ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 3, Issue 4, pp: (39-46), Month: October - December 2015, Available at: www.researchpublish.com

Evaluation of Mucin Histo-Chemical Pattern in Breast, Colonic, Ovarian and Lung Adenocarcinomas

MUAZ OSMAN FAGARE

Assistant Professor Of Histopathology & Cytopathology
Albaha University, Faculty of Applied Medical Sciences, Laboratory Medicine Department, Albaha, KSA
Omdurman Islamic University, Faculty of Medical Laboratory Sciences, Department of Histopathology & Cytopathology
Khartoum, Sudan

Abstract: **This is a retrospective observational case control study aimed to evaluate mucins histo-chemical pattern in breast, colonic, ovarian, and lung adenocarcinomas. The study was conducted in Khartoum state hospitals, from December 2013, to April 2015. Sixty two formalin fixed paraffin embedded tissue blocks from patients with adenocarcinoma used as case samples and twenty formalin fixed paraffin embedded tissue blocks from corresponding normal tissues were used as controls samples. Tissue sections were stained by Mayer's Haematoxylin and PAS-diastase, Phenylhydrazine-PAS, Alcian blue, combined alcian blue-PAS, and combined aldehyde fuchsin-alcian blue techniques. Age group of 61+ were the most observed ages constituted 29(46.8%), of which females were the major subject which constituted 37(59.7%). Breast adenocarcinoma was the most observed which constituted 23(37.1%). Carboxylated Acid mucin was the predominant type with 29(46.8%), followed by neutral, and sulfated acid mucins which constituted 17(27.4%), and 16(25.8%) respectively. The most observed mucin in colonic and breast adenocarcinomas was carboxylated acid mucin which constitute 8(12.9%) , 7(11.2%) respectively, followed by neutral mucin among breast adenocarcinoma, then sulfated acid mucin among lung adenocarcinoma which constitute 5(8%), and 4(6.4%) respectively. Its concluded that mucin histochemical patterns have valuable, cost-effective, and important role in the diagnosis of adenocarcinomas. Acid mucin found to be the most predominant mucin in breast, colonic, ovarian, and lung adenocarcinomas. Its recommended to consider mucin histochemistry in diagnosis of adenocarcinomas. Mucin genes immune-expression techniques will be valuable diagnostic tool to discriminate primary from metastatic tumours. And it can elucidate the patho-physiological significance of mucin in adenocarcinomas.**

Keywords: **Mucin, Neutral Mucin, Acid Mucin, Adenocarcinoma, Sialomucin.**

1. INTRODUCTION

Mucus is the slimy and viscoelastic secretion that covers the epithelial surface of tubular organs such as tracheobronchial, gastrointestinal, reproductive tracts, and other specialized organs. In the body mucus is secreted by connective tissue cells and specialized epithelial cells known as goblet cells and are abundant in the epithelium of the gastrointestinal, respiratory and reproductive tracts, and the secretory epithelial surfaces of the liver, pancreas, gall bladder, kidney, salivary, and lacrimal glands [1]. Mucus secretions adhere to the epithelial surface and serve as a protective diffusion barrier against harmful substances and act as a lubricant between the lumen and the cell surface [2],[3]. The composition of mucus varies with its location and pathophysiological conditions [4],[5], but normally mucus is composed of water, inorganic salts, immunoglobulins, secreted proteins, and mucins. 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 [6],[7]. 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 signalling [8],[9],[10],[11]. In general, mucins are large (well over 106 Daltons) glycoproteins [12],[13] composed of 75% carbohydrate and 25% amino acids linked via O-glycosidic bonds between N-acetylgalactosamine and serine/threonine/proline (Ser-Thr-Pro) residues. The hallmark of the mucin family is the large and polymorphic central domain, which is composed of a variable number of tandem repeats (VNTR) rich in Ser-Thr-Pro residues that can be modified with a large number of O-linked oligosaccharides and a few N-glycan chains [14],[15],[16]. Till now, about twenty mucin (MUC) genes have been identified and these are designated as MUC1-2, MUC3A, MUC3B, MUC4, MUC5B, MUC5AC, MUC6-9, MUC11-13, MUC15-17, and MUC1921 [3]. Based on physiological fate and nature, mucins are categorized into three subgroups: ''secreted/gel-forming'', ''membrane bound'', and ''soluble'' mucins. The first group is composed of strictly secreted, gel-forming mucins including MUC2, MUC5AC, MUC5B, MUC6, and MUC19, which form oligomeric structures. The second group is composed of mucins either tethered at the cell surface or secreted in the mucus. The mucins of this group, MUC1, MUC3A, MUC3B, MUC4, MUC11, MUC12, MUC13, MUC15, MUC16, MUC17, MUC20, and MUC21, harbor a transmembrane domain, a short cytoplasmic tail (CT), and an extensive extracellular domain. The third subgroup, composed of MUC7, MUC8, and MUC9, are exclusively secreted non-gel forming mucins. Adenocarcinoma is a type of cancer that arise from epithelial tissue that has glandular origin or glandular characteristics throughout the body. It can occur in many different places in the body, and is most prevalent in the following cancer types; breast, colon, ovaries, and lung carcinomas. Well differentiated adenocarcinomas tend to resemble the glandular tissue that they are derived from, while poorly differentiated adenocarcinomas may not.

