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www.turkjpath.org
This issue is published as 1000 copies
Printing Date: 16.08.2016

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06500, Ankara, Turkey
Tel : +90 (312) 223 55 44; +90 (312) 222 44 06
Fax: +90 (312) 222 44 07
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INTRODUCTION

Glucose is the basic energy source for the maintenance of human metabolism. In humans, glucose hemostasis is regulated by certain steps that are dependent on each other; glucose transport proteins (GLUT) are the major transport proteins regulating glucose entry into cells (1,2). There are 14 types of GLUT with different sensitivity to insulin stimulus, tissue distribution, and glucose affinity (2,3). GLUT-1 is the most well-known transport protein that is primarily found in erythrocytes, blood-brain barrier, liver and capillary endothelium (1-5).

Malignant cells need a higher glucose transporter gene expression due to their increased metabolic rates and glucose requirements. This expression and activity is regulated by some oncogenes and growth factors (4,6-8). Increased GLUT-1 expression has been reported in many human cancers such as lung, gallbladder, gastric, head and neck, colorectal, ovarian, pancreatic, esophageal, breast, laryngeal and bladder carcinoma (4-7, 9-15). Some studies in the literature have shown GLUT-1 expression in endometrial hyperplasia and endometrial carcinoma; however, only a few studies have scrutinized the impact of GLUT-1 expression on prognostic parameters and survival (16-19).

In this study, we analyzed the expression of GLUT-1 in endometrial hyperplasia to determine its role in endometrioid type adenocarcinoma, in hyperplasia and its correlation with tumor’s prognostic parameters and survival.

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GLUT-1 Expression in Proliferative Endometrium, Endometrial Hyperplasia, Endometrial Adenocarcinoma and the Relationship Between GLUT-1 Expression and Prognostic Parameters in Endometrial Adenocarcinoma

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ABSTRACT

Objective: Malignant cells show increased glucose uptake in in vitro and in vivo studies. This uptake is mediated by glucose transporter proteins. GLUT-1 is the most common transporter protein, and its expression is reported to be increase in many human cancers. The aim of this study is to determine the GLUT-1 overexpression in benign, hyperplastic, and malignant endometrial tissues, to evaluate the usefulness of GLUT-1 expression in endometrial hyperplasia, and to determine its role in the neoplastic progression to endometrioid type adenocarcinoma. We also aimed to analyze prognostic clinical parameters, predict prognosis, and survival.

Material and Method: We examined immunohistochemical expression of GLUT-1 in 91 cases of endometrial hyperplasia, 100 cases of endometrioid type adenocarcinoma, and 10 proliferative endometrial tissues. The percentage of positive cells and staining intensity were assessed in a semi quantitative fashion and scored (1+ to 3+).

Results: GLUT-1 immunoreactivity was not present in proliferative endometrium. Twenty-nine (31.9%) of 91 endometrial hyperplasia cases showed positive immunoreactivity, of which only six were cases of hyperplasia without atypia while 23 of them were cases with atypia. We found GLUT-1 positivity of 95% in endometrioid type adenocarcinoma. GLUT-1 overexpression was not significantly correlated with any of the clinicopathological parameters except histological grade in endometrioid adenocarcinoma; the survival was not found to be correlated with GLUT-1 expression.

Conclusion: GLUT-1 immunostaining may be useful in distinguishing hyperplasia without atypia from hyperplasia with atypia; GLUT-1 overexpression is a consistent feature of endometrioid adenocarcinoma. A correlation between GLUT-1 expression and tumor grade has been found, although other prognostic parameters and survival has no meaningful correlation.

