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Detection of Amyloid among Adenocarcinoma Tissues in Shendi Town 2021

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

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الآيــــة

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

قال تعالى:

" يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ"

صدق الله العظيم

سورة المجادلة: الآية(11)

Dedication

For my son Bebo Hashim my source of my strength

For my dear father &kind hearted mother.

Candle of my life

My brothers (Salim & Gadora) & sisters (Safa, Nosiba & Weam)

My husband

My full gratitude to ali aboud family

My friends

Jaloya, Fanona ,kanosha, Asasa, Mohammed Abo Elgasim who

support me

Thank you for your presence in my

Life

Acknowledgments

List of abbreviations

Abbreviations	Explanation
AA	Amyloid A
AL	Amyloid light chain
ATTR	Amyloid transthyretin
DCIS	ductal carcinoma IN SITU
DPX	Disterene a plasticizer and xylene
FFPE	formalin fixed paraffin embedded tissue blocks
NCI	National cancer institute
NIAD-4	Near-infrared fluorescent
NSCLC	Non-small cell lung cancer
SAA	Serum Amyloid A
SEER	Surveillance epidemiology and end results
SPSS	Statistical Package for the Social Sciences
US	United States

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Abstract

Background: Amyloidosis (am-uh-loi-DO-sis) is a rare disease that occurs when an abnormal protein, called amyloid, builds up in your organs and interferes with their normal function. Amyloid isn't normally found in the body, but it can be formed from several different types of protein. Organs that may be affected include the heart, kidneys, liver, spleen, nervous system and digestive tract. Amyloid tumor of the breast, first reported in 1973, is a rare disease, with only six cases reported in Japan to date. Some varieties of amyloidosis occur in association with other diseases. This study aimed to detect amyloid in adenocarcinoma tissues.

Materials and methods: This was a descriptive cross sectional study conducted in Shendi town during the period from September 2021 to January 2022. A total of 240 tissues were enrolled in this study, 200 of them were adenocarcinoma tissues included as case group and the remainder 40 benign tissues were included as control group. Socio demographic and samples data were collected by using questionnaire. Amyloid was detected using Congo red technique.

Results: Amyloid was detected in 182 cases (91%) of cancerous tissues. Benign tissue sections were showed positive amyloid in 6 cases (15%).

Conclusion: There was extreme amyloid expression in adenocarcinoma tissues. The amyloid density increased with late stage of cancers. The breast cancer tissues showed strong amyloid expression.

المستخلص

خلفية: الداء النشواني هو مرض نادر يحدث عندما يتراكم بروتين غير طبيعي يسمى الأميلويد في أعضائك ويتداخل مع وظيفتها الطبيعية. لا يوجد الأميلويد بشكل طبيعي في الجسم ، ولكن يمكن أن يتكون من عدة أنواع مختلفة من البروتين. تشمل الأعضاء التي قد تتأثر القلب والكلى والكبد والطحال والجهاز العصبي والجهاز الهضمي. يعد ورم الثدي النشواني ، الذي تم الإبلاغ عنه لأول مرة في عام 1973 ، مرضًا نادرًا ، حيث تم الإبلاغ عن ست حالات فقط في اليابان حتى الآن تحدث بعض أنواع الداء النشواني مصحوبًا بأمراض أخرى. هدفت الدراسة للكشف عن الأميلويد فى أنسجة السرطان الغدى.

الطرائق والمواد: أجريت هذه الدراسة الوصفية المقطعية فى مدينة شندى فى الفترة ما بين سبتمبر 2021 ألى يناير 2022، أشتملت هذه الدراسة على 240 نسيج، 200 منها أنسجة سرطان غدى أدخلت كمجموعة الحالة متبقى الأربعين من الأنسجة الحميدة التى أدخلت كمجموعة تحكم. البيانات الأجتماعية الديموغرافية و بيانات العينات جمعت بأستخدام طريقة الأستبيان. الكشف عن الأميلويد تم بأستخدام تقنية الكونقو الأحمر.

النتائج: وجد الأميلويد فى 182 (91٪) من الأنسجة السرطانية. سجلت شرائح الأنسجة الحميدة وجود الأميلويد في 6 (15٪) حالات.

الخلاصة: يفرز الأميلويد بكثرة فى أنسجة السرطان الغدى. تزيد كثافة الأميلويد فى المراحل الأخيرة للسرطان. أظهرت أنسجة سرطان الثدى أفراز قوى للأميلويد.

1.1 Background:

Amyloidosis (am-uh-loi-DO-sis) is a rare disease that occurs when an abnormal protein, called amyloid, builds up in your organs and interferes with their normal function. Amyloid isn't normally found in the body, but it can be formed from several different types of protein. Organs that may be affected include the heart, kidneys, liver, spleen, nervous system and digestive tract. Amyloid tumor of the breast, first reported in 1973 (1) , is a rare disease, with only six cases reported in Japan to date (2). Some varieties of amyloidosis occur in association with other diseases. These types may improve with treatment of the underlying disease. Some varieties of amyloidosis may lead to life-threatening organ failure. Treatments may include chemotherapy similar to that used to combat cancer. Your doctor may suggest medications to reduce amyloid (3) . Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body (4), these contrast with benign tumors, which do not spread (5). Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss, and a change in bowel movements. While these symptoms may indicate cancer, they can also have other causes. Over 100 types of cancers affect humans our main types of cancer are; carcinomas, sarcomas and leukemia. Carcinoma begins in the skin or the tissue that covers the surface of internal organs and glands. Sarcoma begins in the tissues that support and connect the body. Leukemia is a cancer of the white blood cells (6) . Adenocarcinoma develops in cells located in the glands that line your organs (glandular epithelial cells). These cells secrete mucous, digestive juices or other liquids. If your glandular cells begin to change or grow out of control, tumors can form. Some tumors found in glandular cells are not cancerous. These are called adenomas. However, some tumors that form in the glandular cells are cancerous; these are called adenocarcinomas (7)

