Study of Mucins in Epithelial Ovarian Tumors

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Summary:

Background: *Ovarian tumors account for a considerable proportion of clinically important tumors .Many factors affect their prognosis and their response to treatment, including histological types ,and grade and rate of growth. Recently,, the ability of such tumors to secrete mucins was studied and assessed as another possible prognostic factor .*

Objectives: *Histochemical staining for mucins was used in this study to clarify the types of mucins secreted' by the different histological types of these tumors and to demonstrate the changing patterns of mucins in the benign, , borderline, and malignant tumors ,and to be used as a helpful routine diagnostic technique in the diagnosis and to identify the early malignant transformation of these tumors.*

Materials & Methods: *One hundred and six (106) specimens of ovarian surface epithelial cysts and tumors were randomly selected from the files of the pathology department in the Teaching laboratories at the Medical City Hospital ,Baghdad , from 1985 -1999. All the specimens were formalin- fixed and paraffin embedded and 3-5 microns thick sections were stained with Haematoxylene & Eosin ,Periodic Acid Schiff (PAS)stain for neutral mucin, Alcian Blue PH 2.5 & Orcein /Alcian Blue PH 2.5 stains for acidic (sialo & sulfo) mucins.*

Results *: The study showed quantitative and qualitative changes in the mucins production in various cysts & tumors with a statistically significant negative linear correlation in the P.A.S. positive i.e. neutral mucins which is reduced in the malignant tumors compared to the benign tumors . A statistically significant positive linear correlation in the Orcein/ AB stains positive acidic mucin production,which was increased in the malignant tumors compared to the benign tumors.*

Conclusion: *Mucin stains are helpful histochemical techniques that can be used routinely on tissue sections to differentiate between the various histological types of surface epithelial tumors of the ovary and to predict the malignancy potential especially of the mucinous tumors.*

Keywords: *Ovarian tumors, mucin stains.*

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Introduction:

Ovarian cysts and tumors are common in clinical practice (1). Ovarian cancer presents a significant diagnostic and therapeutic challenge to pathologists and gynecologists (2). The surface epithelial tumors constitute 60% of neoplasms arising in the ovary and more than 90% of the malignant variety (3). The epithelial cancers are considered as one of the major causes of cancer deaths among women and first among gynecologic malignancies (4).

Accurate evaluation of the primary tumors is important to predict the prognosis and to determine the appropriate treatment strategies.

Histochemistry is commonly used presently in many centers in the world for diagnosis and identification of many pathological conditions .

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In this study epithelial ovarian cysts and tumors were studied by mucin staining methods which include:-

Periodic Acid Schiff (PAS) : for neutral mucin .

Alcian-Blue PH 2.5 (AB):- For acidic sialomucin .

Orcein/ Alcian -Blue PH 2.5 (O/AB): for acidic sialomucin and sulfomucin.

The aim of the study was to demonstrate the types of mucins secreted by different histological variants of ovarian surface epithelial cysts and tumors, and to identify the changing patterns of mucin secretion in benign and malignant tumors in an attempt to identify early malignant transformation.

Material and methods:

A retrospective and prospective study is done for 106 specimens of randomly selected ovarian cysts and tumors, which were submitted to the surgical pathology department in the Teaching laboratories of the Medical City in Baghdad from January 1985 till the end of December 1999. Including:

42 specimens of serous cysts and tumors were studied, of which 16 cases were benign,

10 cases were borderline, and 16 cases were malignant. Diagnostically, 12 specimens were emdometroid carcinoma, 8 Bremer's tumor, and two clear cell carcinoma of the ovay. All specimens were formalin-fixed paraffin embedded, and 3-5 micron thick sections were cut and stained with Haematoxylene & Eosin stain. Mucin stains including : Periodic Acid Schiff (PAS) for neutral mucin ,Alcian Blue PH 2.5 for acidic sialomucin and Orcein/Alcian Blue PH 2.5 for acidic mucins; the sialo and sulphomucins were also used for all tissue sections(5,26).(See Table 1).