2. MATERIALS AND METHODS

2.1. Study design:

This is a retrospective observational case control study aimed to evaluate the histochemical pattern of mucins histo-chemistry in breast, colonic, ovarian, and lung adenocarcinomas, using different techniques of special stains. The study was conducted in Khartoum state hospitals, during the period from October 2013, to April 2015.

2.2. Study samples:

Sixty two formalin fixed paraffin embedded tissue blocks from patients diagnosed as having adenocarcinoma used as case samples and twenty formalin fixed paraffin embedded tissue blocks from corresponding normal tissues were used as controls samples. the tissue sections were stained accordingly by Mayer's Haematoxylin and different special stains techniques.

2.4. Sample Processing:

Ten thin sections 3-5 u were cut by rotary microtome from case and control sample blocks, tissue sections then transferred to the hot plate for 15 minutes to dissolve the excess wax.

2.5. Staining Procedures

2.5. PAS-technique with diastase:

Sections were de-waxed in 3 changes of xylene for 10 minutes, then rehydrated with descending grades of alcohols (absolute 3 changes for 6 minutes, 90% alcohol 2 changes for 6 minutes, 70% alcohol for 6 minutes), then bring them to distilled water, duplicate sections will be brought to distilled water for diastase treatment , then section were treated with periodic acid for 5 minutes, then section were washed with several changes of distilled water, then covered with Schiff's solution for 15 minutes, then washed in running tap water for 5-10 minutes, to stain nuclei, sections were stained with Harris's haematoxylin from 5-7 minutes 'differentiated as appropriate' in acid-alcohol and blued in running tap water for 10 minutes, then section were rinsed in absolute alcohol, cleared in xylene, and mounted in D.P.X .

2.5.1. Phenylhydrazine-PAS technique for neutral mucin:

Test and positive control sections were de-waxed in 3 changes of xylene for 10 minutes, then rehydrated with descending grades of alcohols (absolute 3 changes for 6 minutes, 90% alcohol 2 changes for 6 minutes, 70% alcohol for 6 minutes), then bring them to distilled, all sections were treated with periodic acid for 5 minutes, then section were washed with several changes of distilled water, one positive control section and one test section were treated with phenylhydrazine for 1 hour at room temperature, duplicate sections were allowed to remain in distilled water, then section were washed in running tap water for 5 minutes, section were treated with Schiff's solution for 15 minutes followed by washing 5-10

minutes, to stain nuclei, sections were stained with Harris's haematoxylin from 5-7 minutes 'differentiated as appropriate' in acid-alcohol and blued in running tap water for 10 minutes, then section were rinsed in absolute alcohol, cleared in xylene, and mounted in D.P.X .

