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Role of Combine Alcian Blue and Periodic Acid Schiff's in Demonstration of Adenocarcinomas and Poorly Differentiated Cancers

A dissertation submitted is in partial fulfillment M.Sc. degree in Histopathology and Cytology

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The Verse

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (١) **خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ** (٢) **اقْرَأْ وَرَبُّكَ** الْأَكْرَمُ (٣) الَّذِي عَلَّمَ بِالْقَلَمِ (٤) عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ (٥)

صدق الله العظيم

سورة العلق الآيات 1-5

Dedication

To who have taught me the meaning of life, who gave me love and respect,,,,,,,,,,,,

My father

To the spring that never stops giving who trained me to change to the best

My Mother

Deepest feeling who supported me always learn me to give

Even without take

To my dear son Ahmed and my dear daughter Fatima

To dears brothers and sisters

To my dear friends

To those help me to complete this research

To all my colleagues in Shendi University

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First of all, thanks to ALLAH for the completion of this dissertation and ask him to bless this effort.

I would like to express my deepest gratitude and appreciation to my supervisor, **Dr**. **Mohammed Abdelgader Elsheikh** for useful comments, guidance and advice which help greatly in making this dissertation to be beneficial.

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Deepest thanks to my family, friends and my colleagues and everyone who encouraged and supported me to perform this work.

Abstract

Background:

Aim: To detect expression of mucins in adenocarcinomas and poorly differentiated cancers.

Mucins are complex carbohydrates secreted by different types of epithelial cells and glandular tissues of many organs. . Pathological expression of mucins has been implicated in cancer development and progression. The diagnosis of adenocarcinomas based on morphological appearance under the light microscope. Mucin demonstration can play a role in distinction between adenocarcinomas and further in identification of poorly differentiated cancers.

Materials and methods: This is hospital based descriptive cross sectional study, Sections were obtained from archival paraffin blocks which included randomly selected 60 cases of adenocarcinomas and poorly differentiated cancers, and used sections were stained for combined Alcian blue — PAS to study the mucin expression . Used master cheat in data collection and SPSS in data analysis .

Results: The commonest age group was ranged from 61-80 years old . 70% of cases were females, mucin was present in 90% of study samples. Mixed types of mucins were the most common type(55%) followed by neutral mucin(35%), all samples free from acidic type alone.

Conclusion: Mucin expression considered as differential material between different grades of tumor of adenocarcinoma type, and further may be distinguish between poorly differentiated adenocarcinoma and poorly differentiated squamous cell carcinoma.

المستتخلص

خلفية:

الهدف :لتحديد إفراز البروتين المخاطي في مختلف مراحل السرطان الغدي وسرطانات غير متمايزة .

يعتبر البروتين المخاطي من الكاربوهيدريدات المركبة وينتج بواسطة أنواع مختلفة من الخلايا الطلائية والخلايا الغدية في عدة أعضاء في جسم الإنسان.وجد أن البروتين المخاطي يفرز بصورة غير طبيعية في حالة نشوء وتطور السرطان.توضيح البروتين المخاطي يمكن أن يلعب دورا في التمييز بين السرطانات الغدية وأيضا في التعرف علي السرطانات غير المتمايزة.

الطرائق والمواد: هذه الدراسة الصفية المقطعية المستشفويه هدفت لتقييم دور صبغة الألشيان الأزرق ومحلول الشف، في تحديد إفراز البروتين المخاطي في مختلف مراحل السرطان الغدي وسرطانات غير متمايزة.شملت هذه الدراسة 60 عينة نسيجية مصابة بالسرطانات الغدية والسرطانات غير المتمايزة.

النتائج:وجدت الدراسة أكثر عم يتراوح بين 61-80 سنه و70%من العينات نساء ، وجد البروتين المخاطي في 90% من عينات الدراسة. أكثر أنواع الميوسين وجودا هو النوع المخلوط (55%)يليه الميوسين المتعادل (35%) بينما لم يوجد ميوسين حمضي علي حدة.

الخلاصة: وجدت الدراسة أن إفراز مادة الميوسين يميز بين مراحل السرطانات الغدية المختلفة وأيضا يمكن أن يميز بين السرطانات الغدية غير المتمايزة والسرطانات الحرشفية غير المتمايزة.

List of abbreviations

Abbreviations	Explanation
AB	Alcian blue
CEA	Carcino embryonic Antigen
CRC	Colorectal cancers
DPX	Disterene Polystyrene and Xylene
FFPE	Formalin Fixed Paraffin Embedded
GC	Gastric cancer
GIT	Gastrointestinal tract
IHC	Immunohistochemistry
IM	Intestinal Metaplasia
MUCs	Mucins
PAS-AB	Periodic acid Schiff- Alcian blue
PAS	Periodic acid Schiff
PDC	Poorly Differentiated Cancer
SPSS	Statistical Package for Social Science
PH	Power of Hydrogen
MUC	mucin
CA-125	Cancer antigen -125
CUP	Cancer of unknown primary

List of contents

List of figures

List of tables

1-1 Introduction:

Mucin like the proteoglycans consist of polysaccharide chains covalently linked to a protein core[1]. Typically, the carbohydrate component is attached via an O-glycosidic linkage to serine or threonine. The serine- and threonine rich protein core may contain anywhere from several hundred to several thousand amino acids. A defining structure of the epithelial mucins is the presence of paired repeated amino acid sequences within the protein core. Mucins are categorized numerically into functionally distinct families based in part upon differences in the paired amino acid sequences and the structure of their protein core [2].

The glycosaminoglycans which are strongly acidic polyanions, the polysaccharide chains of mucins vary from neutral or weakly acidic to strongly acidic sulfomucins. They also demonstrate a more varied composition of monosaccharide units. Neutral mucins contain a high content of uncharged monosaccharides such as mannose, galactose and galactosamine, and are found in high concentrations in the gastric mucosal epithelium, Brunner's glands in the duodenum and the prostatic epithelium. The sialic acids are a diverse group of nine-carbon monosaccharides which contain acarboxylate group at the carbon in position 1[3]. This is ionized at a physiological pH and imparts an overall negative charge on the molecule. The function of the mucins depends on the tissue location of the mucin-producing cell and the mucin type. The secreted mucins usually provide lubrication and protection for the secreting cells and/or tissues in the immediate area. The role of the membrane-bound mucins is not fully understood. These mucins are possibly involved in the regulation of cellular functions such as cell proliferation and cell adhesion [4].

