

PERIODIC ACID-SCHIFF (PAS) STAIN IN SEROUS TUMOURS
AND CLEAR CELL CARCINOMAS OF THE OVARY
A morphometric study

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ABSTRACT

PAS staining of 118 serous tumours and 17 clear cell carcinomas of the ovary were investigated by morphometric means. There were 45 benign, 24 borderline and 49 malignant serous tumours. Serous borderline cystadenomas contained more PAS positive mucin than benign cystadenomas or cystadenocarcinomas. Saliva did not markedly influence the staining in serous tumours as judged by subjective estimation or morphometry. Subjective evaluation showed a decrease in staining of 12 clear cell carcinomas after saliva treatment. Morphometry revealed a change in 11 cases. Of 5 cases with no subjective difference 3 showed difference when estimated with morphometry. The study suggests that differential diagnosis and grading of ovarian tumours may benefit from the quantitative estimation of PAS staining and staining differences before and after saliva treatment.

INTRODUCTION

Many ovarian tumours show positive staining in PAS reaction caused by presence of neutral mucosubstances or glycogen (Bigelow and Blaustein, 1977, Klemi and Nevalainen, 1978, Long and Sommers, 1969, Stenbäck and Kauppila, 1981). In benign serous tumours mucin production is scanty and appears as a thin weakly staining rim along the cell border and extracellularly in the cystic lumen (Garcia-Bunuel and Monis, 1964, Klemi and Nevalainen, 1978). The total amount of mucin in borderline serous tumours was larger than in benign serous tumours but the staining was weak. In serous carcinomas the staining positivity was also weak (Klemi and Nevalainen,

1978). On the other hand, it is well known that ovarian clear cell carcinomas contain glycogen as well as neutral mucosubstances (Klemi and Grönroos, 1979, Nordback and Jauslahti, 1980, Ohkawa et al., 1944, Garcia-Bunuel and Monis, 1964). Glycogen was found only in few serous cystadenocarcinomas (Garcia-Bunuel and Monis, 1964, Klemi and Nevalainen, 1978). This makes PAS staining suitable for differential diagnostics of ovarian tumours. Because quantitative aspects may give additional clues we have analysed the PAS positive material in ovarian tumours by morphometric means.

MATERIALS AND METHODS

118 serous tumours and 17 clear cell carcinomas of the ovary, collected from the files of Kuopio and Tampere University Central Hospitals, were investigated. Two pathologists classified these tumours according to the criteria of WHO (Serov et al., 1973). There were 45 benign, 24 borderline and 49 malignant serous tumours. 17 tumours were classified as clear cell carcinomas of the ovary. 1-5 formalin fixed paraffin embedded samples were used and 5 μ m thick hematoxylin-eosin stained sections were analysed. The tumours were classified according to the dominating part and the most typical area of each tumours was used for analysis.

The staining method was as follows: The sections were deparaffinized with xylene and hydrated by transfer through ethanol to water, treated with 1% periodic acid (4min), rinsed in running tap water (5min), rinsed in distilled water (1min), treated with Schiff's reagent (15min), rinsed in running tap water (10min), treated with Mayer's hematoxylin (5min), rinsed in running tap water (10min) and rinsed in distilled water (1min). Then the sections were transferred through ethanol to xylene and mounted. Successive sections were stained with PAS and with PAS after saliva pretreatment (SPAS) (15min in 37°C).

The staining positivities of PAS and SPAS stained sections were subjectively graded as negative (0), weak (1), moderate (2) or heavy (3), to describe the total staining positivity in the section. The possible difference between PAS and SPAS staining was written down.

The morphometric method was developed on an earlier method for morphometric measurement of CEA staining (Aalto et al., 1982). The sections were analysed at 10x objective and 10x ocular magnification in randomly chosen square fields. The field covered an area of 500 μ m x 500 μ m of the section and had 25 test points. Positive staining was usually seen in the apical parts of the cell and the rest of the cytoplasm was usually negative.