2.5.2. Alcian blue:

Sections were de-waxed in 3 changes of xylene for 10 minutes, then rehydrated with descending grades of alcohols (absolute 3 changes for 6 minutes, 90% alcohol 2 changes for 6 minutes, 70% alcohol for 6 minutes), then bring them to distilled water, then sections were stained in alcian blue stain dissolved in (3% acetic acid) to yield (2.5) pH, for 5 minutes, then sections were blotted in filter paper, then sections were counter stained with 0.5% aqueous neutral red for 2-3 minutes, then washed in water, rinsed in absolute alcohol, cleared in xylene and mounted in D.P.X.

2.5.3. Combined alcian blue-pas technique:

Sections were de-waxed in 3 changes of xylene for 10 minutes, then rehydrated with descending grades of alcohols (absolute 3 changes for 6 minutes, 90% alcohol 2 changes for 6 minutes, 70% alcohol for 6 minutes), then bring them to distilled water, then section were stained in alcian blue dissolved in (3% acetic acid) for 5 minutes, then washed in distilled water, then covered with (1% periodic acid) for 5 minutes, then rinsed in distilled water, then sections were covered with Schiff's solution for 15 minutes, then washed in running tap water for 5-10 minutes, then nuclei were stained lightly with Mayer's haematoxylin for 5-7 minutes , then sections were blued in running tap water for 5-10 minutes, rinsed in absolute alcohol, cleared in xylene, and mounted in D.P.X.

2.5.4. Combined aldehyde fuchsin-alcian blue:

Test and positive sections were de-waxed in 3 changes of xylene for 10 minutes, then rehydrated with descending grades of alcohols (absolute 3 changes for 6 minutes, 90% alcohol 2 changes for 6 minutes, 70% alcohol for 6 minutes), then sections were stained with aldehyde fuchsin solution for 20 minutes, then rinsed in 70% alcohol, then in water, sections were stained with alcian blue for 5 minutes, then rinsed in water, then dehydrated in absolute alcohol for 3 minutes, cleared in xylene, and mounted in D.P.X.

2.6. Statistical analysis:

Analysis was performed using statistical software SPSS version 22 (Statistical package for the Social Sciences). analyses were done such as; descriptive statistics, frequencies, cross tabulation.

2.7. Ethical considerations:

- The aims and benefits of this study were explained to the participants.
- Informed consents were obtained from all members who involved in this study.

2.8. Method of data collection:

Data concerning patients involved in this study such as age, sex, and the results of tissue biopsies diagnosed were collected by check list method.

3. RESULTS AND OBSERVATIONS

In this study, the mucin histochemical pattern in breast, colonic, ovarian, and lung adenocarcinomas were evaluated. These evaluation were assessed among 62 patients referred to different hospitals in the Khartoum State, their ages ranging from 30 to 77 with mean age of 58 years old. As shown in table (1,5), and figure (1,5), the majority of the study population age were among the age group of 61+ which constituted 29(46.8%), followed by the age group of 46-61, then 30-45 years old which constituted (32.2%), and (21%) respectively. As shown in table (2) and figure (2), two groups of individuals were classified according to their gender yields, the majority of the study populations were among females group which constituted 37(59.7%), and the male group constituted 25(40.3%).

Table (3) and figure (3) show the distribution of the study population by malignancy type. Breast adenocarcinoma was the major type of malignancy among the study population which constituted 23(37.1%), followed by colonic, ovarian, and lung adenocarcinomas which constituted 19(30.6%), 11(17.8%), 9(14.5%) respectively. Tables (4), and figure (4), represent the distribution of the study population by the mucin type, acid mucin carboxylated type was the most observed with 29(46.8%), followed by neutral mucin and acid mucin sulfated type which constituted 17(27.4%), and 16(25.8%)

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 3, Issue 4, pp: (39-46), Month: October - December 2015, Available at: www.researchpublish.com

respectively. Table (5), and figure (5) represent the distribution of the study population by malignancy and age, the majority of the study population were among the adenocarcinoma of the breast and their age group were among the of 61+ which constituted 18(29%), followed by colonic adenocarcinoma with age group of 46-61and 61+ with the same frequency which constituted 7(11.3%), followed by the ovarian adenocarcinoma with group age of 46-61 which constitute 4(6.4%).Table (6), and figure (6), show the distribution of the study population by malignancy and mucin type, the most observed mucin was acid mucin (carboxylated type) and that was among colonic and breast adenocarcinoma which constitute 8(12.9%) , 7(11.2%) respectively. Followed by neutral mucin among breast adenocarcinoma, then acid mucin (sulfated type) among lung adenocarcinoma which constitute 5(8%), and 4(6.4%) respectively.