Cancer is one of the leading causes of death worldwide. It is estimated that the global cancer burden will even grow in years to come and will reach 21.4 million new cases [5].The grading of cancer is a histological method intended to help

predict prognosis based on specific morphological features. It typically is based on architectural or cytological features (nuclear grade or number of mitoses), or in some cases, a combination of both. Grading is usually broken down into a spectrum from well differentiated (grade 1) to the most poorly differentiated (grade 3 or 4). The number of grades ranges from two to five [6–8]. The highest grade tumours (grade 3 or 4) lack any specific differentiation.

Adenocarcinoma is a malignant neoplasm arising from epithelial cells of the glands or glandular like structures and can arise in multiple sites of the body. Some of the common sites that develop adenocarcinoma are the breast, lung, prostate, gastrointestinal tract like the colon, rectum, pancreas, stomach, and esophagus. Adenocarcinomas also make 70 percent of cancer of unknown origin [9].

Adenocarcinomas are easily diagnosed and distinguished from other cancer histologies by a light microscopy examination. The diagnosis is usually based on the identification of glandular structures under the light microscopy. As these features are shared by all types of adenocarcinomas, it is impossible to diagnose the primary site of origin of these tumors, especially in a metastatic setting. Also, with poorly differentiated adenocarcinomas, where the minimal glandular formation is seen on light microscopy or no glandular formation, but by special stains can identified mucin, immunohistochemistry (IHC) is an important tool to help further diagnose of the adenocarcinoma type[10].Combine alcian blue and periodic acid Schiff is one of special stains that are used for the evaluation of mucins. This combination of techniques differentiates neutral mucins from acidic mucins within a tissue section[11].

1-2 Rationale:

Adenocarcinomas and poorly differentiated cancers are common cancerous lesion in our histopathology and cytology lab. The diagnosis of adenocarcinomas based on morphological appearance under the light microscope. As these morphological features are shared by all types of adenocarcinomas, it is impossible to diagnose the primary site of origin of these tumors, especially in a metastatic setting and with poorly differentiated adenocarcinomas, where the minimal glandular formation is seen on light microscopy or no glandular formation. The differentiation between poorly differentiated cancers is imposable on routine histopathological as they shared the same features. Mucin demonstration can play a role in distinction between adenocarcinomas and further in identification of poorly differentiated cancers. Combined alcian blue and periodic acid Schiff method is a popular method for identification of all types of mucin, as it is specific, sensitive and clearly demonstrates mucins.

1-3 Objectives:

1-3-1General Objective:

To detect expression of mucins in adenocarcinomas and poorly differentiated cancers.

1-3-2 Specific Objectives:

1. To correlate expression of mucin with adenocarcinomas grades and poorly differentiated cancers.
2. To correlate expression of mucin subtypes with tumor grades among study samples.

2-1 Mucins:

Mucins are glycoproteins of high molecular weight that are synthesized, stored and secreted by the epithelial mucosal cells of several organs[12, 13]. Their general structure and biochemical composition provides protection for the cell surface against pathogens and toxins[14, 15]. Mucins are classified into neutral mucins and acidic mucins; the latter include sulpho and sialo mucins. The neutral mucins can be found primarily in the surface epithelia of the stomach, Brunner's glands of the duodenum and in the prostatic epithelium. The acid mucins are found widely distributed throughout the gastrointestinal tract and the respiratory tract[16]. Increased mucin production is indicative of many cancers, including cancers of the pancreas, lung, breast, ovary, urinary bladder, colon and other tissues[17]. Mucins are the most abundant macromolecules in mucus and are responsible for its biochemical and biophysical properties due to their nature and extent of glycosylation [18, 19]. The mucins are a closely related family of O-glycoproteins that play an important role in the renewal and differentiation of the epithelium, cell adhesions, immune response, and cell signaling [20-24].

Genes coding for the protein component of mucin are designated as MUCs. At present, 14 mucin glycoproteins have been assigned to the MUC gene family [25]. Mucins can be subdivided into membrane-associated and secreted forms [26-28]. In normal tissues, mucins seem to be expressed in a relatively organ- and cell-specific manner [29-32]. Some mucins can be observed in several types of tissues, whereas others exhibit a more limited pattern of expression. Although the characteristic patterns of mucin expression for each organ can be maintained during neoplastic transformation, mucin expression sometimes is altered in carcinomas compared with normal tissues[27, 28, 33-35]. Nevertheless, this relative tissue specificity suggests that differential expression of particular mucins might be a useful means for

discriminating between carcinomas of various sites and might have important applications in diagnostic surgical pathology.

2-2 Adenocarcinoma:

A carcinoma is a cancer arise from the epithelial tissue, from the surface either squamous cell carcinoma or transitional cell carcinoma, and from the gland is adenocarcinoma. Adenocarcinoma is a malignant neoplasm arising from epithelial cells of the glands or glandular likes structures. Adenocarcinoma can arise in multiple sites of the body. Some of the common sites that develop adenocarcinoma are the breast, lung, prostate, gastrointestinal tract like the colon, rectum, pancreas, stomach, and esophagus. Adenocarcinomas also make 70 percent of cancer of unknown origin[9]. A number of environmental as well as lifestyle risk factors are associated with the development of cancers. Different sites have different carcinogens and risk factors. Tobacco smoking, by far, seems to play a major role in most of them.

Breast cancer is the second most common cause of cancer worldwide and the most common cancer in women[36]. The incidence of breast cancer is more in the Caucasian countries compared to the Asian and African nations[37]. Breast cancer mortality has been in decline largely attributed to the screening measures and adjuvant therapy[38, 39].

Adenocarcinoma of the Prostate is the second most common cancer in men worldwide. It is the third leading cause of death in the United States Prostate cancer mortality is on a decline especially in the United States which is attributed to increased screening as well as adjuvant therapies [40, 41].

