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DNA ploidy analysis of borderline epithelial ovarian tumours

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DNA Ploidy Analysis of Borderline Epithelial Ovarian Tumors

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Abstract

Objective: Borderline epithelial ovarian tumors not uncommonly pose a great difficulty to surgical pathologists as morphologically they may show very similar features as those of malignant epithelial tumors except invasion. However it is important to separate these from their invasive counterparts because of their superior prognosis. Recently, attention has been focussed on the prognostic value of flow cytometric analysis of DNA ploidy in borderline epithelial ovarian tumors. The purpose of this study is to investigate whether flow cytometric analysis of cellular DNA content acts as a useful adjunct to the histopathological diagnosis of borderline malignancy.

Materials and Methods: Fifteen histologically confirmed borderline serous epithelial tumors of the ovary were selected. Samples were analyzed on a FACScan flow cytometer using the software MODFIT. A total of 10,000 nuclei were counted each time.

Results: The mean CV for the 15 cases was 3.67 (Range 2.4-5.0). In the DNA histograms a diploid sample was defined as one that had a single Go/Cl peak. An aneuploid tumour was defined as one that displayed an additional distinct peak. All 15 cases of borderline serous epithelial tumors showed a diploid stemlinc with DNA index between 0.9-i .10

Conclusion: This study suggests that aneuploidy if ever demonstrated in histological confirmed borderline tumors should prompt extensive sampling of the tumor and a close follow up (JPMA 50: 349, 2000).

Introduction

Borderline ovarian tumors or turnouts of low malignant potential (LMP) comprise approximately 7.5%¬15% of all epithelial ovarian neoplasms, most of which have serous or mucinous Features[1]. It is important to separate these from the invasive tumors because of their superior prognosis[2]. This distinction however, is not always easy, particularly in non-serous types and there is some controversy regarding the arbitrary diagnostic criteria used to designate the borderline tumors[3]. The presence of stromal invasion is an important histologic criterion for separating ovarian carcinomas from borderline lesions, however tangential sectioning of surface lesions may lead to erroneous results. The focus of present day cancer research to some extent has shifted from the exclusive subjective and semi-quantitative diagnostic and prognostic parameters (stage and grade) to quantitative measurements of fundamental tumor properties directly related to their growth and metastatic potential, like DNA content and ploidy. The development of flow cytometry has introduced a new possibility for routine DNA measurements with high speed and high resolution. Prognostic information of flow cytometric DNA measurements of malignant ovarian tumor samples has been reported[4,5] and aneuploidy recognized as a significant adverse prognostic indicator of ovarian carcinoma[6]. However data on borderline ovarian tumors is scarce. Where most studies identify flow cytometric analysis of cellular DNA content as a useful adjunct to the histopathological diagnosis of borderline malignancy, others have cast doubts on its role in predicting which tumor(s) will behave in a more aggressive fashion[7,14]. The purpose of this study is to further explore whether tumor cell DNA content in borderline ovarian tumors may be used as an adjunct to support the histologic diagnosis.

Materials and Methods

Selection of cases for ploidy analysis
Formalin fixed, paraffin embedded tissue blocks were selected from the pathology record of the Aga

Khan University Hospital. Fifteen cases of histologicaly confirmed borderline ovarian tumors were selected. Only those blocks were selected which on screening showed a good proportion of representative tissue.

Sample preparation

Three to five 25 urn thick sections were obtained/cut from routinely fixed, paraffin embedded tissue blocks for each case. Sections were dewaxed in two changes (2 x 10 minutes) of xylene and rehydrated in 100, 90, 70 and 50% alcohol for 10 minutes each. The sections were then rinsed in PBS x 10 minutes and incubated in 0.5% pepsin solution at pH 1.5 and 37° C for 30 minutes. Hypodermic needles of 40 and 25 bore were used to break up the tissue. Released nuclei were spun, washed and cytopreps made to check their condition staining was done with propidium iodide (P1) in isoton (250 ug/ml) containing I mg/mI RNAase for 30 minutes at 4°C before analyzing on FACScan machine.

Flow Cytometry

Samples were analyzed on a FACScan flow cytometer using the software MODFIT, Flow cytometric data was acquired and displayed in standard two parameter dot plots using FL2 width and FL2 area as the axes. This allowed to draw gates in which debris below the first Go/C I distribution and particles with extended time in flight (presumed doublets) were excluded from analysis using carefully defined and standardized gating criteria. FL2 area signals were then used to generate single parameter DNA histograms. Specimens were rejected if the median half-peak coefficient of variation (CV) of the diploid peak was more than 5. A total of 10,000 nuclei were counted in each case.