TABLE 1: Distribution of the study population by age

Grouped age	frequency	Percentage
30-45	13	21%
46-61	20	32.2%
61+	29	46.8%
Total	62	100%

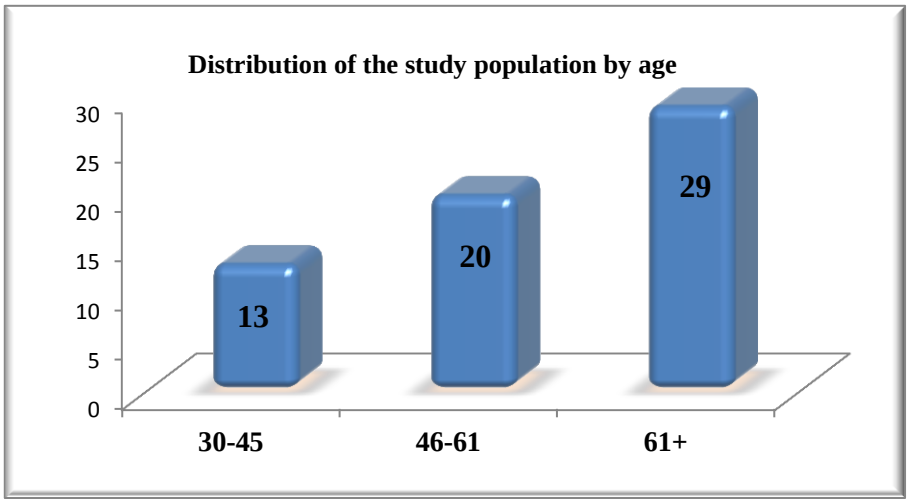

Fig. 1. Distribution of the study population by age

TABLE 2. Distribution of the study population by gender

Gender	frequency	Percentage
Female	37	59.7%
Male	25	40.3%
Total	62	100%

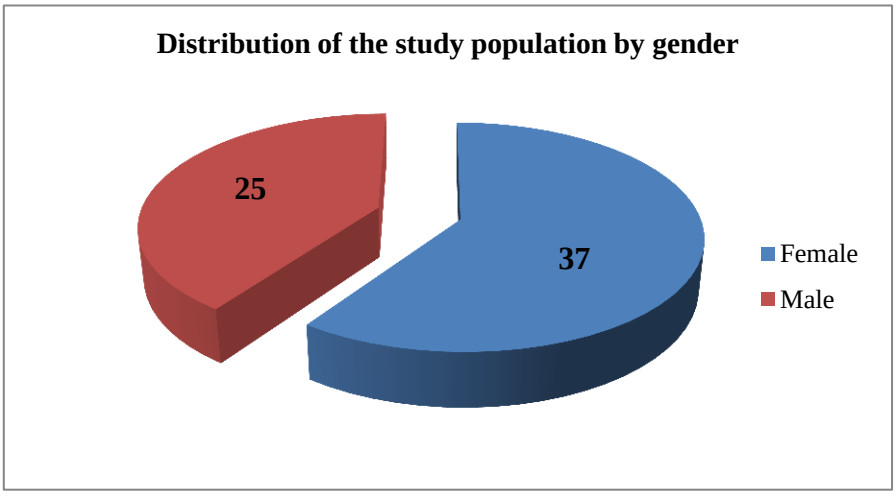

Fig. 2. Distribution of the study population by gender

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 3, Issue 4, pp: (39-46), Month: October - December 2015, Available at: www.researchpublish.com

TABLE 3. Distribution of the study population by type of malignancy

Type of malignancy	frequency	Percentage
Breast Ca	23	37.1%
Colon Ca	19	30.6%
Ovarian Ca	11	17.8%
Lung Ca	09	14.5%
Total	62	100%