Colorectal cancers are the third most common cancer in males and second in females [41]. In the United States, the annual incidence, as well as mortality, is slowly decreasing[42].The incidence of CRC varies globally with the highest incidence rates in Australia, New Zealand, Europe, and North America, whereas

the lowest seen in Africa and South central Asia. People with low socioeconomic status also tend to have an increase in the incidence of colorectal cancer.

Gastric cancer is the fifth most common cancer and the second most frequent cause of cancer death worldwide[43]. GC remains a major cause of mortality and morbidity worldwide, and the total number of gastric cancer cases, contributing to more than 1 million cases per year and 5.7% of all cancer diagnoses, is predicted to rise as a result of population growth.

Ovarian cancer represents the second most common malignant gynecologic neoplasm, in Western countries and it is accounted for more mortality than all other female genital tumors [44].

Adenocarcinomas are easily diagnosed and distinguished from other cancer histologies by a light microscopy examination. The diagnosis is usually based on the identification of glandular structures under the light microscopy. As these features are shared by all types of adenocarcinomas, it is impossible to diagnose the primary site of origin of these tumors, especially in a metastatic setting. Also, with poorly differentiated adenocarcinomas, where the minimal glandular formation is seen on light microscopy or no glandular formation but stain for mucin, immunohistochemistry (IHC) is an important tool to help further diagnose the type of adenocarcinoma[10].

Adenocarcinomas are diverse and can involve any part of the body. The management and treatment of adenocarcinoma differ depending on the primary site of disease as well as the stage of cancer. Prior to the initiation of any treatment, it is very important to first characterize the site and type of adenocarcinoma.

The prognosis of adenocarcinoma depends on the type of adenocarcinoma as well far has the greatest impact on prognosis. Other independent factors that determine prognosis are the performance status of the patient, site of metastasis, tumor burden, eligibility, and tolerance of treatment[45-48].

Complications of adenocarcinoma primarily are related to the cancer site, the extent of the tumor, metastatic area. Other complications associated with adenocarcinoma are related to the management options.

The grading of cancer is a histological method intended to help predict prognosis based on specific morphological features[6].Methods of evaluating malignant tumors are based on different parameters. When grading a tumor, the pathologist is referring to the appearance of the tumor cells, specifically to their degree of anaplasia. Von Hanse man and Broders were leaders in popular izing this approach[49]. For most tumors, four grades are used. Grade I tumors are so well-differentiated that they closely resemble the normal parent cells, whereas Grade IV tumors are so anaplastic that even the recognition of their cell of origin becomes difficult. Grades II and III are intermediate. Instead of using a numerical system, some pathologists prefer to indicate that the tumor is well differentiated, moderately differentiated, poorly differentiated or undifferentiated. These designations would correspond to Grades Ito IV, respectively. If different areas of a given neoplasm show different grades of malignancy, the tumor should be graded according to the more undifferentiated area (prostatic cancer being an exception). Because of this focal variation in the degree of differentiation, the grade assigned to a small biopsy of a tumor may not always be representative of the whole neoplasm[50].

Grading has no prognostic value in certain types of cancers, such as melanoma of the skin, and in others it is of question able value. In some tumors, such as transitional cell carcinoma of the urinary bladder, the grading has a direct relationship to the prognosis. The five-year survival rate of Grade I tumors is 80 percent, whereas for Grade III neoplasms it is only 20 percent[50]. In the central nervous system, grading is useful for astrocytomas, but not for ependymomas or oligodendrogliomas. In bone sarcomas, grading are of value for chondrosarcomas,

since the five year survival rate is 78 percent for the well-differentiated tumors, 53 percent for the moderately differentiated and only 22 percent for the poorly differentiated ones[51].Gleason has shown a remarkable correlation between a five grade system of prostatic carcinoma and patient survival[52].Tumor grading has been a traditional component of the pathologic evaluation, and offers guidance to therapy and patient management in many organ systems [53-57].

2-3 Poor differentiated carcinoma (PDC):

Pathologists use the term poorly differentiated to describe tumours made up of cancer cells that look very abnormal compared to normal cells, noncancerous cells can be described PDC based on their shape, size, or color. When looking at these cancers under a microscope, there is enough detail to tell that they are carcinomas, but the cells are too irregular to classify them further. These cancers make up about 3 of 10 cases of CUP. On further testing, about 10% of these turn out to be lymphoma, melanoma, or sarcoma [58].

2-4 Combine alcian blue-PAS:

The PAS technique is perhaps the most versatile and widely used of the techniques for the demonstration of glycoproteins, carbohydrates and mucin. Unlike the other techniques described thus far, the PAS technique also recognizes neutral mucin. The reactivity of the PAS technique is not based upon the presence of acidic groups among the polysaccharides but instead upon the structure of the monosaccharide units. The combination of the alcian blue and the PAS techniques can be used as a means of distinguishing neutral mucin from acid mucin. In most protocols, sections are stained with the standard alcian blue (pH 2.5) method followed by the PAS technique. The alcian blue at a pH of 2.5 will stain all acid mucin deep blue but will not color the neutral mucin. the subsequent application of the PAS technique will stain the neutral mucin bright magenta [59].

Combine alcian blue and periodic acid Schiff is one of special stains that are used for the evaluation of mucins. It is also valuable as a means of detecting mucins; a lack of staining with the combined alcian blue-PAS technique strongly suggests that the substance in question is not a mucin. Tissues and cells which contain both neutral and acidic mucins will stain varying shades of purple due to the binding of alcian blue and the reactivity with Schiff reagent. This is seen in the goblet cells of the small intestine which contain neutral and sialomucins [(11)].

2-5 Future of mucin staining:

The main practical use of mucin histochemistry lies in the diagnosis of adenocarcinoma, particularly poorly differentiated adenocarcinomas.

The stains in routine use are alcian blue and PAS, either alone or in combination. Clearly this practice will continue long into the future. New developments will arise from an improved understanding of the nature of cancer mucin and this will certainly acquire diagnostic importance. It is generally assumed that cancer 'mucin' is the counterpart (albeit differing qualitatively) of the normal secretions of mucinous cells of crypts, ducts or acini [(60)]. Nevertheless, mucin secreting adenocarcinomas may arise in tissues or organs that do not normally secrete mucin, such as prostate, breast and ovary. Mucinous metaplasia is only a partial explanation for this phenomenon. Intraluminal PAS positive material may not be secretory mucin at all, but rather represent up regulated membrane associated glycoprotein (glycocalyx) that is normally elaborated by non-mucin secreting columnar or cuboidal cells [(61)].