Congo red is an organic compound, the sodium salt of 3, 3′-([1, 1′-biphenyl]-4, 4′- diyl) bis (4-aminonaphthalene-1-sulfonic acid). It is an azo dye. Congo red is water-soluble, yielding a red colloidal solution; its solubility is greater in organic solvents. Histology and microscopy, Congo red is used for staining in amyloidosis. Highman's Congo red stain is used for the detection of amyloid on formalin-fixed, paraffin-embedded tissue sections with amyloidosis, and may be used for frozen sections as well amyloid deposits will be stained red and the nuclei will be stained blue. The thickness of sections is usually 5 um. But in case of inadequate amyloid deposits, 10um thick sections will be more satisfactory (8) . Amyloidosis is a rare and serious illness. Amyloidosis occurs when normal proteins in the body become misshapen and clump together. These misshapen proteins are called amyloid deposits or fibrils. Over time, the amyloid deposits build up in organs and tissues in the body. Eventually, this buildup causes symptoms and organ failure because the organs and tissues are not able to work as well as they should. Amyloid protein deposits can be found in specific organs, such as the lung, skin, bladder, or bowel, or they can be systemic. "Systemic" means that the deposits may be found throughout the body. Systemic amyloidosis is the most common (9) .

1.2 Rationale

Cancer increase in incidence in Sudan with years, prognosis of cancer associated with cancer management, expression of amyloid associated with chronic diseases such as cancer, amyloidosis associated with poor prognosis. Previous studies show strong relation between amyloid deposition and adenocarcinomas, detection of amyloidosis among adenocarcinoma patients may play a role in detection of cancer prognosis and then in directing plan of cancer management. There were very little published studies that correlated amyloid expression with cancer stage and prognosis.

1.3. Objectives

1.3.1. General objective

1. To detect amyloid expression in adenocarcinomas tissues.

1.3.2. Specific objectives

1. To correlate amyloid expression with cancer stage.

2. To compare between amyloid expression and type of tissue with adenocarcinoma

2.1 Cancers:

Cancer is a disease in which some of the body"s cells grow uncontrollably and spread to other parts of the body. Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and multiply (through a process called cell division) to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place (10). In the past several decades, the incidence of adenocarcinoma has increased greatly, and adenocarcinoma has replaced squamous cell carcinoma as the most prevalent type of NSCLC (11) .

2.1.1 Breast cancer: Breast cancer is the most commonly diagnosed cancer among US women, with an estimated 268,600 newly diagnosed women with invasive disease (48,100 cases of ductal carcinoma in situ [DCIS] in 2019, accounting for approximately 15.2%-30% of all new cancer cases among women, depending on the data sources (12) . In men, on the 2019 there were 2,670 new cases of breast cancer, accounting for < 1% of all new cancer cases among men (13) . Each year, nearly 42,000 women die of breast cancer, making it the second-leading cause of cancer deaths among US women after lung cancer (14) . The lifetime risk of dying of breast cancer is approximately 2.6% (15) .

2.1.2 Prostate cancer: About 1 man in 8 will be diagnosed with prostate cancer during his lifetime. Prostate cancer is more likely to develop in older men and in non-Hispanic Black men. About 6 cases in 10 are diagnosed in men who are 65 or older, and it is rare in men under 40. The average age of men at diagnosis is about 66 (16) .

2.1.3 Ovarian cancer:

Ovarian cancer ranks fifth in cancer deaths among women, accounting for more deaths than any other cancer of the female reproductive system. A woman's risk of getting ovarian cancer during her lifetime is about 1 in 78. Her lifetime chance of dying from ovarian cancer is about 1 in 108. These statistics don‟t count low malignant potential ovarian tumors. This cancer mainly develops in older women. About half of the women who are diagnosed with ovarian cancer are 63 years or older. It is more common in white women than African American women (17)

. 2.1.4 Thyroid cancer:

Thyroid cancer is the most common endocrine malignancy, accounting for 1.9% of all new malignant tumors (excluding skin cancer and in situ carcinomas) diagnosed annually in the United States (0.92% of cancers in men; 2.9% in women (18) .

2.2 Amyloid:

Amyloids are aggregates of proteins characterized by fibrillar morphology of 7- 13 nm in diameter, a beta sheet secondary structure and ability to be stained by particular dyes, such as Congo red. In the human body, some types of amyloidosis are life threatening and others are less harmful. The type of amyloidosis depends on the type of protein that accumulates. The most common types of amyloidosis are; AL (primary) amyloidosis, AA (secondary) amyloidosis, familial (ATTR) amyloidosis and wild-Type (senile) amyloidosis (18). AL amyloidosis (immunoglobulin light chain amyloidosis is the most common type and used to be called primary amyloidosis. AL stands for "amyloid light chains," which is the type of protein responsible for the condition. There‟s no known cause, but it happens when your bone marrow makes

abnormal antibodies that can"t be broken down. It"s linked with a blood cancer called multiple myeloma. It can affect your kidneys, heart, liver, intestines, and nerve.