Key Words: GLUT-1, Prognosis, Endometrial hyperplasia, Endometrioid adenocarcinoma
MATERIAL and METHOD

This study was approved by Çukurova University Faculty of Medicine Department of Pathology. Paraffin-embedded tissue blocks of 91 previously diagnosed cases of endometrial hyperplasia (EH), and 100 previously diagnosed endometrioid type endometrial adenocarcinoma (ETEA) cases were included. These patients underwent total abdominal hysterectomy, with bilateral salpingo-oophorectomy and pelvic lymph node dissection and omentectomy after diagnosis of endometrial cancer based on specimens obtained from curettage. Information was present on survivals, stages and clinical data. Exclusion criteria included unknown survival information and stages. The control group consisted of incidental endometrial tissue samples with proliferative endometrium characteristics that belong to patients who underwent curettage or underwent to hysterectomy for leiomyoma. In all cancer cases, age, clinical stage, histologic grade, myometrial invasion (>50%), lymph node metastases, lymphovascular space invasion, cervical and ovarian involvement, and peritoneal cytology positivity were determined. Surgical procedure and detailed pathologic reports were obtained and recorded.

All ETEA cases were reviewed. Clinical stage was assessed based on the evaluation of the surgical specimens, radiological and physical examination findings belonging to patients by two independent pathologists according to International Federation of Gynecology and Obstetrics (FIGO) 2009 system (20). Since the statistical evaluation of a single case will not be meaningful, the only Stage 4 case was included in Stage 3 cases. Subjects with no myometrial invasion were labeled as the first group; those with the involvement of ½ of superficial layer of myometrium as the second group; and those with the involvement of ½ of deep layer of myometrium as the third group. The subjects were categorized into 2 groups based on the status of lymphovascular invasion, lymph node metastasis, cervical and adnexal involvement, and positive peritoneal cytology. In all cancer patients, overall survival was determined in months. Only, cases with proven death related to cancer were analyzed. The-follow-up time was between 60 to 82 months.

EH cases were evaluated according to WHO 1994 classification at the time of their diagnosis. There was only one case diagnosed as “hyperplasia with simple atypia”, and this single case was included in the group of cases with “hyperplasia with complex atypia”. Hence, our hyperplasia cases were grouped as hyperplasia with or without atypia according to the latest, 2013 WHO classification (21). There were 51 EH cases without atypia and 40 EH cases with atypia.

Hematoxylin-Eosin stained preparations of the cases were examined in light microscope and suitable paraffin blocks were selected for each case. The immunohistochemical staining was performed on selected formalin fixed paraffin embedded tissues. Five μm thick sections were taken from the paraffin blocks, and the immunohistochemical studies were performed on this sections using Mouse monoclonal antibody GLUT-1 (Cat RB-078-A1 Neo Markers and dilution 1:50) with positive and negative control blocks. Placental tissue was used as a positive control.

GLUT-1 expression was determined immunohistochemically and all H.E stained slides were evaluated using the light microscope at 20x magnification with Nikon (Eclipse 800) microscopes used by two independent pathologists who were blind to patients’ clinical data. Only linear membranous staining was considered positive for GLUT-1 expression. The percentage of positive stained tumor cells in tissue samples were semi quantitatively determined (0-3).

Staining in at least 1% to 10% of tumor cells was considered as positive staining with GLUT-1. 0: negative staining, 1: 1-10% positive staining, 2: 10-50% positive staining, 3: ≥ 50% positive staining. The correlation between GLUT-1 staining score, ETEA, EH, and ETEA’s histopathological prognostic parameters, FIGO grade, myometrial invasion, lymphovascular invasion, cervical involvement, adnexal involvement, lymph node metastasis, peritoneal cytology positivity and survival was statistically evaluated.

Statistical analysis was performed by using the SPSS (Version 17.0, SPSS Inc., Chicago, IL, USA) statistical software package. Normally distributed continuous variables were presented as mean±standard deviation (normality was assessed using Kolmogorov-Smirnov test or, when n<30, Shapiro-Wilk’s test); non-normally distributed continuous variables were presented as median. Relationship between tissue GLUT-1 expression and clinical parameters was analyzed using χ² method, and Student’s t test. A p value of less than 0.05 was considered statistically significant.