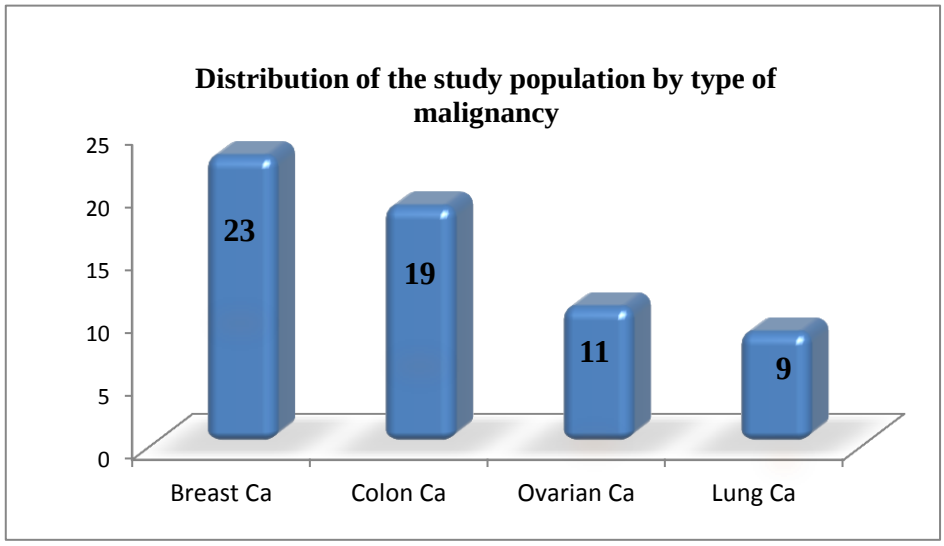

Fig. 3. Distribution of the study population by type of malignancy

TABLE 4. Distribution of the study population by the mucin type

Type of mucin	frequency	Percentage
Acid mucin (carboxylated)	29	46.8%
Neutral mucin	17	27.4%
Acid mucin (sulfated)	16	25.8%
Total	62	100%

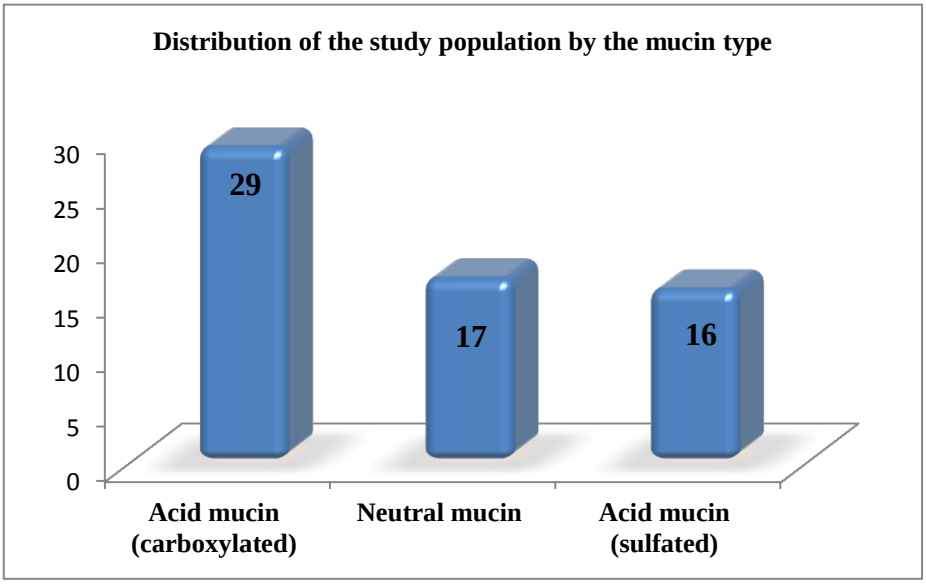

Fig. 4. Distribution of the study population by the mucin type

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 3, Issue 4, pp: (39-46), Month: October - December 2015, Available at: www.researchpublish.com