2-6 Previous studies:

Eiman and Esam studied demonstration of mucins in Gastrointestinal Tract (GIT) Carcinoma lesions in Sudanese patients they found that; neutral mucin was the most prominent in esophageal and gastro esophageal carcinomas while acid mucin was the most prominent in carcinomas of colon, rectum and stomach. Carboxylated mucin was the most prominent type of acid mucins in all GIT carcinomas. They were concluded that; demonstration of different mucins in GIT carcinomas may assist in their classification and predicting prognosis and behavior of the tumor [(62)].

Agrawal *et al*., study the role of mucin histochemistry in benign and malignant lesions of prostate, they found that; carcinoma of prostate showed presence of both neutral and acidic mucins. Prostatic carcinomas showed positivity for acidic mucins (46.66%) in addition to the positivity for neutral mucins (56.66%). All the cases of low grade prostatic carcinomas showed positivity for acidic mucins but none of the high grade carcinomas showed positivity for the same. They were concluded that Positivity for acidic mucins with Alcian Blue (2.5 pH) technique can be used to differentiate well differentiated adenocarcinomas of prostate from benign hyperplasia [(63)].

Sumana and his colleagues study the mucin histochemistry in normal and adenocarcinoma of colorectum tissues. They found that; mucin histochemistry of normal colon mixture of mucins was observed with predominance of neutral and sulphomucins. In colon adenocarcinoma sialomucins were seen predominant than neutral and sulphomucins. They concluded that, mucin histochemistry is a valuable and cost-effective tool for determining the types of mucins which is one of the important prognostic markers in early detection of colorectal cancers [(64)].

Another study done by Mandal *et al.*, they were study the mucin histochemistry of stomach in metaplasia. Their study included gastric biopsy specimens (total 70). They summarized that; after obtaining clinical history, radiological and endoscopic

findings were noted and after macroscopic study of the specimen, Hematoxylin and eosin, periodic acid Schiff-alcian blue (PAS-AB) staining were performed, intestinal metaplasia (IM) was found in 9 (12.9%) cases by Hematoxylin and eosin , periodic acid Schiff-alcian blue (PAS-AB) positive staining in 15 (21.4%) cases [65].

Another study done by Testsuo *et al*., they were study mucinous carcinoma of the thyroid ,their study concluded, after microscopically, the tumor was composed entirely of strands or solid clusters accompanied by extensive extra cellular mucin these mucin was positive with alcian blue stain and negative with periodic acid-Schiff stain [66] .

Jain *et al.*, studied diagnostic and prognostic significance of different mucin expression, preoperative CEA, and CA-125 in colorectal carcinoma in Aclinicopathological study, they found that the combine Alcian blue-periodic acid Schiff (PAS) staining was positive for both stains in 68.88% of cases indicating that both neutral and acidic mucins are increased in CRC. They concluded that; mucin evaluation in CRCs remains one of the valuable methods as mucinous variants correlate with worse prognosis [67].

3-1 Study design:

This is hospital based descriptive cross sectional study, aimed to evaluate the role of combine alcian blue–PAS, in the detection of mucin expression in different grades of adenocarcinoma and poorly differentiated cancers.

3-2 Study area:

This study was conducted in River Nile State-Shendi locality at El-mek Nimir University Hospital at Histopathology lab during the period from August to December 2021.

3-3 Study populations:

The study populations were paraffin tissue blocks taken from patients with adenocarcinomas with different grades of tumor and poorly differentiated cancers.

3-3-1 Inclusion and exclusion criteria:

All samples recruited in this study were paraffin tissue blocks with adenocarcinomas at different grade of tumor and poorly differentiated cancers. Other cancer types were excluded from this study.

3-4 Study samples and sample size:

Sixty formalin fixed paraffin embedded tissue blocks (FFPE) diagnosed with adenocarcinomas and poorly differentiated cancers were involved in this study.

3- 5 Tools of data collection and variable:

Information from Archived blocks was obtained from data records, included patient age, gender and diagnosis. Mucin expression was detected by using combine alcian blue & PAS method.

3-6 Sample processing:

All blocks were cut into 3 microns by using rotary microtome. And spread in water bath then put in frosted end coated glass slides, then all sections were deparaffinized in xylene and rehydrated through descending grade of alcohols, then washed in water. Then sections were stained by combine alcian blue & PAS.

3-7 Combine alcian blue & PAS method:

Each section was dewaxed in xylene for 10 minutes, then rehydrated in descending grades of ethanol (absolute, 90% and 70% for 2 minutes in each change), and washed in water. After hydration; sections were stained in alcian blue for 5 minutes and washed with tap water, after that oxidized with periodic acid for 5 minutes and washed with water, then each section was covered with Schiff reagent for 30 minutes and washed in running tap water for 5 minutes, then counter stained with Mayer's Hematoxylin for 3 min, and washed with water, then blued with running tap water for 10 min. Lastly each section was dehydrated in ascending grades of ethanol, then cleared in xylene and mounted with Disterene Polystyrene and Xylene (DPX) [68].

3-8 Result interpretations:

Stained slide was observed by microscope by lenses 10 x/0.25 and 40x/0.65, the present of intracellular blue color indicated positive acid mucin, the present of intracellular red magenta color indicated positive neutral mucin, varying shades of purple color inside the cell indicated positive for both acid and neutral mucins.

3-9 Quality controls:

Quality control of sectioning, staining and cover slipping was performed as Llewellyn [68].

3-10 Data analysis and presentation:

Data analysis was done using computerized Statistical Package for Social Science SPSS (16.0). Frequencies, person's chi-square test and other variables were calculated and presented in form of figures and tables.

3-11 Ethical considerations:

The study was performed after written approval from Department of Histopathology & Cytology at Faculty of Medical Laboratory Science and approval also taken from Faculty of Higher Studies and Scientific Research at

Shendi University. Sample collection and processing were performed after taking ethical acceptance from hospital administration. Benefits and results of this study will be published with the assurance on confidentiality.