RESULTS

The patient group was consisted of 91 EH cases diagnosed between the years 1985 and 2004, and 100 ETEA cases diagnosed between the years 1985 and 2003 at Çukurova University Faculty of Medicine Department of Pathology. The mean age of the ETEA cases was 57.81±10.957 years, with the youngest patient being 28 and the oldest 79 years of age. The EH cases had a mean age of 49.31 ±10.214, with the youngest patient being 27 and the oldest 79 years of age. The mean age of the control group was 47.30±2.830 years.

Patients with hyperplasia without atypia had a mean age of 48.30 ±10.497 years and those with atypia had a mean age of 50.9±9.675 years (p=0.104).
The study group of ETEA included 66 patients with stage 1 endometrioid carcinoma, 16 with stage II endometrioid carcinoma, 17 with stage III endometrioid carcinoma, and 1 patient with stage IV endometrioid carcinoma. Histological grade 1 (G1) was noted in 36 (36%) women, G2 in 44 (44%) women, and G3 in 20 (20%) women.

Among 91 EH cases, 51 (56%) had no atypia and 40 (44%) had atypia. While there was no GLUT-1 expression in 62 (68.1%) of the EH cases, 29 (31.9%) EH cases showed immunohistochemical GLUT-1 expression. GLUT-1 was not expressed more than 50% of cells in any of the cases.

Of the GLUT-1 positive EH cases, 6 (20.7%) had complex hyperplasia without atypia, 23 (79.3%) had hyperplasia with atypia. There was a significant difference between hyperplasia cases with and without atypia with respect to GLUT-1 expression (p=0.0001) (Figure 1,2).

A significant correlation was found between GLUT-1 expression and grade in ETEA cases (p=0.007). No statistically significant differences were observed between GLUT-1 expression and myometrial invasion (p=0.667), lymph node metastases (p=0.776), cervical involvement (p=0.460), adnexal involvement (p=0.335), vascular invasion (p=0.775) and positive peritoneal cytology (p=0.570). The correlation between GLUT-1 expression and prognostic parameters in ETEA was shown on (Table I).

While there was no GLUT-1 expression in 5 (5%) of ETEA cases, it was positive in 95 (95%) of ETEA cases (Figure 3,4). None of the tissue samples of the proliferative endometrium

**Figure 1:** Immunohistochemically staining GLUT-1 in hyperplastic endometrium without atypia (GLUT-1; x200).

**Figure 2:** Immunohistochemically staining GLUT-1 in hyperplastic endometrium with atypia (GLUT-1; x400).

**Figure 3:** GLUT-1 staining in endometrioid type endometrial adenocarcinoma, Grade II (GLUT-1; x200).

**Figure 4:** GLUT-1 staining in endometrioid type endometrial adenocarcinoma, Grade I (GLUT-1; x200).
in the control group showed GLUT-1 expression. The distribution of GLUT-1 expression among EH, ETEA, and control groups was shown on (Table II).

The correlation between GLUT-1 expression and survival was analyzed and the survival time was assessed. The patients’ median overall survival when GLUT-1 was expressed in more than ≥50% of cells was 60 months. The patients’ median overall survival when GLUT-1 was expressed in less than <50% of cells was 82 months. No statistically significant differences were observed between GLUT-1 expression and survival (p=0.5).

<table>
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<th>Endometrial carcinoma Stage</th>
<th>GLUT-1(-) n(%)</th>
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<td>1</td>
<td>66</td>
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<th>GLUT-1&gt;50% n(%)</th>
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<tr>
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<tr>
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<th>GLUT-1&gt;50% n(%)</th>
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<td>67 (74.4%)</td>
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<tr>
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<td>7 (77.8%)</td>
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<th>GLUT-1&gt;50% n(%)</th>
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</thead>
<tbody>
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<td>2 (3%)</td>
<td>52 (77.6%)</td>
<td>13 (19.4%)</td>
</tr>
<tr>
<td>(+)</td>
<td>9</td>
<td>0 (0%)</td>
<td>6 (66.7%)</td>
<td>3 (33.3%)</td>
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</table>

Table I: The prognostic parameters of endometrioid type endometrial adenocarcinoma cases and correlation between GLUT-1 expression and prognostic parameters.

