RESEARCH ARTICLE

Tumour Markers in Peritoneal Washing Fluid - Contribution to Cytology

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Abstract

  Background: Peritoneal washing cytology (PWC) that shows the microscopic intra-peritoneal spread of gynaecologic cancers is not used in staging but is known as prognostic factor and effective in planning the intensity of the therapy. False negative or false positive results clearly affect the ability to make the best decision for therapy. In this study we assessed levels of tumour markers, carcinoembryonic antigen (CEA), cancer antigen 125 (CA-125) and carbohydrate antigen (CA19-9), in peritoneal washing fluid to establish any possible contribution to the peritoneal washing cytology in patients operated for gynaecologic cancer. Materials and Methods: Preoperative tumour markers were studied in serum of blood samples obtained from the patients for preoperative evaluation of a gynaecologic operation. In the same group peritoneal tumour markers were studied in the washing fluid obtained for intraoperative cytological evaluation. Results: This study included a total of 94 patients, 62 with malignant and 32 with benign histopathology. The sensitivity of the cytological examination was found to be 21% with a specificity of 100%. When evaluated with CEA the sensitivity of the cytological examination has increased to 37%. Conclusions: In addition to examination of PWC, the level of CEA, a tumour marker, in peritoneal washing fluid can make a diagnostic contribution. Determining the level of CEA in peritoneal washing fluid will be useful in the management of gynaecologic cancers.

Keywords: Tumor markers - peritoneal washing fluid - cytology - diagnostic significance

Introduction

  Gynaecologic cancers are a group of disease including vulvar, vaginal, cervical, endometrial and ovarian cancers as well as gestational trophoblastic neoplasm. Gynaecologic cancers are still an important problem of public health in developing countries and constitute 19% of new cancer cases (Sankaranarayanan et al., 2004). Intra peritoneal spread of gynaecologic cancers is an important reason of mortality and morbidity and presents as malignant ascites. Microscopic tumour spread may be detected by PWC before formation of malignant ascites and detection of these malignant cells gives information about the prognosis of the disease (Shiel et al., 2004). Low rates of positive PWC may be seen in occult spread of benign diseases. In many studies, positive PWC has been shown in the progress of benign diseases (Sharifi et al., 1999).

Tumour markers are soluble glycoproteins. Levels of these markers can be evaluated in blood, urine and other body fluid samples of the patients. The ideal tumour marker of gynaecologic cancers has still not been determined. However many tumour-related antigens are determined frequently by monoclonal antibody technique in serum samples of the patients. The tumour markers used most frequently in gynaecologic cancers are CEA, CA 125 and CA 19-9.

  The limitations of PWC examination are the possibility of false positivity in benign diseases and false negativity in the early stage of disease. Although positive PWC does not seem to change the stage of the disease in the latest guidelines, it is a poor prognostic factor and effective in determining the intensity of the treatment (Selvaggi et al., 2003). In this study, CEA, CA 125 and CA 19-9 in peritoneal washing fluid were evaluated in patients operated due to a gynaecologic cancer.

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Materials and Methods

Patients

This study includes the patients who were operated due to either a malignant or a benign gynaecologic pathology in Obstetrics and Gynaecology Clinic of Antalya Education and Research Hospital between 2010 and 2012. Inoperable patients with advanced stage disease or metastasis and patients with inadequate sample for evaluation and clinically detected ascites were excluded from the study. A standard preoperative evaluation for staging the disease and determining the prognosis was carried out for all of the patients. The local ethic committee gave approval for the study. Patients' files were analysed and information on demographic data such as age, gender and disease stage were obtained.

Obtaining the samples

Preoperative tumour markers were studied in serum of blood samples obtained from the patients for preoperative evaluation of a gynaecologic operation. PWC was obtained by aspiration after injecting 100 mL saline solution to the peritoneal cavity just after penetration to peritoneum surgically. Cytopathological examination was performed by two pathologists (DS, ASA). After completing the cytopathological examination, remaining samples were saved in -80⁰C. Peritoneal washing fluid tumour markers were quantified in these samples.