4- Results:

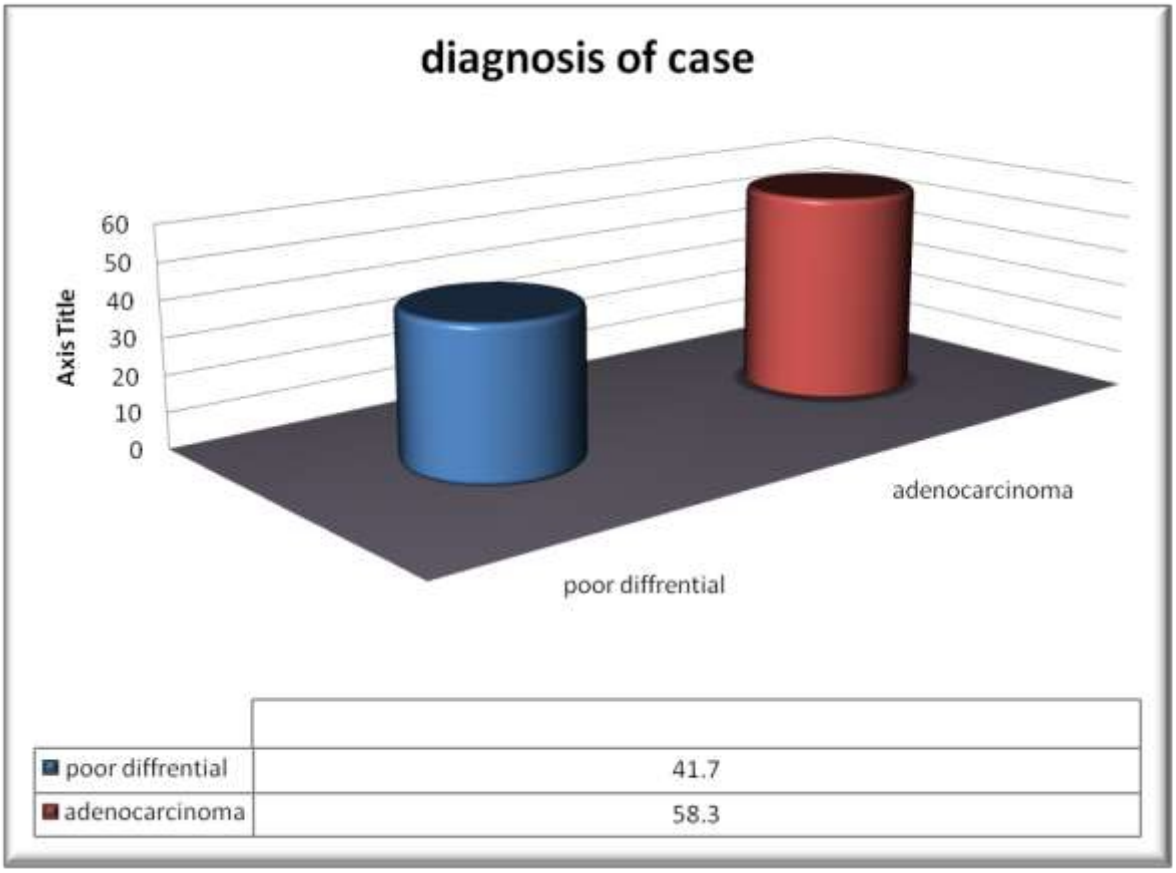

Figure 1: Shows percentages distribution of study cases.

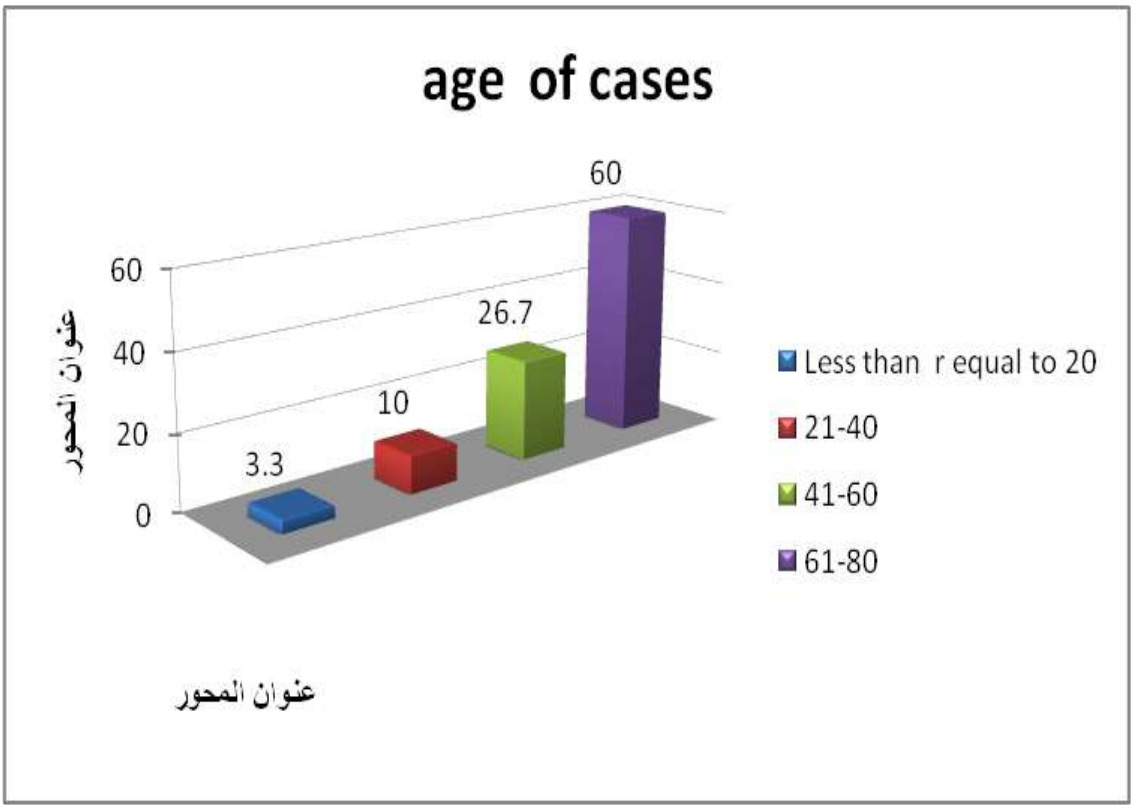

Figure 2: Shows percentages of age groups (mean of age is 62 years old).