Table II: GLUT-1 expression in endometrial hyperplasia, endometrioid type endometrial adenocarcinoma, and control groups.

DISCUSSION

Endometrial cancer is the most common gynecological cancer in Europe and North America and the seventh most common cause of death worldwide; more than 80% of endometrial cancers are ETEA (22,23). To date, the pathogenesis of endometrial cancer remains unclear, and it is considered to represent a multi-step, multi-stage, multi-factor complex biological process involving a number of genetic variations (24,25). Molecular, endocrinological, and epidemiological alterations typical for epithelial tumors differ between the two mentioned types of epithelial cancers. In other words, a concrete aberration can be different for a type I and a type II tumor. Type I tumors are estrogen-
dependent, and associated with endometrial hyperplasia, whereas type II tumors are estrogen-independent and associated with endometrial atrophy. Type I tumors are the most common histological type and comprise low-grade adenocarcinomas (25, 26).

EH is the major preneoplastic lesion for type I tumors (27, 28). EH is the most common overdiagnosed condition that is surgically treated (29). WHO classification dated 1985 is the oldest and most widely used classification system and it separates endometrial proliferation into simple or complex hyperplasia on the basis of architectural features, and typical or atypical on the basis of cytological features that are originally defined by Kurman et al. in 1985. The classification system was based on both structural and cellular properties, and has been shown to be associated with intraobserver variation. Furthermore, it has been reported that cellular atypia rather than structural properties increases carcinoma risk (30, 31). In the latest WHO’2013 classification that replaced the WHO’s 1994 classification, EHs were classified in a two–group system as hyperplasia without atypia and hyperplasia with atypia (21). Hyperplasia cases were also assessed according to WHO 2013 classification in our study. For diagnosing ETEA cases at an early stage, it is highly important to define EH cases and differentiate hyperplasia cases with atypia that constitute a high risk group from other hyperplasia’s, and distinguish well-differentiated ETEAs from other lesions in order to provide necessary appropriate treatments and to avoid over diagnosis. The main problem of the current and the previous classification systems is their subjectivity in the determination of atypia. Therefore, searching for adjunctive diagnostic methods, that would be helpful in addition to current morphological criteria to define hyperplasia with atypia gains increasing importance. It is a major unmet need to determine the presence of atypia by an objective criterion and to differentiate well-differentiated adenocarcinoma and hyperplasia especially in small endometrial biopsy and curettage samples. Although some cytometric, immunohistochemical, and molecular genetic alterations have been studied for use in the differential diagnosis, a method for routine practice that is more useful than light microscopic findings is yet to be found.

To date, many studies have indicated that malignant neoplasms have greater metabolic activity and variable glucose need compared to normal tissues. It has been shown that glucose utilization is increased in vivo and in vitro conditions (1-4,16). This increase is the result of the increased expression of glucose transport proteins that mediate glucose entry into cells. GLUT-1 is a well-known member of 14 glucose transfer proteins (4-19, 32). Since GLUT-1 protein is found in cellular membrane, its increased expression can immunohistochemically be detected in the form of membranous staining (5,12,16,19).

A number of studies have shown that GLUT-1 is overexpressed very early in preneoplastic and premalignant lesions including colonic adenoma, borderline ovarian tumors, cervical intraepithelial neoplasia, and prostatic intraepithelial neoplasia (9,10,33,34). Kalir et al. (10) showed that GLUT-1 expression predicted malignant progression in benign borderline and malignant tumors of ovary; they also reported that all of the invasive serous borderline implants were GLUT-1 positive whereas 3 noninvasive implants were GLUT-1 negative. It is quite difficult to differentiate this in routine H&E stained preparations. These studies support the notion that GLUT-1 may take part in carcinogenesis of colonic and ovarian carcinomas and may be useful in detecting preneoplastic lesions (9,10).