TABLE 5. Distribution of the study population by malignancy and age

	Breast Ca		Colon Ca		Ovarian Ca		Lung Ca		Total	
	NO	%	NO	%	NO	%	NO	%	NO	%
30-45	2	3.2%	5	801%	4	6.4%	2	3.2%	13	21%
46-61	3	4.8%	7	11.3%	6	9.6%	4	6.4%	20	32.2%
61+	18	29%	7	11.3%	1	1.6%	3	4.8%	29	46.8%
Total	23	37.1%	19	30.6%	11	17.8%	9	14.5%	62	100%

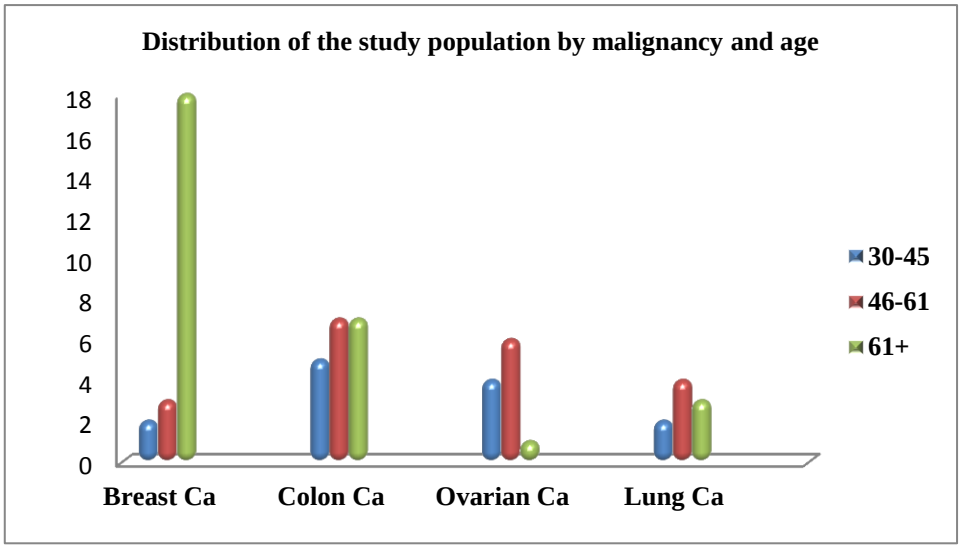

Fig. 5. Distribution of the study population by malignancy and age

TABLE 6. Distribution of the study population by malignancy and mucin type

	Neutral Mucin			Sulphated Mucin			Carboxylated Mucin		
	Strong	Moderate	Weak	Strong	Moderate	Weak	Strong	Moderate	Weak
Breast.Ca.	6(9.7%)	3(4.8%)	1(1.6%)	0(0%)	0(0%)	2(3.2%)	7(11.3%)	4(6.4%)	0(0%)
Colon.Ca.	3(4.8%)	0(0%)	0(0%)	3(4.8%)	3(4.8%)	0(0%)	8(12.9%)	2(3.2%)	0(0%)
Ovarian.Ca.	3(4.8%)	1(1.6%)	0(0%)	0(0%)	1(1.6%)	0(0%)	3(4.8%)	2(3.2%)	1(1.6%)
Lung.Ca.	0(0%)	1(1.6%)	0(0%)	4(6.4%)	2(3.2%)	0(0%)	0(0%)	2(3.2%)	0(0%)