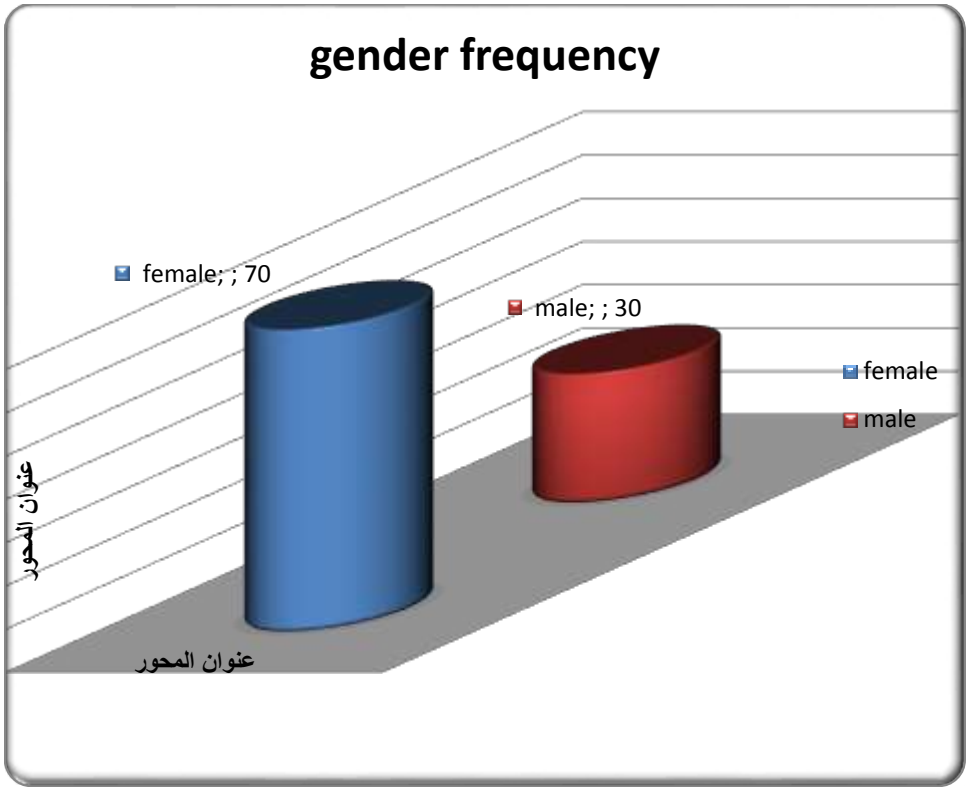

Figure 3: Shows study population's gender.

Table 1: Diagnosis of tissue types.

	Tissue type								
Diagnosis/grade	Prostate	Ovary	Breast	Colon	Gastric	cervix	esophagus	endometrium	Total
Poor diff. ca.	9	2	0	1	3	4	4	2	25
Moderate diff. adenoca.	8	6	1	3	2	0	2	3	25
Well diff. adenoca.	3	1	1	1	2	0	0	2	10
Total	20	9	2	5	7	4	6	7	60
Percentage	33.3%	15%	3.3%	8.3%	11.7%	6.7%	10%	11.7%	100%

Table2: Correlation of mucin expression with study samples.

	Mucin expression			**p. value**
Study sample/grade	Present	Absent	Total	
Poor diff. ca.	19	6	25	
Moderate diff. adenoca.	25	0	25	
Well diff. adenoca.	10	0	10	
Total	54	6	60	
Percentage	90%	10%	100%	0.009

Table 3: Correlation of mucin type expression with the tumor grade.

	Mucin type expression					p. value
Study sample/grade	Neutral	Acid	Mixed	Absent	Total	
Poor diff. ca.	10	0	9	6	25	
Moderate diff. adenoca.	9	0	16	0	25	
Well diff. adenoca.	2	0	8	0	10	
Total	21	0	33	6	60	
Percentage	35%	.0%	55%	10%	100%	0.015

Table 4: Correlation of mucin expression degree with tumor grade.

	Degree of mucin expression					p. value
Study sample/grade	Strong	Moderate	weak	Absent	Total	
Poor diff. ca.	3	6	10	6	25	
Moderate diff. adenoca.	7	16	2	0	25	
Well diff. adenoca.	8	1	1	0	10	
Total	18	23	13	6	60	
Percentage	30%	38.3%	21.7%	10%	100%	0.000

5-1 Discussion:

This is a descriptive cross sectional study performed during the period from August to December 2021, aimed to detect expression of mucin in adenocarcinomas and poorly differentiated cancers. Our study contained 60 cancerous tissues, 35 samples were adenocarcinomas, while the remainders 25 samples were poorly differentiated cancers.

Regarding to the distribution of age among study populations; our study revealed tow third of patients were with advanced age, the age is one of the risk factors associated with the development of cancer, the commonest age among cases was ranged between 61-80 years old with average mean of age 62 years old, this result near to that result in study done by Agrawal and his colleagues, they concluded that; the incidence of carcinoma was found to be increase with the age as the peak incidence was above to the age of 70 years old [63], another studies conducted by Kelsy and Bernstein on 1996 and Howell *et al*., on 2014 they concluded that cancer incidence increased with age [69,70] .

Regarding to the distribution of gender among study populations our study showed that; more than two thirds of patients were females and less than one third of patients were males, on study conducted by Siegel *et al*., on 2016, they summarized that; the mortality of cancer is reported to be greater in men than in women, especially, lung, colorectal and stomach cancers, which are the leading causes of cancer deaths[71]. Sun *et al*., performed a retrospective cohort study on 1,422 patients with histologically proven CRC in which 55.4% were males and 44.6% were females[72]. Regarding gender variation in incidence of cancer may be due to the geographical distribution and poor cancer epidemiological data in our country.