To detect amyloid A tissue sample may be taken and checked for signs of amyloidosis. The biopsy may be taken from the fat under the skin on your abdomen (fat aspirate), bone marrow, or an affected organ such as your liver or kidney. Specialized testing of the tissue can help determine the type of amyloid deposit (19) .

2.3 Pathology of amyloid:

Amyloids are aggregates of proteins characterized by a fibrillar morphology of 7– 13 nm in diameter, a β-sheet secondary structure (known as cross-β) and ability to be stained by particular dyes, such as Congo red (20) . In the human body, amyloids have been linked to the development of various diseases Pathogenic amyloids form when previously healthy proteins lose their normal structure and physiological functions (misfolding) and form fibrous deposits in plaques around cells which can disrupt the healthy function of tissues and organs (20) . Such amyloids have been associated with (but not necessarily as the cause of) more than 50 (21) , amyloidosis may play a role in some neurodegenerative disorders (22) . Some of these diseases are mainly sporadic and only a few cases are familial disorders. Others are only familial, some are iatrogenic as they result from medical treatment. Prions are an infectious form of amyloids that can act as a template to convert other noninfectious form (23) . Amyloids may also have normal biological functions; for example, in the formation of fimbriae in some genera of bacteria, transmission of epigenetic traits in fungi, as well as pigment deposition and hormone release in humans (24) . Amyloids have been known to arise from many different proteins (25) . These polypeptide chains generally form β-sheet structures that aggregate into

long fibers; however, identical polypeptides can fold into multiple distinct amyloid conformations (26) . The diversity of the conformations may have led to different forms of the prion diseases (27) . 7 Congo red positivity remains the gold standard for diagnosis of amyloidosis. In general, binding of Congo red to amyloid plaques produces a typical apple-green birefringence when viewed under cross-polarized light. Recently, significant enhancement of fluorescence quantum yield of Near-infrared fluorescent probes (NIAD-4) was exploited to super-resolution fluorescence imaging of amyloid fibrils and oligomers (28) , to avoid nonspecific staining, other histology stains, such as the hematoxylin and eosin stain, are used to quench the dyes' activity in other places such as the nucleus, where the dye might bind. Modern antibody technology and immunohistochemistry has made specific staining easier, but often this can cause trouble because epitopes can be concealed in the amyloid fold; in general, an amyloid protein structure is a different conformation from the one that the antibody recognizes (29)

. 2.4 Paraffin sectioning method:

Paraffin sectioning method is the procedure of cutting thin slices of tissue that has been dehydrated and infiltrated with wax using specialized equipment. This tissue is then embedded in wax before being cut on a microtome. Paraffin sections are more physically stable and superior to frozen sections in maintaining tissue morphology .with less damage. But due to the wax infiltration process, paraffin sections are not optimal for some staining processes. Histopathologist provide a diagnostic service for cancer; they handle the cells and tissues removed from suspicious „lumps and bumps‟ identify the nature of the abnormality and, if malignant, provide information to the clinician about the type of cancer, its grade and, for some cancers, its responsiveness to certain treatments. With the help of sophisticated imaging techniques,

biopsy tissue can now be obtained from previously inaccessible sites such as the pancreas or retroperitoneum (behind the peritoneum, the membrane lining the abdominal cavity). Tissue is then processed, usually overnight, before being examined under a microscope (30) .

2.5 Binding of Congo red by amyloid:

Congo red in alkaline 80% ethyl alcohol solution saturated with NaCl stains amyloid selectively. The distribution of the dye is very similar to that observed in serial sections stained with Highman's Congo red method. In sections stained with Congo red in aqueous or alcoholic solutions without differentiation all tissue structures are intensely colored, but only amyloid is dichroic. The rapid removal of dye by alkaline solutions from structures other than amyloid suggests ionic or salttype bonds between Congo red and these structures. In contrast, the binding of Congo red by amyloid from alkaline alcoholic solutions and the increase in intensity of staining upon addition of NaCl indicate a non-ionic type linkage between amyloid and dye (31) . The effects of deamination, acetylation and various oxidation procedures on the binding of Congo red indicate interaction of the dye with hydroxyl groups of amyloid. According to data on the direct dyeing of cellulose in textile dyeing, Congo red is adsorbed by hydrogen bonding between hydroxyl groups of the polysaccharide chains and the amino groups of the dye. It seems probable that a similar mechanism is involved in the binding of Congo red by amyloid. It is suggested that the selective staining of amyloid with Congo red is due to steric configurations of amyloid or one of its components which favor non-ionic binding of the eye (32) .