Mucin stains were also used for sections from the endocervix, fallopian tube, and the endometrium and used as control. PAS with diastase was also used for the demonstration of glycogen that usually stains strongly positive with PAS stain and disappears after the use of diastase enzyme to differentiate it from the neutral mucin.
Table (2,) demonstrates the various staining results for mucin stains used on 106 ovarian tumors.
The amount of mucin secreted was scored from 3-0 as follows: score 3 strongly positive means 70% of cells in section shows intracytoplasmic mucin or 70% of cysts showing intraluminal mucin per high power field. Score 2 Positive means 30-70% of cells showing intracytoplasmic mucin or 30-70 % of cysts showing intraluminal mucin per high power field. Score1 weakly positive means 30 % or less of cells show intracytoplasmic mucin or 30 % of cysts showing intraluminal mucin per high power field.

Score 0 negative means no mucin at all in the cells or in cysts. Statistical Analysis: After scoring mucin content statistical analysis was applied including:

P-Value (Anova): For assessing the statistical significance of the difference between the mean of more than two groups.

P-Value (Tuckey's LSD): Least significant differences assessing the statistical significance for the differences between the mean of two groups, adjusting for type 1 error in case of multiple paired comparisons to the same -0.05 .

r- and its P-Value: Spearmann's correlation coefficient: and its assessing the statistical significance of the correlation coefficient.

Results:

As shown in table (2), there were different scores for the three stains: PAS , Alcianblue and Orcein / Alcian blue applied on different histological types of 106 epithelial ovarian tumors .

These scores indicate the amount of mucins secreted by these tumors with especially mucinous tumors where there was obvious secretion of different types of mucin (neutral,sialo and sulphomucin) with high scores for mucin stains .This observation was also seen inside cystic lumen of the benign Brenner tumor that is different from the epithelium of solid nest.(Fig 10).

PAS positive material in the cells of clear cell tumor Fig.(12) and Brenner tumor indicates the presence of glycogen and/or mucin of which the first one is confirmed by diastase digestion. It is diastase sensitive while the mucin is diastase resistant. Table (3) shows that the difference in the mean score for the three stains between the three grades of ovarian tumors was generally small and statistically insignificant .No obvious trend was observed and the calculated correlation coefficients were very small and statistically insignificant.

In endometrioid carcinoma, moderate amount of neutral and acidic mucins, mainly at the luminal border of the cells and intraluminally were seen .As shown in Table 2 & Figure (11).Trace amount of glycogen is present in the cytoplasm of cells. However, mucin stains are not helpful to differentiate endometrioid from serous and clear cell carcinomas.

Serous Ovarian Tumors : As shown in Table (4) and graphs (1,2,3) the differences in mean score for the three stains between the three grades of serous ovarian tumors was small and statistically insignificant .No obvious trend was observed and the calculated correlation coefficients are very small and statistically insignificant.(See Figures 1,2,3 &4.)

Mucinous Ovarian Tumors : As shown in table (5) and graphs (1,2&3), there was a moderately strong and statistically significant negative linear correlation between the three grades of mucinous ovarian tumors and PAS score (r= -0.6).The mean PAS score decreased from(2.8) in mucinous adenoma (benign) to(2.2) in mucinous borderline tumors, and reached (1.8) in mucinous carcinoma (malignant) This negative trend and the difference in mean PAS score between the three grades was statistically significant. However ,the difference in PAS score observed between mucinous borderline tumors and mucinous carcinoma failed to attain the level of statistical significance.

The difference in the mean Alcian blue stain score between three grades of mucinous ovarian tumors was small and statistically insignificant. The negative trend observed was weak (r=-0.2) and not significant statistically. There was also moderately strong and statistically significant positive linear correlation between the grades of mucinous ovarian tumors and Orcein/Alcian blue stain scores (r=0.43).

The mean Orcein score increased from (1)in mucinous adenoma to (1.6) in mucinous borderline and reached its highest value (2.25)in mucinous carcinoma .This positive trend and the difference in mean Orcein score between the three

grades of tumor was statistically significant, However, the difference in mean Orcein score observed between mucinous borderline and mucinous carcinoma ,failed to attain the level of statistical significance. See Figures(5,6,7,8&9).