As far as we know, the study by Wang et al. (16) is the first to show GLUT-1 expression in EH and tumors. In that study, GLUT-1 expression was detected in all EHs with atypia and endometrial carcinomas. Studies in subsequent years have demonstrated varying rates of GLUT-1 expression in EH (71%, 58.3%, 58%) and ETEA (90%, 70.8 %, 71%) (17-19). Ma et al. in 2015 (35) found positive GLUT-1 expression in 25% of EH cases and 70% of tumors. Although GLUT-1 expression in that study was lower in EH cases compared to that found in our study, it was not specified whether hyperplasia cases had atypia or not. In our study, GLUT-1 expression rate was 79.3% in hyperplasia with atypia and 20.7% in hyperplasia without atypia. All of our tumor cases had GLUT-1 expression. Statistically, this finding seems to support the idea that GLUT-1 immunostaining could be useful as an additional marker distinguishing cases of hyperplasia with atypia from those without atypia. This distinction would be guiding to detect EH with atypia, which is a strong risk factor for endometrial carcinoma that is known to play an important role in the genesis of endometrial tumors, especially Type 1. GLUT-1 is highly expressed in endometrial cancer, which can be used to differentiate benign endometrium from atypically hyperplastic endometrium (17-19,35). Since increased expression of GLUT-1 is already known in many neoplasms, its relationship with prognostic parameters has been studied. The earliest and the most striking study on this subject to date is the one that was conducted on colon cancer. In addition to indicate GLUT-1 as a good marker to determine aggressive biological behavior of colorectal carcinomas, it also showed a direct correlation between
lymph node metastases and GLUT-1 expression (9). GLUT-1 positivity rate was found to be 74% in lung tumors. It was shown to be associated with poor differentiation and correlated to a larger tumor size and lymph node positivity (36). Kawamura et al. (37) demonstrated that GLUT-1 was correlated to the tumor’s invasion depth, lymphatic spread, venous invasion, lymph node metastasis, liver metastasis, and stage of gastric carcinoma. In endometrial tumors, on the other hand, the correlation between GLUT-1 expression and clinical characteristics, i.e. increasing stage, decreasing degree of differentiation, and lymphatic metastasis, was significant (P<0.05) (35). Among ETEA’s prognostic parameters, grade was found to be significantly correlated to GLUT-1 expression in our study. GLUT-1 expression was not significantly correlated to other prognostic factors (myometrial invasion, lymphovascular invasion, cervical and adnexal involvement, lymph node metastasis, positive peritoneal lavage fluid). In 2010 Xiong et al. (19) similarly failed to show a correlation between the prognostic parameters and GLUT-1 expression in endometrioid adenocarcinoma. In accordance with the literature data, our results suggest that GLUT-1 is a marker of early stages of endometrial neoplastic transformation (17-19,35,37).

Haber et al. reported that the mortality rate of colon carcinoma increased 2.4 folds in patients with a GLUT-1 expression of ≥50% compared to ones with a GLUT-1 expression of <50% (9). Another study demonstrated that GLUT-1 expression was inversely proportional to survival in gallbladder carcinoma (38). Overexpression of GLUT-1 and increased glucose metabolism in tumors are associated with a poor prognosis in patients with oral squamous cell carcinoma, and GLUT-1 has been shown to be a significant negative biomarker of prognosis and overall survival (39). In our study, mean survival was 60 months in patients with a GLUT-1 expression of more than 50% versus 82 months in those with an expression of less than 50%. That is, as GLUT-1 expression increases, survival decreases, although this correlation was not significant.

In conclusion, the expression of GLUT-1 in EH with atypia and ETEA may play a role in the diagnosis, and may be helpful in distinguishing EH without atypia from EH with atypia. GLUT-1 may guide physicians in determining cases with atypia who are at high risk for cancer development. GLUT-1’s position in this field can be determined by close clinical follow-up of cases that have both atypia and a high GLUT-1 expression rate. GLUT-1 plays a role at the early stage of endometrial carcinogenesis, and it may be used as a subsidiary parameter to show the correlation of prognostic parameters and histological grade in ETEA cases.