Results

Tumour staging and typing

Of the fifteen patients with a histological diagnosis of borderline ovarian epithelial malignancy, majority had FIGO Stage I, typed mostly as serous.

Histogram interpretation

The mean coefficient of variation (CV) for the IS cases was 3.67(range 2.4-5). In the DNA histograms a diploid sample was defined as one that had a single Go/GI peak (Figure Ia).
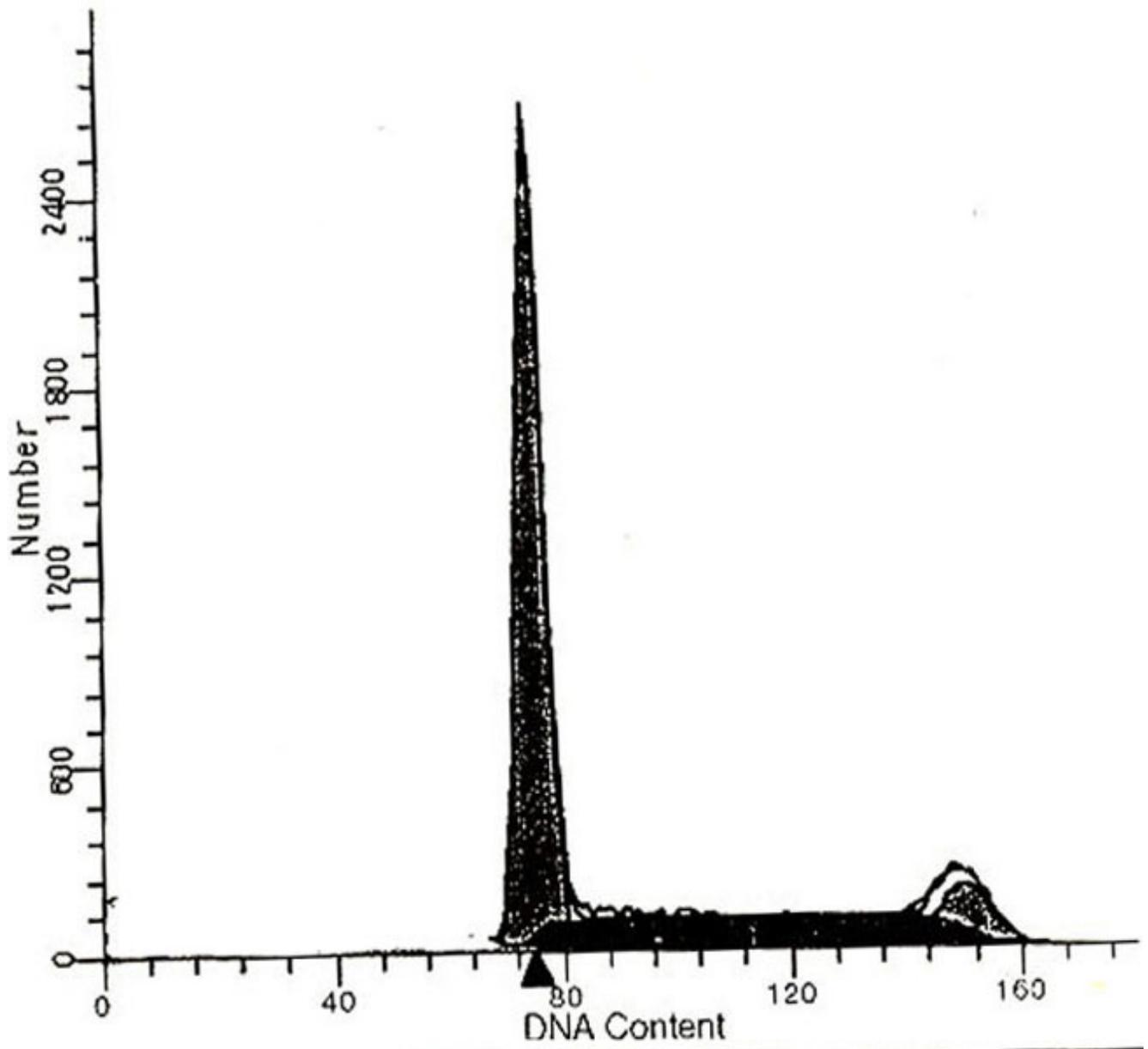
Figure 1a