2.6 Previous studies:

On study conducted by Röcken et al., on 2002 on three cases of amyloidosis of the breast, two of which coincided with breast cancer. Patient no. 1, a 60-year-old woman, presented with two mass lesions measuring 2 cm in diameter, one in each breast. Histologically, a tubulo-lobular carcinoma was found in the left breast accompanied by vascular, interstitial, and periductal amyloid deposits; the lesion in the right breast consisted of amyloid deposits only. Patient no. 2, an 86-year-old 9 woman, presented with an ulcerated breast tumor measuring 5 cm in diameter on the left side. A poorly differentiated invasive ductal carcinoma was found in the mastectomy specimen, and it coincided with interstitial and vascular amyloid deposits. In both patients, tumor cells had invaded the amyloid deposits. Patient no. 3, a 73-year-old woman, presented with a mass measuring $5\times3\times3$ cm in her left breast. Fibrocystic changes, as well as interstitial and periductal amyloid deposits, were found histologically. In each case electron microscopy showed rigid, nonbranching fibrils of indefinite length and measuring 10–12 nm in diameter; these were consistent with amyloid fibrils. Clinical data, immunohistochemistry, and/or amino acid sequencing of the fibril proteins extracted from formalin-fixed and paraffin-embedded tissue specimens provided evidence that the amyloid deposits were of immunoglobulin light chain origin in all three cases (33) .A review of the literature revealed that κ-light chain has been described more frequently than λlight chain in the breast and that there are no specific clinical or radiological symptoms of amyloidosis affecting the breast; a diagnosis can be achieved only by histology (34) . A particularly substance by the authors termed "elastic amyloid" occurs in cases of mammary cancer and cutaneous basal-cell carcinomas. The substance displays some properties similar to those of classic amyloid, such as staining with Congored, responsiveness to methyl-violet, iodine green and Lugol's solution. It is however not birefringent after staining with

Congo-red and is stained by elastica stains. A maturation process of the material seems to develop which suggests similarities to changes observed in experimental amyloidosis. The relation of the "elastic amyloid" to immunological processes around nests of mammary cancer and basal cell carcinoma is discussed (35) . Among the 208 invasive breast cancer samples, SAA protein was found expressed in tumor cell in 44.2% (92/208) cases and expressed in macrophage in 62.5% 10 (130/208) cases. A positive correlation with a high correlation coefficient was found between SAA protein expression levels in tumor cells and macrophage (35) . One study published by Jin et al., on 2016 they detect positive role of serum amyloid A proteins on metastasis and immune biology in cancer, they concluded that; cancer triggers the systemic release of inflammatory molecules that support cancer cell metastasis and immune evasion. Notably, this biology shows striking similarity to an acute phase response that is coordinated by the liver. Consistent with this, a role for the liver in defining cancer biology is becoming increasingly appreciated. Understanding the mechanisms that link acute phase biology to metastasis and immune evasion in cancer may reveal vulnerable pathways and novel therapeutic targets. Herein, we discuss a link between acute phase biology and cancer with a focus on serum amyloid A proteins and their involvement in regulating the metastatic cascade and cancer immunobiology (36) . Systemic amyloidosis causes a variety of symptoms, such as fatigue, weight loss, anemia cardiac symptoms (congestive heart failure, arrhythmia), renal symptoms (nephrotic syndrome, kidney failure), gastrointestinal symptoms (malabsorption syndrome, macroglossia, hepatomegaly, splenomegaly), neurological symptoms (polyneuropathy, carpal tunnel syndrome, orthostatic hypotension, constipation, diarrhea, dysuria), and bleeding symptoms Examinations used to check for systemic amyloidosis include electrocardiography, echocardiography, blood

analysis (renal dysfunction, M protein, free light chain, autoimmune antibody, chronic inflammatory findings), urine analysis (Bence-Jones protein), nerve conduction test, bone marrow biopsy, and biopsy of sites suspected of amyloid deposition The diagnosis of amyloidosis is confirmed by Congo red staining which stains amyloid red, and the stained amyloid also shows green birefringence under a polarizing microscope . A report of 15 patients with amyloid tumor of the breast at the Mayo Clinic showed that amyloid tumor of the breast, when a manifestation of 11 systemic amyloidosis, is mostly found as a late presentation, and none of the patients with a localized amyloid tumor of the breast developed systemic amyloidosis (37) .

3.1 Study design:

This was a descriptive cross sectional study.

3.2 Study area:

This study was conducted in Almek Nemer University Hospitals in River Nile state, Northern of Sudan.

3.3 Study duration:

This study was done in period from August 2021 to February 2022

. 3.4 Study populations:

Populations involved in this study were Sudanese patients with adenocarcinoma

. 3.4.1 Inclusion criteria:

Cancerous patients with breast, prostate, ovarian and thyroid adenocarcinoma were recruited in this study.

3.4.2 Exclusion criteria:

Patients presented with other types of cancer, benign lesion patients were excluded in this study.

3.5 Study sample and sample size:

This study was included 240 formalin fixed paraffin embedded tissue blocks (FFPE). 200 Cancerous tissues were selected as case group, another 40 noncancerous benign FFPE were enrolled as control group.

3.6 Data Collection tools and variables:

Questionnaire sheet was used for collecting sociodemographic and other patient information. All sample data was also involved in questionnaire sheet. Amyloid was detected by using Congo red staining method.

3.7 Sample processing:

Tissue section measured 3 microns from each FFPE tissue block was obtained by using SLEE-MAINZ,-CUT4060 microtome. The cut section was spread in water bath, then put in coated glass slides, followed by drying in dry oven then all sections were deparaffinized in xylene and rehydrated through descending grades of ethanol, then rinsed in water. Then sections were stained by Congo red stain.

3.8 Congo red stain:

After hydration in water, each section was stained in Congo red solution for 15-20 minutes, rinsed in distilled water. Then differentiations take place quickly (5-10 dips) in alkaline alcohol solution, and then each section was rinsed in tap water for 1 minute, section was then counterstained with Mayer"s hematoxylin for 30 seconds, blued in tap water for 2 minutes. After that section was dehydrated through 95% alcohol, 2 changes of 100% alcohol 3 minutes each. The stained section was then cleared in xylene or xylene substitute, 2 changes, 3 minutes each. Finally section was mounted with resinous mounting medium DPX.