Discussion:

The almost universal ability of ovarian neoplasm to secret mucins and the histochemical similarities between the secretion of many of otherwise quite different neoplasms, serve to underline the basic kinship of all tumors in the ovary. The fmding of mucinous secretions in all tumors of presumed celomic origin and its absence in those of sex cord-stromal and germ cell tumors would seem to indicate a common histuchemical denominator for all neoplasms in the first group, and suggests the value of an embryological classification of ovarian neoplasms.(6).

Many studies for mucin histochemistry of various organs in the body were done especially of gastrointestinal tract (7,8,9,10,11,12) and less commonly on breast (12,15) salivary gland (13) and neural tissue (14). A limited attempt has been made to investigate mucin in the ovary which was previously called pseudomucins. Makay D.G. et al used PAS stain on normal ovary and they showed that in the ovarian follicles there was little amount of glycogen and glycoprotein.(16) . To the best of our knowledge no previous Iraqi study was done in this field.

Serous Tumors: In the present study, the results of mucin staining obtained from studying table (4) and graphs (1,2&3) show that a small amount of mucin of different types are seen in the upper part of the epithelial cells and intraluminally in different score values (Figures 1,2,3&4). Application of mucin staining on serous tumors show no significant correlation between grades of this tumor and difficulty in identifying borderline from benign and malignant tumors. These results are similar to that shown by Klemi PJ et al. (17) on serous tumors and they suggest that these mucin secretions were increased with increasing malignancy similar to what was reported by Garasia-Bunnel R.et al.(6).

Mucinous Tumors: As expected, the presence of mucin is the most conspicuous here as shown in table (5) & graphs 1,2 3.In mucinous cystadenoma neutral and acidic mucins ,intracellular and extracellular are seen in different proportions with sialomucin predominating but sulfated mucin can be seen in some cases. With increasing malignancy, the amount of neutral mucin decreases but the sulfated mucin become predominant (Figure 5,6,7,8&9), that is there is negative correlation between PAS and AB pH 2.5 stains and grades of mucinous tumors, but moderate to strong positive correlation between orcein/alcian blue pH 2.5 stain and these grades can also be seen. Accordingly, the highest score of orcein stain (2.25) in mucinous cystadenocarcinoma differed significantly from borderline and benign tumor scores. This can help in differentiation between them .Similar results were obtained by previous studies (18,19) Shi Z.L. in 1989 (20) mentioned that sulfated mucin was increasing from 3.73% in the mucinous cystadenoma to reach 8.33% in malignant tumors.

In endometrioid carcinoma, a moderate amount of neutral and acidic mucins mainly at the luminal border of the cells and intraluminally were seen (Table 2)& graphs (1,2&3).Trace amount of glycogen is present in the cytoplasm of cells(Figure 11). These results are similar to those obtained by Klemi PJ. Et al. (21) in his study on endometrioid tumors as well as to Czenobisky B. et. Al. (22).

In Brenner tumors (Table 2&3) and graph (l,2&3) show that there is a significant difference of mucin scores seen between the epithelial cells and inside the spaces. It appears that PAS positive glycogen and diastase resistant PAS positive mucin could be detected in the cytoplasm of epithelial cells, while intracystic spaces showed similar reactivity to that of mucinous cystadenoma with neutral and sialomucin predominance (Figure 10).These results are similar to those shown by Aguirre P et al. In 1989(23)and Klemi PJ ,(24)on Brenner's tumors .

Clear Cell Carcinoma (Mesonephroma): As shown in table (2)and graphs (1,2&3) presence of large amount of glycogen in the cytoplasm gave moderate PAS scoring, but diastase resistant PAS positivity indicated the presence of sialomucin and sulfated mucin in trace amount giving low scoring by AB pH 2.5 and orcein/AB pH 2.5 stains (Figure 12).These results are similar to Klemi et al fmding(25)in their study on mesonephroid tumors and similar to that obtained by Grasia-Bunnet R. et al. (6).