Quantification of tumour markers

Levels of CEA, CA- 125 and CA 19,9 were quantified in preoperative serum samples and peritoneal washing fluid samples obtained intraoperative by chemiluminescence method using Bechman DX-800. Reference values for CEA, CA-125 and CA 19-9 were as follows respectively; <2.5ng/ml, <35 U/ml and <39 U/ml.

Statistical analysis

Statistical analyses were performed using the SPSS ("Statistical software system for Windows") software version 15. Variables either normal or not, were detected by visual (histograms and probability calculations) and analytical methods (Kolmogorov-Simirnov/Shapiro-Wilk's test). Since CEA, CA-125, CA 19-9 and CA 15-3 are not present in preoperative serum samples and peritoneal washing fluid of patients ordinarily, Mann-Whitney "rank sum test" was used for comparison of malignant and benign groups. A p value of <0.05 was considered significant for all of the tests.

Results

This study includes 94 patients operated for a gynaecologic pathology. Median age of the patients was 48.5±12.3 (range 14-78). Histopathological examination revealed malignancy in 62 patients (66%), and a benign disease in 32 (34%) patients. Median age of the patients with a benign disease was significantly lower (median value: 6 years) than the patients with malignancy. Benign diseases originated most frequently from ovary (54.8%) whereas malignant ones originated from the uterus (59.4).

Table 1. Comparison of Tumor Markers in Patients with a Malignant and Benign Pathology

	Benign	Malignant	p value
Age	46.4±11.6	52.6±12.9	0.03
Tissue of origin			
Cervix	3 (4.8%)	2 (6.3%)	
Uterus	25 (40.3%)	19 (59.4%)	
Ovary	34 (54.8%)	11 (34.4%)	
Preoperative serum level			
CEA (ng/ml)	1.4±0.9	2.5±3.9	0.881
CA 19-9 (U/ml)	11.0±7.7	26.5±53.9	0.994
CA 125 (U/ml)	35.6±59.5	69.5±143.7	0.360
Peritoneal washing fluid level			
CEA (ng/ml)	0.2±0.95	36.3±170.9	0.009
CA 19-9 (U/ml)	92.7±430.8	102.6±371.2	0.615
CA 125 (U/ml)	252.6±257.9	348.9±501.1	0.219

The most frequent benign ovarian disease was simple ovarian cyst and the most frequent malignant uterus disease was endometrioid carcinoma.

In patients with a histopathological diagnosis of malignancy, T1, T2 and T3 lesions were determined in 74.2%, 3.2% and 22.6% of patients, respectively. Patients with a cytopathological diagnosis of malignancy had a tumour with a more advanced stage (p<0.001).

Histopathological findings of the patients with a cytopathological diagnosis of malignancy were all consistent with malignancy. Sensitivity and specificity of the cytological examination was found as 21% and 100% respectively. The patient group with a diagnosis of malignancy after cytopathological examination included patients with ovarian and uterine malignancies. Malignancies with an origin of cervix constituted 5% of all the patients. When all the cytological examination was evaluated with pathological level of CEA in peritoneal washing fluid, the sensitivity of the cytological examination increased to 37%.

PWC was found negative in all patients (3 malignant and 2 benign) with a cervix disease. Many of the patients with a malignant cytology had a malignancy of ovary and uterus. Among patients with cervical malignancy, three of the preoperative tumour markers were found positive in one patient whereas only CEA was found high in the peritoneal washing fluid.

No significant difference was found between benign and malignant groups with respect to CEA, CA 125 and CA 19-9 levels quantified in preoperative serum. While no difference was found between the two groups in terms of CA125 and CA19-9 levels in peritoneal washing fluid, levels of CEA was found significantly higher (p:0.009) (Table 1).