Concerning mucin expression in our study we found that; all adenocarcinomas were showed positive expression of mucin, while 6 out of 25 poorly differentiated cancers were showed negative expression for mucin, and this suggest the positive role of mucin in detection of adenocarcinomas specially when the adenocarcinoma graded poorly differentiated, the well trained pathologist suggest that the negative sample for mucin were not adenocarcinomas and explained they might be with squamous cell carcinoma, this finding similar to that obtained in national study of Eiman and Esam, who studied demonstration of mucins in Gastrointestinal Tract carcinoma lesions (adenocarcinomas) in Sudanese patients, they found that; mucin was present in all studied samples [(62)] .

Agrawal, *et al*., study about role of mucin histochemistry in distinction between prostate carcinoma and benign prostate lesions, they concluded that; prostatic carcinomas showed positive expression of both neutral and acidic mucins while the benign lesions expressed only neutral mucin, our result similar to the above study result because all prostate carcinomas were showed positive expression of both types of mucin [(63)]. Sumana *et al*., studied mucin histochemistry in normal and adenocarcinoma of colorectum, in colon adenocarcinoma sialomucins were seen predominant than neutral and sulphomucins. They concluded that, mucin histochemistry is a valuable and cost-effective tool for determining the types of mucins which is one of the important prognostic markers in early detection of colorectal cancers[(64)].

Regarding to the degree of mucin expression our results indicated that; degree of mucin expression was generally increased with good differentiation and decreased with poor differentiation, our study agree with that study conducted by Sumana and his colleagues, they study mucin histochemistry in normal and adenocarcinoma of colorectum in colon adenocarcinoma and they concluded that; among 25 cases with carcinoma, 18 colon tissues showed moderate reaction (++)

and 7 cases showed weak reaction (+) with PAS stain. PAS with diastase stain was applied on all 50 cases of colon carcinomas and normal colon specimens and they observed that all cases of normal colon showed very strong positive reaction (++++) [64].

5-2 Conclusion:

According to the obtained results in this study, we concluded that:

- Adenocarcinoma and poorly differentiated cancers were increase with age.
- The most frequent gender among study populations was females.
- Mucin helps in confirmation of adenocarcinomas.
- Mucin plays a role in distinction of poorly differentiated adenocarcinoma.
- Mucin and mucin subtypes also play an important role in differentiated between benign and malignant glandular lesions as in case of prostate lesions.

5-3- Recommendations:

On the base of the result we recommended that:

- Identification of poorly differentiated cancers can be carried out through mucin demonstration using combine alcian blue PAS technique.
- Differentiation between benign and malignant tissues can be easier when demonstration mucin subtype as in prostate.
- Future similar studies are needed with larger sample size, and should comprise different types of carcinomas at different grades.

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6- Appendix

6-1Master cheat :

Number of specimen	Age	Gender	Diagnosis	Tumor grade	Mucin	Type of mucin	Degree of mucin expression

:

6-2 Equipments used :

- Rotary microtome
- Microtome knife
- Frosted slides
- Pencil
- water bath
- Coplin jars.
- Oven
- Disposable gloves.
-pastier pipette
- Staining racks
- Cover glass
-Microscope
-wooden stick

6-3 Solution and preparation

- Distilled water

-20% alcohol

- Xylene

- Ethanol (Absolute, 90%,70%)

- Mayer's hematoxylin

- Tape water for bluing.

- DPX

PAS Solutions :

Periodic acid solution

Periodic acid 1 g

Distilled water 100 ml

Preparation of Schiff reagent :

Dissolve 1 g of basic fuchsin and 1.9 g of sodium metabisulfite ($Na_2S_2O_5$) in 100 ml of 0.15 M hydrochloric acid (HCl). Shake the solution at intervals or on a mechanical shaker for 2 hours. The solution should be clear and yellow to light brown in color. Add 500 mg of activated charcoal and shake for 1 to 2 minutes. Filter the solution through a No. 1 Whatman filter into a bottle. The filtered solution should be clear and colorless. If the solution is yellow, repeat the charcoal

decolorization using a fresh lot of activated charcoal. Store at 4°C. Solution is stable for several months. colorless. If the solution is yellow, repeat the charcoal decolorization using a fresh lot of activated charcoal. Store at 4°C. Solution is stable for several months.

Alcian blue pH 2.5 Solutions :

Alcian blue solution

Alcian blue 8GX 1 g

3% acetic acid solution 100 ml

Mayer's hematoxylin Solutions :