Table (1) Special staining methods for mucin and their interpretation

Biochemical stain	Positive stain result	Interpretation
Periodic Acid Schiff (PAS)	Magenta	Neutral mucin
Alcian Blue (AB), ph 2.5	Blue	Acidic mucin sialomucin + sulphated mucin
Orcein/Alcian Blue, ph 2.5	Brown	Sulphated mucin
Orcein Alcian Blue	Blue	Sialomucin

Table (2) Mucin pattern in 106 epithelial ovarian tumors

Diagnosis	No. of cases	Periodic Acid Schiff (Neutral mucin)				Alcian Blue (Sialomucin)				Orcein/Alcian Blue (Sulphomucin)			
		0	1	2	3	0	1	2	3	0	1	2	3
1. Serous tumors	42												
Serous adenoma	16	7	6	2	1	7	7	2	-	8	8	-	-
Serous borderline	10	2	4	4	-	3	3	4	-	5	5	-	-
Serous carcinoma	16	6	6	4	-	4	10	2	-	5	11	-	-
2. Mucinous tumor	42												
mucinous adenoma	16	-	-	3	13	2	4	4	6	6	6	2	2
mucinous borderline	10	-	2	4	4	-	6	2	2	4	-	2	4
mucinous carcinoma	16	1	3	10	2	2	5	8	1	2	1	4	9
3. Endometrioid	12	2	6	3	1	2	4	6	-	7	5	-	-
4. Brenner tumor													
Solid	8	-	4	4	-	4	4	-	-	6	2	-	-
Intracystic		-	-	2	6	-	2	4	2	2	4	2	-
5. Clear cell tumor	2	-	-	2	-	1	1	-	-	-	2	-	-

Table (4) Association between different grades of serous ovarian tumors and mucin stain scores

Grade of the tumor	Mean score	P value (ANOVA)	P value (Tuckey's LSD)	P value (tuckey's LSD)	(r)	P value for (r)
1. PAS						
Serous adenoma (n=16)	0.81	0.5 (NS)	Control	------	0.05	0.7(NS)
Serous borderline (n=10)	1.2		***(NS)	Control		
Serous carcinoma (n=16)	0.88		***(NS)	***(NS)		
2. Alcian Blue						
Serous adenoma (n=16)	0.69	0.4 (NS)	Control	------	0.1	0.4(NS)
Serous borderline (n=10)	1.1		***(NS)	Control		
Serous carcinoma (n=16)	0.88		***(NS)	***(NS)		
3. Orcein/Alcian Blue						
Serous adenoma (n=16)	0.5	0.7 (NS)	Control	------	0.2	0.3(NS)
Serous borderline (n=10)	0.5		***(NS)	Control		
Serous carcinoma (n=16)	0.69		***(NS)	***(NS)		

***(NS): When P value for ANOVA test is not significant, the LSD (Least Significance Difference) is not calculated since it is by default not significant also.
(NS): not significant statistically
n = sample size

Table (3) Association between different grades of ovarian tumors in general and mucin stain scores

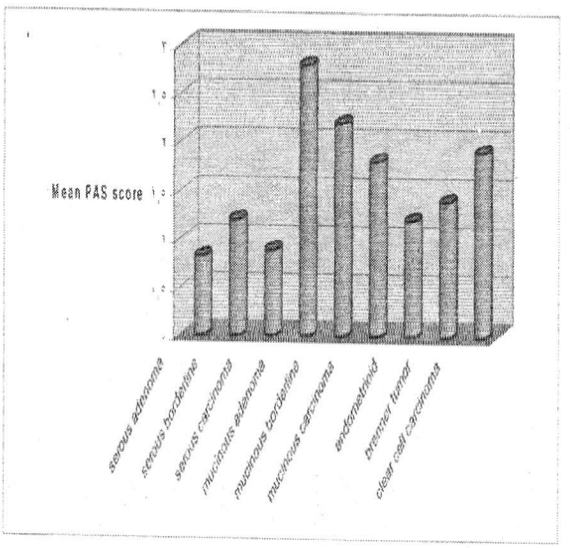
Grade of the tumor	Mean score	P value (ANOVA)	P value (Tuckey's LSD)	P value (tuckey's LSD)	(r)	P value for (r)
1. PAS						
Benign tmours (n=32) (Adenomas + Brenner's)	1.81	0.12 (NS)	Control	***(NS)	-0.2	0.03(NS)
Borderline tumors (n=20)	1.7		***(NS)	Control		
Malignant tumors (n=46) (Carcinomas, endometrioid)	1.35		***(NS)	***(NS)		
2. Alcian Blue						
Benign tmours (n=32) (Adenomas + Brenner's)	1.28	0.95 (NS)	Control	------	0.02	0.8(NS)
Borderline tumors (n=20)	1.35		***(NS)	Control		
Malignant tumors (n=46) (Carcinomas, endometrioid)	1.28		***(NS)	***(NS)		
3. Orcein/Alcian Blue						
Benign tmours (n=32) (Adenomas + Brenner's)	0.75	0.2 (NS)	Control	------	0.2	0.09(NS)
Borderline tumors (n=20)	1.05		***(NS)	Control		
Malignant tumors (n=46) (Carcinomas, endometrioid)	1.17		***(NS)	***(NS)		