An aneuploid tumour was defined as one that displayed an additional distinct peak (Figure I b).
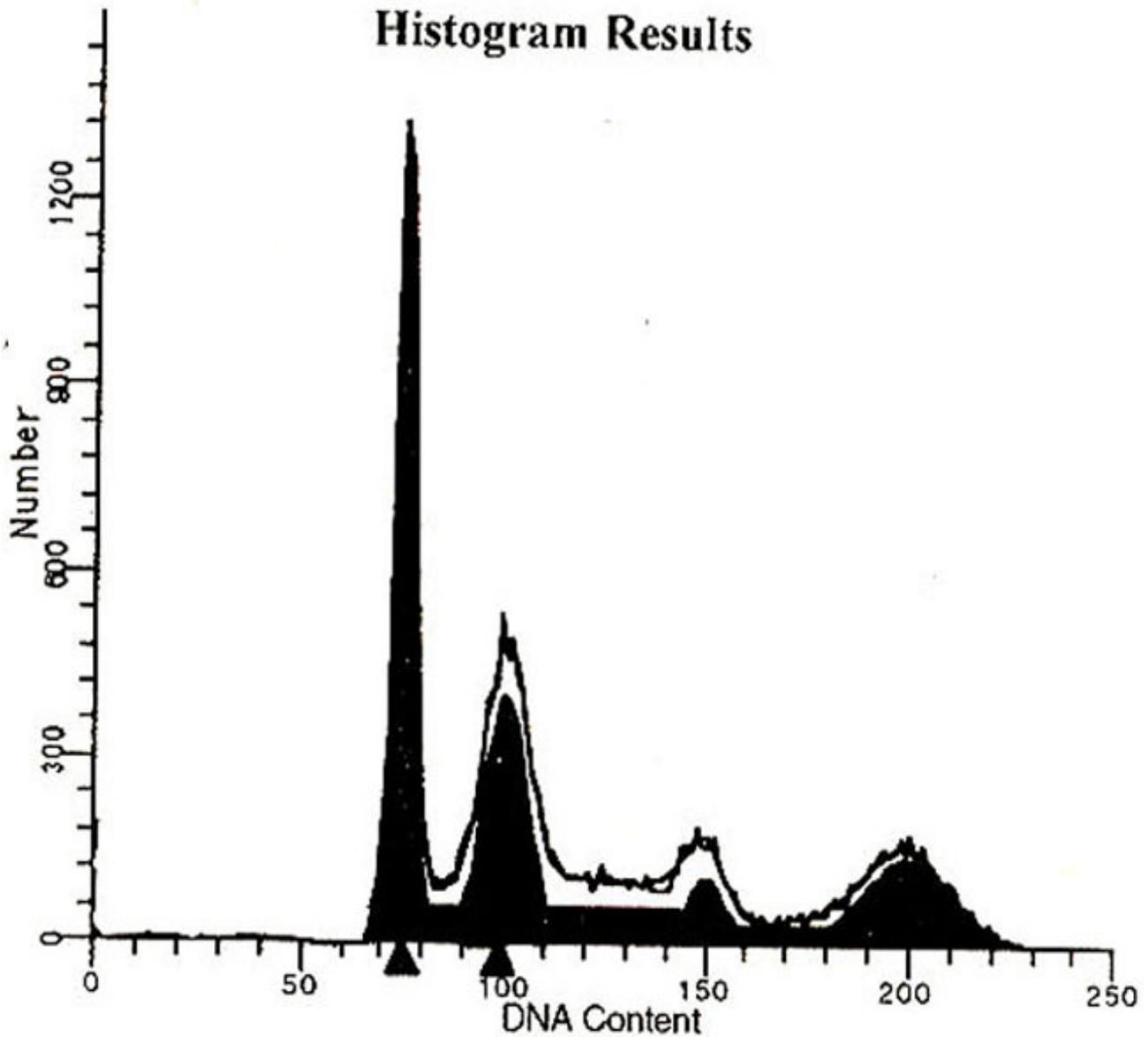
Histogram Results

Figure 1b

The DNA index was calculated as the ratio of the mean channels of the aneuploid Go/G I peak to the diploid Go/C I peak.