Discussion

PWC is used for detecting the microscopic tumour foci that cannot be observed in intraoperative gross examination. Although the presence of malignant cells in the peritoneal cavity does not change the stage of the disease, it has a prognostic significance (Anastasiadis et al., 2011). In our study, we found that CEA, quantified as a tumour marker in the peritoneal fluid has an additional

diagnostic benefit to the diagnostic value of PWC. Specificity and sensitivity of PWC in gynaecological pathologies were searched in many studies. Zuna et al. (1996) determined the role of PWC in gynaecological malignancies. They found a positive cytology result in 80.4% of 112 ovarian carcinoma, 31.2% of 16 borderline ovarian tumour, 12.6% of 135 endometrial carcinoma and 8.7% of 92 cervical carcinoma. They suggested that PWC is highly specific (98.1%) but less sensitive (82.9%) (Zuna et al., 1996). In our study we found a less sensitivity rate in comparison with these rates. We think that the reason of this finding is including the patients with a benign disease in our study.

Tumour markers can be determined in peritoneal washing fluid in progress of both malignant and benign diseases (Ismail et al., 1994). Levels of tumour markers quantified in peritoneal fluid and serum samples may be different (Barbati et al., 1992). Studies performed in patients with ascites, showed that tumour markers quantified in ascites fluid does not play a role in distinction of benign and malignant diseases (Sari et al., 2001). Tüzün et al. (2009) showed just the contrary in a study determining the levels of tumour markers including CA125, CEA and CA19.9 in samples of serum and ascites fluid. In this study, they found the levels of tumour markers correlated in serum and ascites fluid and revealed that the tumour markers were high in patients with a malignancy (Tuzun et al., 2009). In our study patients with ascites were excluded from the study and the study group was different from the study mentioned above in which only patients with a gynaecological pathology were included. CEA was found to be the only tumour marker providing an additional benefit to PWC

Positive PWC is found rarely in vulvar cancers. It ranges between 2.5% and 11% in cervix cancer (Takeshima et al., 1997). CEA and CA125 can be used in diagnosis and follow-up of patients with cervix cancer (Yoon et al., 2007; Gadducci et al., 2008). Positive PWC was not determined in patients with a cervical pathology in our study. We found a high level of CEA in peritoneal washing fluid of a patient with high preoperative tumour markers and this indicates that quantifying CEA in peritoneal washing fluid may be useful in case of negative PWC.

Positive PWC is determined in 7-22% of patients with endometrial cancer (Mlyneek et al., 2005). Positive cytology rate is lower in early-stage patients when compared with advanced stage patients. Kashimura et al. found this rate as 9% in stage I and 25% in stage II endometrial cancer (Kashimura et al., 1997).

Positive cytology is found in 45-60% of patients with ovarian cancer. Presence of peritoneal implants in ovarian cancers with a low malignant potential indicates a high risk of recurrence. PWC may be used to establish this risk (Sneige et al., 2012). In our study there was no patient with an ovarian cancer of low malignant potential. While high levels of serum CA-125 are found in 50% of patients with ovarian cancer, the rate increases to 90% in patients with an advanced stage (Gadducci et al., 1992). High levels of CA125 are determined not only in peritoneal fluid but also in pleural fluid in ovarian cancer (Topalak et al., 2002). In a study searching the levels of CA125 and CEA in ascites

fluid and serum samples in ovarian pathologies, levels of CA125 and CEA in ascites fluid were found statistically higher than serum levels independent of the histology of the ovarian cancer. Levels of CA125 and CEA are found higher in malignant diseases when compared with benign diseases (Harłozińska et al., 1991). In patients with ovarian cancer, high levels of CA19-9 are found in advanced stage more frequently (Yurkovetsky et al., 2010).

In conclusion, CEA, one of the tumour markers quantified in peritoneal washing fluid has a diagnostic contribution to PWC. Quantification of CEA in peritoneal washing fluids seems to be useful in management of gynaecologic cancers.

References

Anastasiadis P, Koutlaki N, Liberis V, et al (2011). The contribution of rapid intraoperative cytology in the evaluation of endometrial cancer spread. *Ann Acad Med Singapore*, **40**, 80-3.