The points falling on the epithelium were graded positive or negative according to staining intensity. The staining result was regarded as positive if the colour of PAS or SPAS staining was heavier than that of the adjacent, usually pink, connective tissue. The PAS (or SPAS) point score of epithelium was calculated by summing up the scores of individual points. The points falling on mucin were graded like epithelial points above. The sum of these scores was the point score of mucin. In practice the positive points were counted with a hemocounter.

RESULTS

In Table 1 the mean values and standard deviations of subjective estimates in different tumour groups are seen. The mean values of borderline serous tumours were higher than those of benign serous cystadenomas or serous cystadenocarcinomas but the difference is not statistically significant. The mean values of benign serous cystadenomas, serous cystadenocarcinomas and clear cell carcinomas were quite near each other. The difference between PAS and SPAS staining was not statistically significant, either.

In clear cell carcinomas subjective evaluation suggested a decrease in staining intensity after saliva treatment in 12 cases. Morphometry showed the change in 11 cases. Of the 5 cases without apparent change as judged by subjective means 3 showed a change in epithelial score after morphometric study.

Table 1. Results of subjective estimation (grading of staining applied. 0=negative, 1=slight, 2=moderate, 3=heavy) of PAS and SPAS staining of 45 benign serous cystadenomas, 24 borderline serous cystadenomas, 49 serous cystadenocarcinomas and 17 clear cell carcinomas of the ovary. The results are expressed as mean \pm SD.

	PAS	SPAS
Serous cystadenomas	1.0 \pm 0.5	1.0 \pm 0.5
Serous borderline cystadenomas	1.5 \pm 0.5	1.5 \pm 0.5
Serous cystadenocarcinomas	1.2 \pm 0.4	1.1 \pm 0.4
Clear cell carcinomas	1.2 \pm 0.5	1.1 \pm 0.5

Table 2. Morphometric estimates of PAS and SPAS staining of 45 benign serous cystadenomas, 24 borderline serous cystadenomas, 49 serous cystadenocarcinomas and 17 clear cell carcinomas of the ovary (the amount of positive staining in 25 randomly chosen fields estimated with the point score method). The results are given as mean \pm SD.

	Serous cystade- nomas	Serous borderline cystade- nomas	Serous cystade- nocarci- nomas	Clear cell carci- nomas
<u>Point score</u>				
epithelium (PAS)	0.7 \pm 2.0	2.9 \pm 3.6	1.3 \pm 3.1	4.7 \pm 9.0
(SPAS)	0.6 \pm 1.9	2.1 \pm 3.3	1.0 \pm 2.2	1.0 \pm 2.0
mucin (PAS)	1.6 \pm 5.5	8.5 \pm 12.5	1.8 \pm 11.6	6.6 \pm 11.2
(SPAS)	2.6 \pm 9.5	9.0 \pm 14.2	2.2 \pm 13.2	2.4 \pm 4.7

In serous cystadenomas, serous borderline cystadenomas and serous cystadenocarcinomas the differences between PAS and SPAS staining results of epithelium were small and statistically not significant. In clear cell carcinomas, on the other hand, the difference was significant for positive cases. The mean decrease of epithelial scores was 5.25 and the corresponding decrease in mucin scores was 6.64. The mean value of PAS mucin point score in serous borderline cystadenomas was 8.5 ± 12.5 and in serous cystadenocarcinomas 1.8 ± 11.6 . The difference was statistically significant ($0.02 < p < 0.05$).

DISCUSSION

The study shows that the amount of PAS positive mucin in serous borderline cystadenomas is significantly higher than that in serous cystadenocarcinomas. The result suggests that PAS staining may be useful in distinguishing between serous borderline cystadenomas and carcinomas, if distinct invasion is not seen. In serous benign cystadenomas the amount of mucin is scanty too, but it is easy to distinguish between serous cystadenomas and carcinomas on histological criteria. The low mucin score in benign cystadenomas may be artificial because mucin is often washed out into the fixative from the large cysts of the benign tumours.

It is well known that clear cell carcinomas contain glycogen as shown by the difference between staining posi-

tivities of PAS and SPAS stainings. In serous cystadenocarcinomas the staining positivity does not change clearly. So the morphometric approach may be helpful in situations where it is difficult to decide after other evidence of the classification of these tumours.

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