1gm hematoxylin

50gm aluminum ammonium sulfate

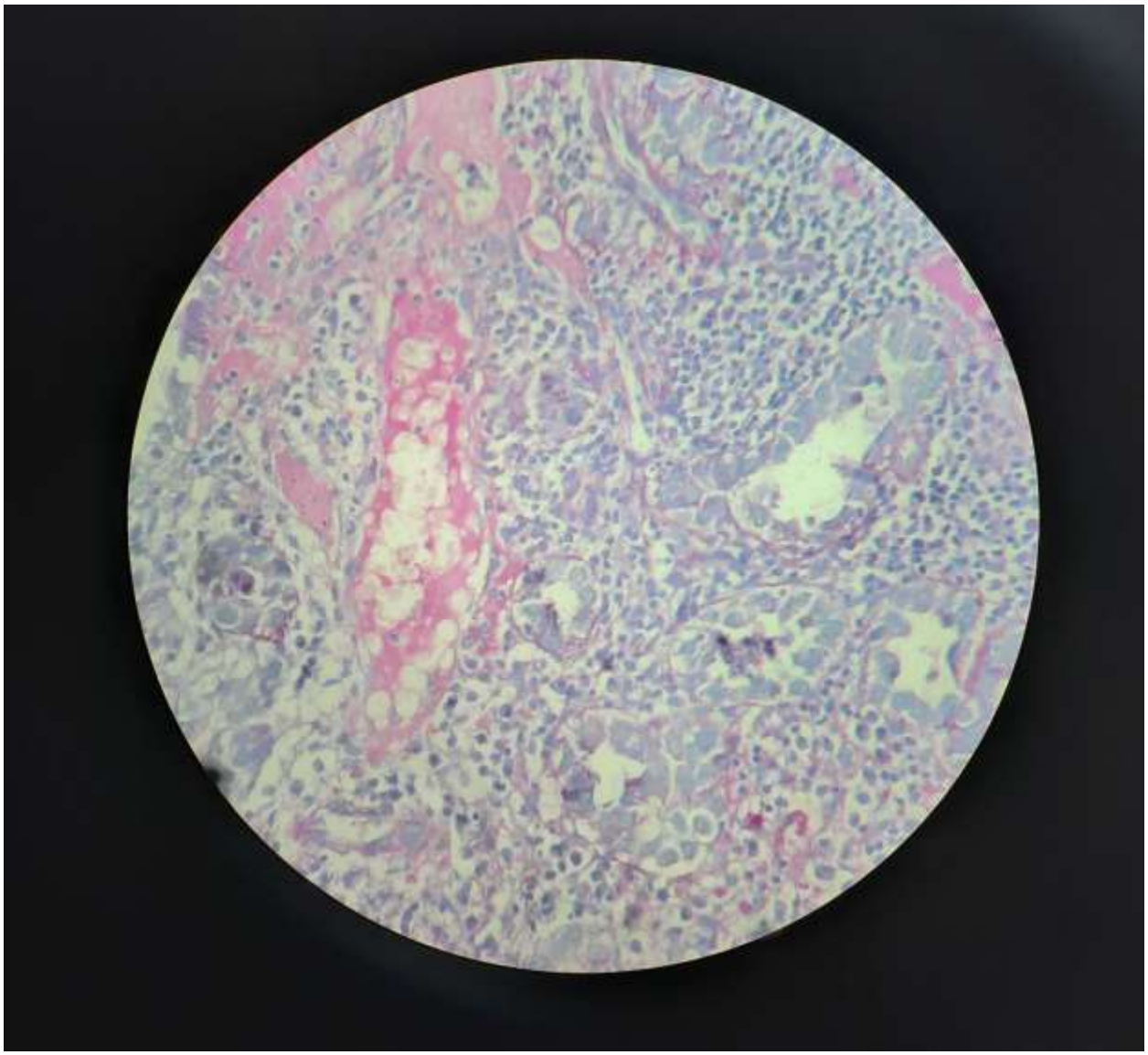
0.2 sodium iodate

50gm chloral hydrate

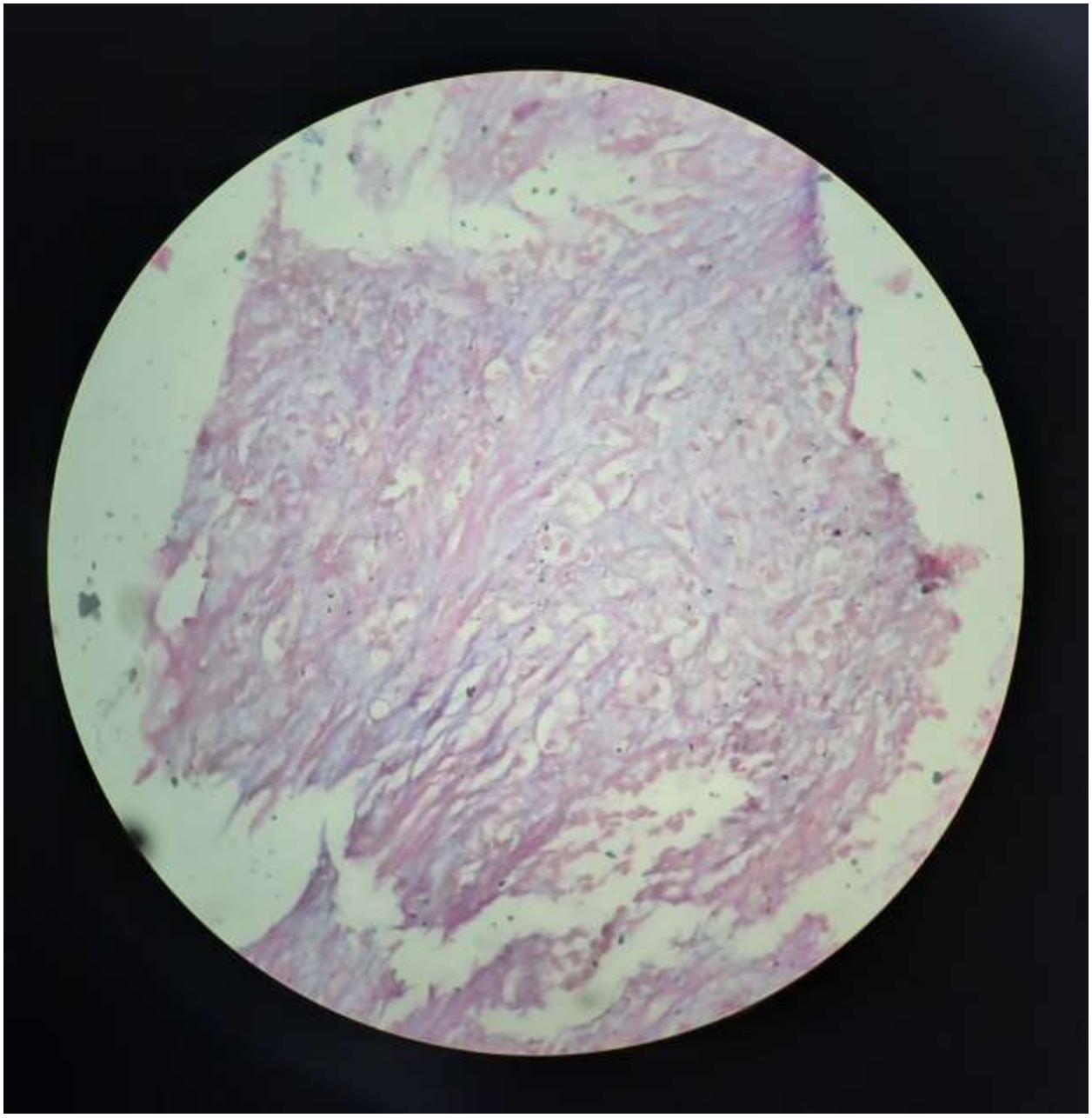
1gm citric acid

1litre distilled water

6-4 Photo :



Endometrial adenocarcinoma (PAS positive) .



Prostatic adenocarcinoma (combine posative) .