3.9 Result interpretations:

Stained slide was observed by microscope by lenses 10 x/0.25 and 40x/0.65. The presence of amyloid was indicated by the observing red extracellular amorphous substance.

3.10 Quality control:

Quality control was performed at each step and procedures during this study to ensure the reliable performance and correct reporting of results.

3.11 Reference management:

Citation was done by End Note reference manager program.
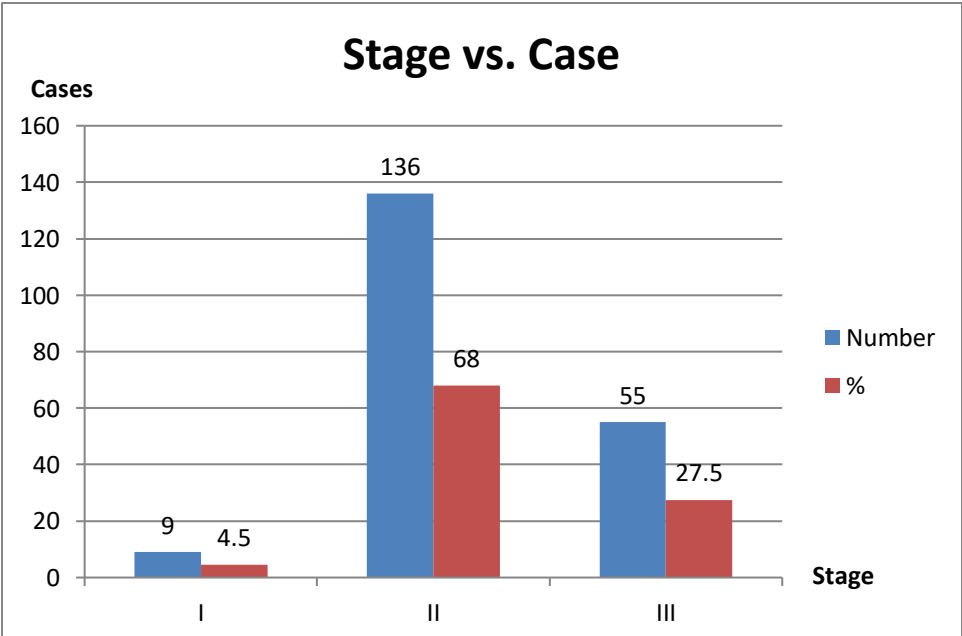
3.12 Data Analysis:

The data obtained from the sheet and the results of the laboratory analysis were analyzed using Microsoft Excel (2010) and Statistical Package for Social Science (SPSS) version 25. Chi Square was used to assess the intergroup significant, frequencies, percentages, mean and other variables were calculated and presented in form of figures and tables.

14 3.13 Ethical considerations:

The study was performed after taking permission from Histopathology and Cytology and Research Council at Faculty of Medical Laboratory Science and Faculty of Higher Study and Scientific research at Shendi University. Collection of samples was done after taking ethical acceptance from hospital administration. All benefits of this study were explained and published in the future with assurance on confidentiality

4. Result:

flowchart (1)

Show relation between stages and cases

Stage vs. Case

Cases

- 9 (4.5) — Stage I
- 136, 68 — Stage II
- 55, 27.5 — Stage III

Legend: ■ Number ■ %

Stage

flowchart (2): Show Compare between Age and cases
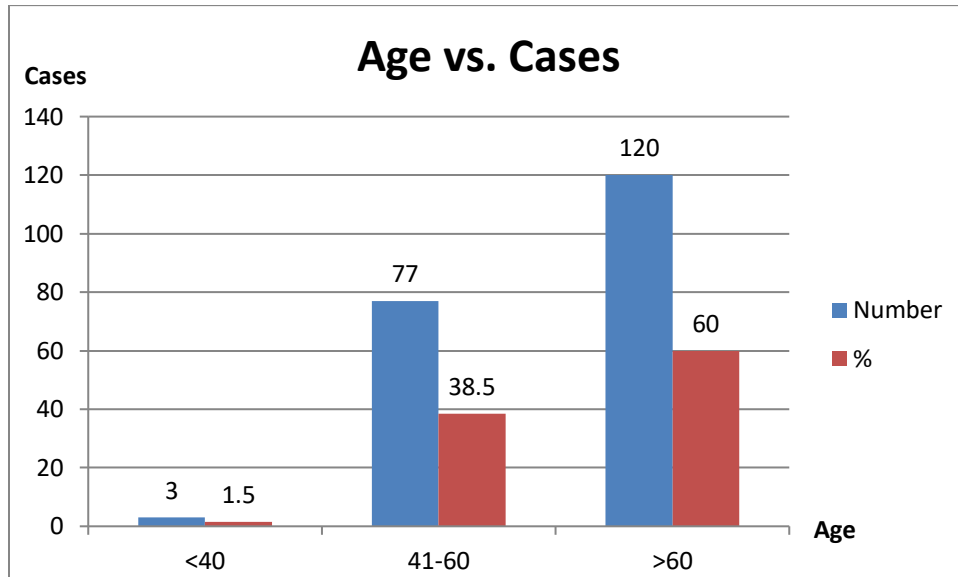
Table (1): Correlation of amyloid expression with adenocarcinoma.

