter Five

Discussion, Conclusion and Recommendations

Chapter five

Discussion, conclusion and recommendations

5.1 Discussion:

The present study includes 50 samples of gastric lesions stained by immunohistochemistry for CEA. Concerning the age groups of the patients, the study revealed that most of gastric tumors patients were more than 50 years. This result is compatible with Guan *et al*. (2019), who reported that mostly GC patients were above 50 years of age.

In this study most of patients were male representing 32 (64%) and the remaining 18 (36%) were female, with a male to female ratio of 1.8:1.0, a result that agreed with Camargo, *et al*. (2012), who found that GC rates lower in females than males, he attributed this result to protective effect of estrogen that may lower the risk of gastric cancer in women.

In this study malignant tumors revealed positive CEA expression in 17 (56.7%) samples and negative expression in 13 (43.3%) samples, while benign gastric tumors showed positive expression in 3 (15%) samples and negative expression in 17 (85%) samples. This result showed significant statistical association (P.value 0.003), a similar result obtained by Shafaghi, *et al*. (2017) who found significant statistical differences between the expression of CEA and types of lesion (P = 0.0001). Also agreed with Virgilio, *et al*. (2017) who found that there was a significant difference between e expression of CEA in gastric lesions ($p<0.01$).

In this study CEA positive expression was found in 8 (26.7%) samples of grade I, 5 (16.67%) samples of grade II, and 4 (13.3%) samples of grade III, while negative CEA expression found in 2 (6.6%) samples of grade I, 5 (16.67%) samples of grade II and 6 (20%) samples of grade III. This result

showed no significant association between CEA expression and tumor grade (P.value = 0.171), This result is compatible with Park, *et al*. (2008), who found that tissue CEA expression was not correlated with the degree of differentiation.

5.2 Conclusion:

On basis of the result this study concluded:

- The age of gastric lesions patients is commonly more than 50 years.
- Gastric lesions found more in male rather than in female.
- CEA expression is more frequent expressed in gastric adenocarcinoma compared with gastric lesions, and no significant association between CEA expression and tumor grade.

5.3 Recommendations:

According to the results, the study recommended:

Further study should be done for expression of CEA in gastric tumors with large sample size and stratified by tumor stage and lymph nodes involvement.

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Appendices

Appendices

Materials and instruments:

Materials and instruments used for processing and staining of the specimens include:

Disposable gloves.

Rotary microtome.

Microtome knives.

Coated slides.

Cover glasses.

Water bath.

Dry oven.

Coplin jars.

Humidity chamber.

Xylene.

Ethanol (100%, 90%, 70%, 50%).

DW.

Mayer's haematoxylin.

Tris EDTA buffer (pH 9.0).

Phosphate buffer (pH 7.4).

Peroxidase blocker (0.3% hydrogen peroxide in methanol).

Primary antibody CEA (anti- human CEA).

Secondary antibody (dextran polymer conjugated secondary antibody-HRP).

Substrate.

Chromogen.