***(NS): When P value for ANOVA test is not significant, the LSD (Least Significance Difference) is not calculated since it is by default not significant also.
(NS): not significant statistically
n = sample size

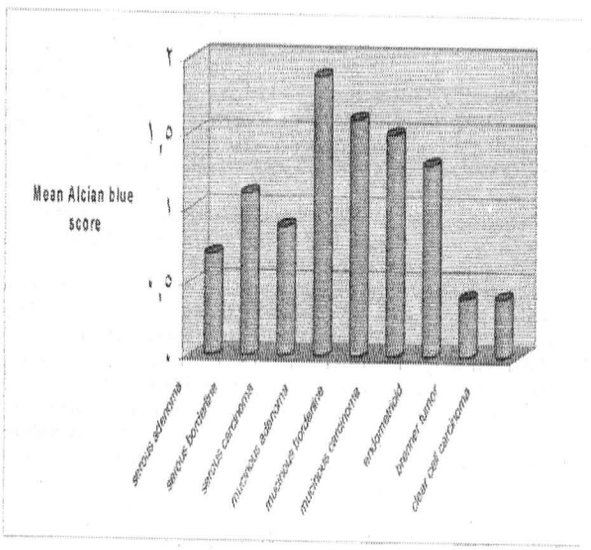
Table (5) Association between different grades of mucinous ovarian tumors and mucin stain scores

Grade of the tumor	Mean score	P value (ANOVA)	P value (Tuckey's LSD)	P value (tuckey's LSD)	(r)	P value for (r)
1. PAS						
Mucinous adenoma (n=16)	2.8	<0.001	Control	------	-0.6	<0.001
mucinous borderline (n=10)	2.2		0.04	Control		
mucinous carcinoma (n=16)	1.8		<0.001	0.26		
2. Alcian Blue						
Mucinous adenoma (n=16)	1.88	0. 5 (NS)	Control	------	-.02	0.3(NS)
mucinous borderline (n=10)	1.6		***(NS)	Control		
mucinous carcinoma (n=16)	1.5		***(NS)	***(NS)		
3. Orcein/Alcian Blue						
Mucinous adenoma (n=16)	1	0.01	Control	------	0.43	<0.01
mucinous borderline (n=10)	1.6		0.34(NS)	Control		
mucinous carcinoma (n=16)	2.35		<0.01	0.4(NS)		

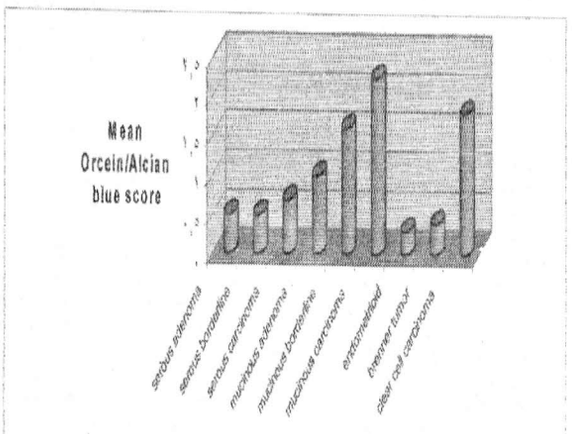
***(NS): When P value for ANOVA test is not significant, the LSD (Least Significance Difference) is not calculated since it is by default not significant also.
(NS): not significant statistically
n = sample size