Participants	Amyloid			P.value
Adenocarcinoma patients	Positive	Negative	Total	
Benign samples	15	36	51	0.0
Total	200	40	240	

Table (4.2) : Amyloid and cancer stage :

Cancer stage	postive	Negative	p.value
I	8	1	
II	124	13	
III	53	1	0.18
Total	185	15	

Table (3): Comparison between amyloid expression and adenocarcinoma organ.

		organ					P.value
		Breast	prostate	overy	thyroid	Total	
AMYLOID	Positive	159	13	9	4	185	
	negative	15	0	0	0	15	0.4
Total		174	13	9	4	200	

5.1. Discussion:

This was a descriptive cross sectional study included 200 FFPE adenocarcinoma tissues divided into 174 breast, 13 prostate, 9 ovarian and 4 thyroid samples. Common cancer in United States was breast cancer with 284,200 new cases of breast cancer expected in the United States in 2021. The next most common cancers are prostate cancer and lung cancer (38) . Regarding distribution among age groups in patients with adenocarcinoma, our study showed that; adenocarcinoma was most frequently observed among elderly people with an age over 60 years old. According to the most recent statistical data from NCI''s [surveillance epidemiology and end results (SEER)] program, the median age of a cancer diagnosis is 66 years (39). Another study concluded; prostate cancer is more likely to develop in older men and in non-Hispanic Black

men. About 6 cases in 10 are diagnosed in men who are 65 or older, and it is rare in men under 40. The average age of men at diagnosis is about 66 (40) . Another study summarized; between 1% and 2% of people will get thyroid cancer at some point during their lifetime. It affects three times as many women as men and is most common after age 30, though it can occur in any age group. Thyroid cancer is more likely to be aggressive in older adults (41) . Similar result was also observed by DeSantis et al., who summarized that; the median age at diagnosis is 62 years for breast cancer, 67 years for colorectal cancer, 71 years for lung cancer, and 66 years for prostate cancer (42) . Concerning relation between genders of patient with the development of adenocarcinoma; our study indicated that adenocarcinoma was dominant among females and this due to the involvement of breast and ovarian cancer. Despite this; in study achieved by Yang et al., on 2012 they concluded that; it is possible that a difference in the expression of estrogen, or its signaling axes, may contribute to this gender bias (43) . 22 In respect to the expression of amyloid among adenocarcinoma samples, our study showed that; there was strong statistical significant different and that was mean patient with adenocarcinoma strongly under risk of developed amyloid, and this matched with the fact that amyloid being expressed among patients with chronic diseases. In report on three cases of amyloidosis of the breast, two of which coincided with breast cancer. Patient no. 1, a 60-year-old woman, presented with two mass lesions measuring 2 cm in diameter, one in each breast. Histologically, a tubulo-lobular carcinoma was found in the left breast accompanied by vascular, interstitial, and periductal amyloid deposits; the lesion in the right breast consisted of amyloid deposits only. Patient no. 2, an 86-year-old woman, presented with an ulcerated breast tumor measuring 5 cm in diameter on the left side. A poorly differentiated invasive ductal carcinoma was found in the mastectomy specimen, and it coincided with interstitial and

vascular amyloid deposits. In both patients, tumor cells had invaded the amyloid deposits. Patient no. 3, a 73-year-old woman, presented with a mass measuring 5×3×3 cm in her left breast. Fibrocystic changes, as well as interstitial and periductal amyloid deposits, were found histologically. In each case electron microscopy showed rigid, non-branching fibrils of indefinite length and measuring 10–12 nm in diameter; these were consistent with amyloid fibrils. Clinical data, immunohistochemistry, and/or amino acid sequencing of the fibril proteins extracted from formalin-fixed and paraffin-embedded tissue specimens provided evidence that the amyloid deposits were of immunoglobulin light chain origin in all three cases. A review of the literature revealed that κ-light chain has been described more frequently than λ-light chain in the breast and that there are no specific clinical or radiological symptoms of amyloidosis affecting the breast; a diagnosis can be achieved only by histology (42) . This finding also similar to those findings in other studies performed by Malle E, Sodin-Semrl S, Kovacevic A., they concluded that; amyloid was present among cancer patients (44) . 23 Regarding association between stage of cancer and amyloid expression, this study indicated there was not statistical significant difference as the p value was recorded more than 0.05, and that mean amyloid expression was not associated with the stage of cancer. Previous studies indicated that; serum amyloid A (SAA) was determined in 160 patients with cancer. Active disease was associated with high titer compared with the titer in non-active condition (31.8 v 5.8 micrograms/ml, respectively; p = 0.0002). SAA value showed a direct correlation with the stage of the disease: it was lowest at stages 1 and 2 and highest at the metastatic stage 4 (stage 1 v 4, p = 0.001; stage 2 v 3, p = 0.05). Cancers of the lung and unknown primary site were characterized by highly increased SAA concentration. Initial SAA value had prognostic significance: a value below 10 micrograms/ml correlated

with survival advantage, whereas a higher initial value indicated a greater likelihood of a poor outcome (actuarial survival analysis p less than 0.001). When stage was accounted for, initial SAA value had significant prognostic bearing on survival of patients with advanced disease (stages 3 and 4) but not on that of patients with limited disease (stages 1 and 2). Serial testing showed good concordance between changes in SAA titer and clinical course (45) .

24 5.2 Conclusion:

In this study, we concluded that: • Adenocarcinoma commonly developed in elderly. • Amyloid frequently expressed in adenocarcinoma patients. • The amyloid incidence was increased as the cancer stage progress.

25 5.3 Recommendations:

On the base of the obtained result we concluded: • Detection of amyloid in adenocarcinoma tissue may indicate progression of cancer. • Further studies with larger sample size involved all adenocarcinoma tissues should be carried out targeting amyloid subtypes. • For further future studies, we recommend using advance technique to detect specificity and sensitivity of Congo red stain.