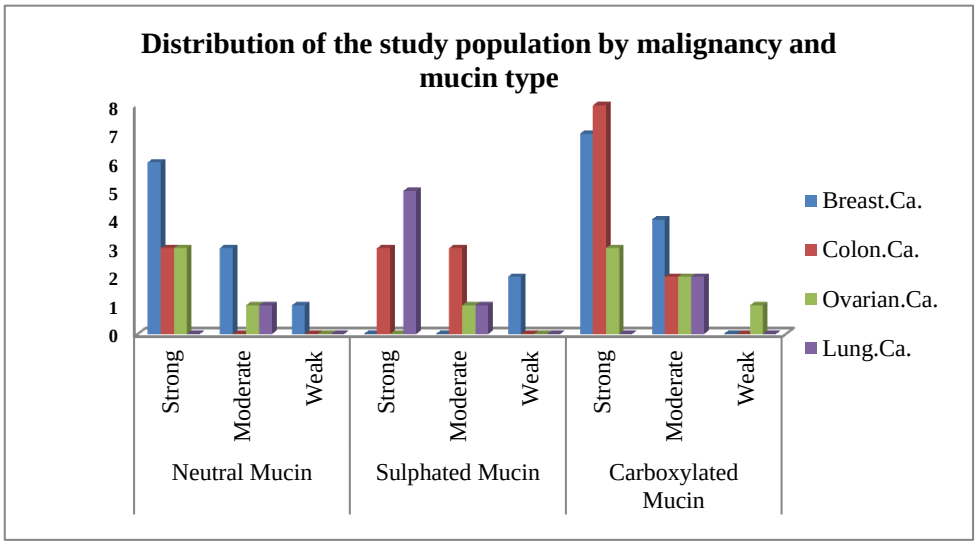

Fig: 6. Distribution of the study population by malignancy and mucin type

5. DISCUSSION

Mucin is a high molecular weight glycoprotein that is synthesized, stored and secreted by connective tissue and epithelial mucosal cells, especially the goblet cells [17]. In normal tissues, mucins seem to exhibit tissue- and cell-specific histochemistry patterns of expression. The patterns of distribution exhibited might be quite complex, with several different mucins often expressed in the same organ and, at times, the same cell [18]. However, under pathologic conditions this distinct expression patterns be modified. In adenocarcinomas, dys-regulation of mucin expression can occur, including increased, loss of, and aberrant expression of various mucin glycoproteins[19] .This study evaluate mucin histochemical pattern in breast, colonic, ovarian, and lung adenocarcinomas. Out of 62 (100%) patients with breast, colonic, ovarian and lung adenocarcinomas, breast adenocarcinoma was the most frequently observed type among the study population, this finding support the result by the study [20] which find that breast cancer was the most commonly diagnosed cancer during the study period .The present study showed that acid mucin was the most predominant type of mucin observed among samples this finding support many studies by [21,22] these studies observed the predominance of acid mucins over neutral mucins.The present result show that, acid mucin carboxylated type was the most encountered type in the colonic adenocarcinoma samples this finding support the result by the study[23] which observed a slightly predominance of sialomucins over other type of mucins. During this study, in colonic adenocarcinoma samples acid mucin carboxylated type found to be expressed more than neutral mucin, this finding are consistent with the results of previous study [24] which observed the predominant of sialomucins than neutral mucin in colonic adenocarcinoma.

6. CONCLUSION AND RECOMMENDATIONS

On the basis of this study and review of other studies we conclude that:

1. Patterns of mucin histochemistry can play valuable, cost-effective and important role in the diagnosis adenocarcinomas.
2. Acid mucin found to be the most predominant expressed mucin in breast, colonic, ovarian, and lung adenocarcinomas.
3. Carboxylated acid mucin found to be the most observed mucin in breast, and colonic adenocarcinomas.
4. Neutral mucins were observed quit more frequently in breast, and ovarian adenocarcinomas.

Recommendations:

1. Its recommend that mucin histochemistry should be considered in the diagnosis and differentiation of adenocarcinomas.
2. It's recommended to accompany Immune-expression tests of mucin gene which will be more valuable and helpful diagnostic tool and it can elucidate the patho-physiological significance of mucin in adenocarcinomas.
3. Further studies at molecular level of mucin gene should be conducted to use it as marker to discriminate primary from metastatic tumours.

REFERENCES

[1] Forstner, J. F. (1978). Intestinal mucins in health and disease. Digestion 17, 234–263.

[2] Hollingsworth, M. A. and Swanson, B. J. (2004) Mucins in cancer: protection and control of the cell surface. Nat Rev 4, 45 60.

[3] Moniaux, N., Escande, F., Porchet, N., Aubert, J. P., and Batra, S. K. (2001) Structural organization and classification of the human mucin genes. Front Biosci 6, D1192–D1206.

[4] Brockhausen, I. (1999). Pathways of O-glycan biosynthesis in cancer cells. Biochim Biophys Acta 1473, 67–95.