All 15 cases of borderline serous ovarian tumors showed a diploid stemline with DNA index between 0.9-.10. None of the cases demonstrated aneuploidy despite multiple sampling (3-5 of each case).

Discussion

Common epithelial ovarian tumors are subgrouped into benign, borderline and invasive malignant categories under recommendations of FIGO and WHO[14]. The usual finding of an indolent clinical course and generally improved prognosis in patients support the value of this categorization with borderline tumors compared to their invasive counterparts[15]. However the identification of borderline tumors are subject to considerable interobserver variability [16] and the validity of their arbitrary categorization can be challenged on a number of grounds[17].

The flow cytometric analysis of cellular DNA content is relatively simple to perform and is highly reproducible. The most extensive work on its clinical usefulness in gynecological pathology has been in association with prognosis of epithelial carcinomas of the ovary and in the differential diagnosis and prognosis of possible molar gestations. Much less data is available on the DNA content of borderline tumour and its biological significance. However a large number of studies have demonstrated prognostic significance of flow cytometric analyses of cellular DNA content in general. Kuhn et al[6] observed that borderline tumors in 7 of 8 women were diploid and all but one of the women was alive after a mean follow up period of 48 months. Likewise Friedlander et al[7] studied ploidy status in 44 women with borderline tumors and reported that 42 were diploid. Of the 2 women with aneuploid tumors one died 7 months after diagnosis. All 42 women with diploid DNA status were alive after an average of 3 years of follow up. As part of a larger follow up study of borderline ovarian tumors survival Kaern et. al[10], Determined ploidy status in a nested case control study of 64 women with mucinous and borderline ovarian tumors. Of the 30 women who died and 4 women with disease recurrence after 7 years of follow tip (odds ratio 1.l, pc 0.0l). Aneuploidy was also more strongly associated with poor survival in women with mucinous tumors. It has been shown in a very large series from the Norwegian Radium Hospital in Oslo, with long term follow up. that DNA aneuploidy of serous (P160 stage> I) and mucinous borderline tumors is clearly associated with poor prognosis[18,19]. There are several other small series that have confirmed the value of DNA cytomeiry in this respect.

Some recent studies have however cast doubts. liarlow et. al[20] reported a case control study negating any significant association between ploidy and prognosis of borderline ovarian tumors. Data from women who had died of borderline ovarian tumors were compared to an age, histology and histologic stage matched sample of women with the same diagnosis still living after atleast five years of follow up. 25% of the women who died and 24% of those still alive had aneuploid DNA tumors.

Seidman et. al.[12] reported a similar finding. They analyzed 40 serous tumors of low malignant potential (STLMP) and compared with 26 serous carcinomas, The mean follow up periods were 14.3 years for stage I STLMP and 8.3 years for stage III STLMP 40% of STLMP as compared to 54% of serous carcinomas were aneuploid. 50% of the STLMPs, which progressed. had aneuploidy, as did 38% of those that did not. Correlation between diploidy and superior prognosis was rejected on the basis that 3 of 4 DNA diploid tumors progressed and 3 of 6 that did not progress were aneuploid. False diploid and aneuploid sample samples may offer an explanation here. The samples used in both the studies were de-parallelized. When analyzing de-paraffinized material it is imperative to examine the microscopic slides to assure that a substantial amount of normal tissue is present to provide the normal internal diploid control. Samples containing necrosis may similarly result in false aneuploid peaks[21].

The nature of the tissue analyzed may also affect the accuracy of the DNA analysis. It has been shown that considerable hcterogeaeity in DNA content is present in most neoplasms. It is thus recommended that multiple samples (generally 3 or more) from a neoplasm be examined. Seidman et. al. had a few eases ia their study with one sample only. which could have resulted in the erroneous results.

The subjectivity of the interpretation of the DNA histograms[22] and the absence of standardized criteria for histogram diagnosis most probably account in part for the variations in DNA ploidy results for the same types of tumors that are reported in literature.

In the light of our results and the already existing data, we conclude that aneuploidy if ever demonstrated in histologically borderline tumors should prompt extensive sampling of the tumor and a close follow up. We thus believe that flow eytometric analysis of cellular DNA content may complement conventional histopathological diagnosis by providing an objective parameter that correlates with the biological behavior and may identify the few genuine aggressive borderline ovarian epithelial neoplasms that show clinical progression.

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