• References:

1. CLARK JR, Fridman TR, Odell MJ, Brierley J, Walfish PG, Freeman JL. Prognostic variables and calcitonin in medullary thyroid cancer. The Laryngoscope. 2005;115(8):1445-50
2. . WESTERMARK Per. Localized AL amyloidosis: a suicidal neoplasm?. Upsala journal of medical sciences. 2012;117(2): 244-50.

3. XIANG L, Xin W, Zengqi T, Si C. Role of glycans in cancer cells undergoing epithelial–mesenchymal transition. Frontiers in oncology. 2016; 6: 33.
4. 4. National Cancer Institute. 17 September 2007. Retrieved 28 March 2018
5. SAINI A, Manish K, Shailendra Bhatt, Vipin S, Anuj M. Cancer causes and treatments. Int J Pharm Sci & Res. 2020; 11(7):3109-22.
6. MIKAMI Y, McCluggage W. Endocervical glandular lesions exhibiting gastric differentiation: an emerging spectrum of benign, premalignant, and malignant lesions. Advances in anatomic pathology. 2013; 20(4): 227-37
7. CAMPBELL JL. Elemental Analysis and Imaging by Proton-Induced XRay Emission. Quantitative Microbeam Analysis. 1993; 40: 303.
8. PANDA UN. Concise Pocket Medical Dictionary. 1st edition, JP Medical Ltd, Anshan 2008.
9. SHELDRAKE AR. The ageing, growth and death of cells. Nature. 1974; 250(5465):381-5.

WYNDER EL, Muscat JE. The changing epidemiology of smoking and lung cancer histology. Environmental health perspectives. 103(8):143 -8.
11.CHEN W, Rongshou Z, Peter DB, Siwei Z, Hongmei Z, Freddie B, Ahmedin J, Xue QU, Jie H. Cancer statistics in China, 2015. CA: a cancer journal for clinicians. 2016; 66(2):115-32. 27

12.WANG F, Xiang S, Ingrid M, Tuya P, Ingrid AM, Zhigang Y, Wei Z, Christina EB, Xiao-Ou S. Overall mortality after diagnosis of breast cancer in men vs women. JAMA oncology. 2019; 5(11):1589-96.

13.BRAY F, Jacques F, Isabelle S, Rebecca LS, Lindsey AT, Ahmedin J. Global cancer statistics 2018: GLOBOCAN estimates of incidence

and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018; 68(6): 394-424.

14.EWERTZ M, Jensen AB. Late effects of breast cancer treatment and potentials for rehabilitation. Acta Oncologica. 2011; 50(2): 187-93.

15.MILLER KD, Rebecca LS, Chun CL, Angela BM, Joan LK, Julia HR, Kevin DS, Rick A, Ahmedin J. Cancer treatment and survivorship statistics, 2016. CA: a cancer journal for clinicians. 2016; 66(4): 271-89.

16.CHORNOKUR G, Ernest KA, Joellen MS, Catherine MP. Global ovarian cancer health disparities. Gynecologic oncology. 2013; 129(1): 258-264.

17.FIGGE JJ. Epidemiology of thyroid cancer. Thyroid Cancer: A comprehensive Guide to Clinical Management, 2nd edition, Humana Press. 2006; 9-13.

18.KHOOR A, Colby, Thomas V. Amyloidosis of the lung. Archives of pathology & laboratory medicine. 2017; 141(2): 247-54.

19.DUBREY SW; Hawkins PN, Falk RH. Amyloid diseases of the heart: assessment, diagnosis, and referral. Heart. 2011; 97(1): 75-84.

20.Fandrich M. On the structural definition of amyloid fibrils and other polypeptide aggregates. Cellular and Molecular Life Sciences. 2007; 64.16: 2066-78.

21.VAN HJ, Pieter W, Lambert PW, Robert MW, Marcel MV. Heparan sulphate proteoglycans in Alzheimer's disease and amyloid-related disorders. The Lancet Neurology. 2003; 2(8): 482-92. 28

22.BENSON MD, Kincaid JC. The molecular biology and clinical features of amyloid neuropathy. Muscle & Nerve: Official Journal of the

American Association of Electrodiagnostic Medicine. 2007; 36(4): 411-23.

23.MUCHOWSKI, Paul J. Protein misfolding, amyloid formation, and neurodegeneration: a critical role for molecular chaperones?. Neuron. 2002; 35(1): 9-12.

24.MAKARAVA N, Gabor GK, Regina S, Irina a, Herbert B, Robert G, Ilia V. Genesis of mammalian prions: from non-infectious amyloid fibrils to a transmissible prion disease. PLoS pathogens. 2011; 7(12): e1002419.

25.COBB NJ, Surewicz WK. Prion diseases and their biochemical mechanisms. Biochemistry. 2009; 48(12): 2574-85.

26.SELKOE DJ. Cell biology of the amyloid beta-protein precursor and the mechanism of Alzheimer's disease. Annual review of cell biology.1994; 10(1): 373-403.

27.TOYAMA BH, Weissman JS. Amyloid structure: conformational diversity and consequences. Annual review of biochemistry. 2011; 80: 557-585.

28.ALIYAN A, Cook NP, Marti AA. Interrogating amyloid aggregates using fluorescent probes. Chemical reviews. 2019; 119(23): 11819-56.