Barbati A, Anceschi MM, Di Renzo GC, et al (1992). CA 125 in peritoneal fluid: reliable values at high dilutions. *Obstet Gynecol*, **79**, 1011-5.

Gadducci A, Ferdeghini M, Prontera C, et al (1992). The concomitant determination of different tümör markers in patients with epithelial ovarian cancer and benign ovarian masses: relevance for differential diagnosis. *Gynecol Oncol*, **44**, 147-54.

Gadducci A, Tana R, Cosio S, et al (2008). The serum assay of tumour markers in the prognostic evaluation, treatment monitoring and follow-up of patients with cervical cancer: a review of the literature. *Crit Rev Oncol Hematol*, **66**, 10-20.

Harłozińska A, Bar JK, Gawlikowski W, et al (1991). CA 125 and carcinoembryonic antigen levels in cyst fluid, ascites and serum of patients with ovarian neoplasms. *Ann Chir Gynaecol*, **80**, 368-75.

Ismail MA, Rotmensch J, Mercer LJ, et al (1994). CA-125 in peritoneal fluid from patients with nonmalignant gynecologic disorders. *Reprod Med*, **39**, 510-2.

Kashimura M, Sugihara K, Toki N, et al (1997). The significance of peritoneal cytology in uterine cervix and endometrial cancer. *Gynecol Oncol*, **67**, 285-90.

Mlyneek M, Uhareek P (2005). Peritoneal cytology in endometrial cancer. *Neoplasma*, **52**, 103-8.

Sankaranarayanan R, Ferlay J (2006). Worldwide burd of gynaecological cancer; the size of the problem. *Bailliere's Best Pract Res*, **20**, 207-25,

Sari R, Yildirim B, Sevinc A, Bahceci F, Hilmioglu F (2001). The importance of serum and ascites fluid alpha-fetoprotein, carcinoembryonic antigen, CA 19-9, and CA 15-3 levels in differential diagnosis of ascites etiology. *Hepatogastroenterology*, **48**, 1616-21.

Selvaggi SM (2003). Diagnostic pitfalls of peritoneal washing cytology and the role of cell blocks in their diagnosis. *Diagn Cytopathol*, **28**, 335-41.

Sharifi S, Ducatman BS, Wang HH, Fraser JL (1999). Peritoneal washing cytology is unnecessary in gynecologic surgery for benign diseases. *Cancer Cytopathol*, **87**, 259-62.

Shield P (2004). Peritoneal washing cytology. *Cytopathology*, **15**, 131-41.

Sneige N, Thomison JB, Malpica A, et al (2012). Peritoneal washing cytologic analysis of ovarian serous tumors of low malignant potential to detect peritoneal implants and predict clinical outcome. *Cancer Cytopathol*, **120**, 238-44.

Takeshima N, Katase K, Hirai Y, et al (1997). Prognostic value of peritoneal cytology in patients with carcinoma of the

uterine cervix. *Gynecol Oncol*, **64**, 136-40

Topalak O, Saygili U, Soyturk M, et al (2002). Serum, pleural effusion, and ascites CA-125 levels in ovarian cancer and nonovarian benign and malignant diseases: a comparative study. *Gynecol Oncol*, **85**, 108-13.

Tuzun Y, Celik Y, Bayan K, et al (2009). Correlation of tumour markers in ascitic fluid and serum: are measurements of ascitic tumour markers a futile attempt? *J Int Med Res*, **37**, 79-86.

Yoon SM, Shin KH, Kim JY, et al (2007). The clinical values of squamous cell carcinoma antigen and carcinoembryonic antigen in patients with cervical cancer treated with concurrent chemoradiotherapy. *Int J Gynecol Cancer*, 17, 872-8.

Yurkovetsky Z, Skates S, Lomakin A, et al (2010). Development of a multimarker assay for early detection of ovarian cancer. *J Clin Oncol*, **28**, 2159-66.

Zuna RE, Behrens A (1996). Peritoneal washing cytology in gynecologic cancers: long-term follow-up of 355 patients. *J Natl Cancer Inst*, **88**, 980-7.