[5] Lamblin, G., Degroote, S., Perini, J. M., Delmotte, P., Scharfman, A., Davril, M., Lo-Guidice, J. M., Houdret, N.,Dumur, V., Klein, A., and Rousse, P. (2001). Human airway mucin glycosylation: a combinatory of carbohydrate determinants which vary in cystic fibrosis. Glycoconj J 18, 661–684.

[6] Carraway, K. L. and Hull, S. R. (1989). O-glycosylation pathway for mucin-type glycoproteins. Bioessays 10, 117–121.

[7] Hanisch, F. G. (2001). O-glycosylation of the mucin type. Biol Chem 382, 143–149.

[8] Fukuda, M. (1996) possible roles of tumor-associated carbohydrate antigens. Cancer Res 56, 2237–2244.

[9] Fukuda, M. (2002). Roles of mucin-type O-glycans in cell adhesion. Biochim Biophys Acta 1573, 394–405.

[10] Satoh, S., Hinoda, Y., Hayashi, T., Burdick, M. D., Imai, K., and Hollingsworth, M. A. (2000). Enhancement of metastatic properties of pancreatic cancer cells by MUC1 gene encoding an anti-adhesion molecule. Int J Cancer 88, 507–518.

[11] Wesseling, J., van der Valk, S. W., Vos, H. L., Sonnenberg, A., and Hilkens, J. (1995). Episialin (MUC1) over expression inhibits integrin-mediated cell adhesion to extracellular matrix components. J Cell Biol 129, 255– 265

[12] Gindzienski, A. and Zwierz, K. (1987). The effect of SDS and 2-mercaptoethanol on the supramolecular structure of human gastric mucus gel. Biochem Med Metab Biol 38, 347–354.

[13] Paszkiewicz-Gadek, A., Gindzienski, A., and Porowska, H. (1995). The use of preparative polyacrylamide gel electrophoresis and electroelution for purification of mucus glycoproteins. Anal Biochem 226, 263–267.

[14] Corfield, A. P., Myerscough, N., Gough, M., Brockhausen, I., Schauer, R., and Paraskeva, C. (1995). Glycosylation patterns of mucins in colonic disease. Biochem Soc Trans 23, 840–845.

[15] Gendler, S. J. and Spicer, A. P. (1995). Epithelial mucin genes. Annu Rev Physiol 57, 607–634.

[16] Strous, G. J. and Dekker, J. (1992). Mucin-type glycoproteins. Crit Rev Biochem Mol Biol 27, 57–92.

[17] Kim YS, Gum JR, Byrd JC, Toribara NW. (1991). The structure of human intestinal apomucins. Am Rev Respir Dis;144:S10–14.

[18] Copin MC, Devisme L, Buisine MP, et al. (2000). From normal respiratory mucosa to epidermoid carcinoma:expression of human mucin genes. Int J Cancer.;86:162-168.

[19] Baldus SE, Hanisch FG. (2000). Biochemistry and pathological importance of mucin-associated antigens in gastrointestinal neoplasia. Adv Cancer Res.;79:201-248.

[20] Intisar E. Saeed1, Hsin-Yi Weng, Kamal H. Mohamed & Sulma I. Mohammed (2014). Cancer incidence in Khartoum, Sudan: first results from the Cancer Registry, 2009–2010 Cancer Medicine; 3(4): 1075–1084.

[21] Ali U, Nagi AH, Naseem N, Ullah E (2012). Mucin Histochemistry in Tumours of Colon, Ovaries and Lung. J Cytol Histol 3: 163. doi:10.4172/2157-7099.1000163.

[22] Ionila M, Margaritescu CL, Pirici D, Mogoanta SS (2011). Mucinoous adenocarcinoma of the colon – a histochemical study Rom. J Morphol Embryol 52: 783–790.

[23] Rutgers JL, Baergen RN (1994). Mucin histochemistry of ovarian borderline tumors of mucinous and mixed-epithelial types. Mod Pathol 7: 825-828.

[24] Roopali D.Nikumbh1, Dhiraj B.Nikumbh, B.N.Umarji. (2012). Mucin Histochemical Study of the Colon in Normal and Malignant Lesions. IJHSR, Vol.2; Issue: 7.