29.KAYED R, Glabe CG. Conformation-dependent anti-amyloid oligomer antibodies. Methods in enzymology. 2006; 413: 326-44.

30.GRAHAM L, Orenstein J M. Processing tissue and cells for transmission electron microscopy in diagnostic pathology and research. Nature protocol. 2007; 2(10): 2439-50.

31.PUCHTLER H, Sweat F, Levine M. On the binding of Congo red by amyloid. Journal of Histochemistry & Cytochemistry. 1962; 10(3): 355-64.

32.HELLQUIST H, Jan O, Hannibal S, Lars M. Amyloidosis of the larynx. Acta oto-laryngologica. 1979; 88(1-6): 443-50.

33.ROCKEN C, Kronsbein H, Sletten K, Albert R, Roland B. Amyloidosis of the breast. Virchows Arch. 2002; 440(5): 527–35.

34.WAKUGAWA M, Yoko O, Haruko H. A Case of Adenoid Basal Cell Epithelioma with Amyloid Deposition. Nishi Nihon Hifuka. 1994; 56(6):1154-57.

35.YANG M, Fangfang L, Kayoko H, Jinko S, Xiaoying F, Li Z, Lanjing Z, Li F, Zhongsheng T, Kiichi H. Serum amyloid A expression in the breast cancer tissue is associated with poor prognosis. Oncotarget. 2016; 7(24): 35843.

36.JIN H, Pinheiro PS, Xu J, Amei A. Cancer incidence among A sian A merican populations in the U nited S tates, 2009–2011. International journal of cancer. 2016;138(9):2136-45.

37.HAYAT MJ, Howlader N, Reichman ME, Edwards BK. Cancer statistics, trends, and multiple primary cancer analyses from the Surveillance, Epidemiology, and End Results (SEER) Program. The oncologist. 2007;12(1):20-37.

38.MILLER KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, Stein KD, Alteri R, Jemal A. Cancer treatment and survivorship statistics, 2016. CA: a cancer journal for clinicians. 2016;66(4):271-89.

39.TUTTL RM. Risk-adapted management of thyroid cancer. Endocrine Practice. 2008;14(6):764-74.

40.DESANTIS CE, Lin CC, Mariotto AB, Siegel RL, Stein KD, Kramer JL, Alteri R, Robbins AS, Jemal A. Cancer treatment and survivorship statistics, 2014. CA: a cancer journal for clinicians. 2014;64(4):252-71.

41.YANG H, Sukocheva OA, Hussey DJ, Watson DI. Estrogen, male dominance and esophageal adenocarcinoma: is there a link?. World Journal of Gastroenterology: WJG. 2012;18(5):393. 30

42.ROCKEN C, Kronsbein H, Sletten K, Roessner A, Bässler R. Amyloidosis of the breast. Virchows Archiv. 2002;440(5):527-35.

43.BIRAN H, Friedman N, Neumann L, Pras M, Shainkin-Kestenbaum R. Serum amyloid A (SAA) variations in patients with cancer: correlation with disease activity, stage, primary site, and prognosis. Journal of clinical pathology. 1986;39(7):794-7.

44.LIN, Hai-yingjie, et al. The prognostic value of serum amyloid A in solid tumors: a meta-analysis. Cancer Cell International, 2019, 19.1: 1-10.

45. BIRAN, H., et al. Serum amyloid A (SAA) variations in patients with cancer: correlation with disease activity, stage, primary site, and prognosis. Journal of clinical pathology, 1986, 39.7: 794-797.

Appendix-1:

- **Preparation of Congo red solutions:**

1. **Congo red:**

   - Congo red (Sigma, Cat# C-6277) -------- 0.5 g.

   - 50% Alcohol --------------------------------100 ml.

2. **1% Sodium Hydroxide:**

   - Sodium hydroxide ------------------------ 1 g.

   - Distilled water----------------------------- 100 ml.

3. **Alkaline Alcohol Solution:**

   - 1% Sodium hydroxide ---------------------- 1 ml.

   - 50% alcohol ---------------------------------- 100 ml.

- **Preparation Mayer's Hematoxylin :**
  - Hematoxylin---------------------------------- 1 g.
  - Distilled water-------------------------------- 1000 ml.
  - Aluminum potassium sulfate---------------- 50 g.
  - Sodium iodate--------------------------------- 0.2 g.
  - Glacial acetic acid--------------------------- 20 ml.

Appendix-2:

- **Reagents and equipment:**
- Distilled water.
- Alcohols.
- Xylene.
- DPX.
- Filter papers.
- Frosted end microscope slides.
- Slide storage boxes.
- Soft pencil.
- Light microscope.
- Cover glass.
- Coplin jars.
- Rotary microtome.
- Dry oven.
- Water bath.

Appendix-3:

<div align="center">

Questionnaire

Shendi University

Faculty of Graduate Studies and Scientific Research Detection of Amyloid among Adenocarcinoma Tissues in Shendi Town 2021

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1-   No. ……………………………………………………………………

2-   Gender: Male ( )   Female( )

3-   Age: 18-40 ( )   41-60 ( )   over 60 ( )

4-   Organ: Breast ( )   Prostate   ( ) Ovary   ( )   Thyroid ( )

5-   Cancer stage: Stage I ( )Stage II ( )   Stage III ( )   Stage IV ( )

6-   Amyloid result: Positive ( )   Negative ( )

7-   Amyloid intensity: Weak ( )   Moderate ( )   Strong ( )

Appendix-4:

- **Micrographs:**