Graph (1) Mean PAS score for different epithelial ovarian tumors



Graph (2) Mean Alcian blue score for different epithelial ovarian tumors



Graph (3) Mean Orcein / Alcian blue score for different epthelial ovarian tumors

Fig. 1: Serous cystadenoma PAS stain showing negative staining for neutral mucin (X100)

Fig. 2: Serous cystadenoma Orcein/AB stain showing weak positvity for sialo and sulphomucins (X100)

Fig.3: Serous cystadenoma borderline PAS stain showing weak positivity on brush borders of epithelial cells and intraluminal (X100)

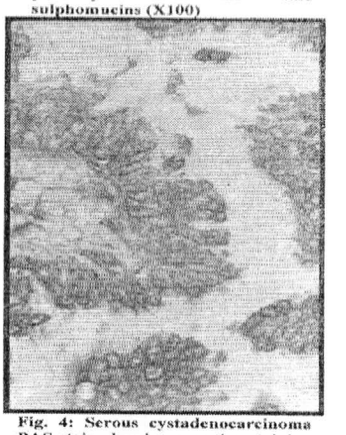


Fig. 4: Serous cystadenocarcinoma PAS stain showing negative staining for neutral mucin (X400)

Figure 5 : Mucinous adenoma PAS Stain shows strong positivity for Neutral mucin X400

Figure 6 : Mucinous borderline PAS Stain shows strong positivity for Neutral mucin X400

Figure 7 : mucinous adeno carcinoma PAS stain show less positivity for neutral Mucin X100

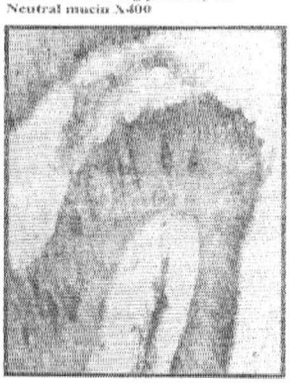


Figure 8 : mucinous borderline Orcein /AB stain shows positivity for both sialo & sulpho mucins X400.

Figure 9 : mucinous adeno carcinoma Orcein / AB stain shows strong positivity For sulpho mucin intracellulary & weak Sialo mucin intraluminally X100.

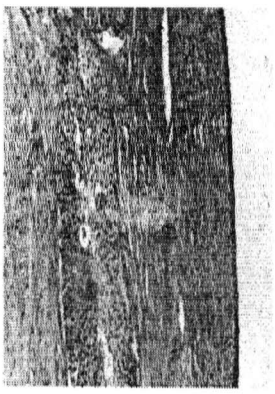


Figure 10 : Benign Brenner tumor PAS stain shows weak positivity for neutral mucin in solid nest & strong positivity Lining of cyst X400.

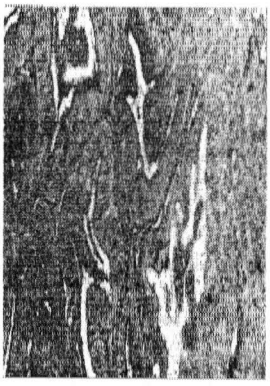


Figure 11 : Endometrioid carcinoma PAS stain show positivity for neutral mucin X100.

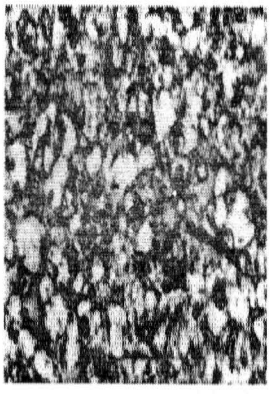


Figure 12: Clear cell carcinoma PAS stains shows strong positivity for Glycogen & neutral mucin X400.

Conclusion:

1. All epithelial ovarian tumors secret all types of mucin and show positivity to mucin stains in different proportions .
2. Mucinous tumors show strong positivity to mucin stains and this technique can help to differentiate them from other epithelial tumors of the ovary and to predict the malignant potential of borderline tumors.
3. Mucin stains can be used to identify the glycogen content in some ovarian tumors as in Brenner tumors and in Clear cell carcinoma.
4. In non-mucinous tumors these stains were not helpful in the differentiation between these tumors and their grades.

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