Our study or other previous studies could not demonstrate any significant relationship between GLUT-1 expression and other prognostic parameters and survival.

CONFLICT OF INTEREST
The authors declared no conflict of interest.

FUNDING SOURCE
The authors declared no funding source was involved in the creation of this manuscript.

REFERENCES


Circulating Tumor Cells in Breast Cancer: Correlation with Clinicopathological Parameters, Hormone Profile and MicroRNA Polymorphisms

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1Department of Surgical Oncology, AIIMS, Jodhpur, RAJASTHAN, INDIA,
*Department of Pathology, King George Medical University, Lucknow, UTTAR PRADESH, INDIA

ABSTRACT

Objective: Circulating tumor cells are isolated tumor cells in the peripheral blood that serve as important prognostic indicators for many kind of tumors. The study was conducted to know the rate of detection of circulating tumor cells among breast cancer patients in comparison with benign breast diseases and control subjects and to know the association between CTC positivity and various clinicopathological parameters, hormonal profile and microRNA polymorphisms.

Material and Method: In the present case control study, we included 182 healthy controls, 108 cases of benign breast disease and 114 breast carcinoma cases. Various clinicopathological details of cases were recorded. Immunohistochemistry was performed for estrogen (ER) and progesterone receptors (PR) and Her-2 neu. Circulating tumor cells were analyzed using flow cytometry (EPC, carcinoma cases). Various clinicopathological details of cases were recorded. Immunohistochemistry was performed for estrogen (ER) and progesterone receptors (PR) and Her-2 neu. Circulating tumor cells were analyzed using flow cytometry (EPC). Genotypic frequency of micro RNA polymorphisms was determined by PCR-RFLP assay.

Results: Circulating tumor cell positivity was observed in 11/114 (9.64%) breast cancer cases but absent in benign and control groups, and was significantly associated with tumor size, histologic type, tumor grade, metastasis and skin infiltration (p<0.05). Circulating tumor cell positivity did not show any correlation with the immunohistochemical profile. No significant associations between pre-miRNA genetic variations miR-196a2 C/T (rs11614913), miR-146a G/C (rs2910164) and miR-499 T>C (rs3746444) polymorphisms and circulating tumor cell positivity were observed.

Conclusion: The flow cytometry protocol for detection and molecular characterization of circulating tumor cells is a time and cost-effective technique, suitable for routine clinical use. However, more elaborate studies are needed to establish the findings as our study was limited by small sample size.

Key Words: Breast cancer, Circulating tumor cells, Hormone receptors, microRNA, Flow cytometry

INTRODUCTION

Breast cancer is by far the most common cancer among women worldwide. According to the incidence of cancers, breast cancer ranks second in the world (1). According to the National Cancer Registry Program’s (NCRP) recent report for 2008, the load of breast and cervical cancers together was 23.6-38.7% of total cancers in the Northeastern states of India, while in all the other states these two cancers contributed 35.2-57.7% of the total cancers (2). Different published reports of cancer registries in India indicate rising trends in breast cancer incidence (3).

The tumor cells shed into blood circulation from primary or metastatic cancers are referred to as circulating tumor cells (CTC). Although rare, CTC serves as a biomarker to evaluate the tumor genotypes during the course of treatment and progression of the disease. A proportion of CTC are capable of initiating a metastatic clone. CTC have been identified in a variety of epithelial cancers, predominantly breast, prostate, lung and colon. CTC are more likely to be detected in patients with metastatic disease, and they have also been reported in localized cancers (4,5).

For detection of CTC, a number of techniques are currently available, but none of these approaches constitute a desired optimal level to serve as a gold standard. Available techniques for CTC isolation and detection include either nucleic acid based detection (free DNA or RNA) (cell free circulating DNA, cfDNA) or intact CTC detection based on their physical properties (large cell size, differences in density, charge, migratory properties, granules etc.) or detection of CTC by directing antibodies against cell surface antigens (Cell Search System- FDA approved method, Isoflux and Flow cytometry). Among the cell
surface antigens used with these technologies, the most widely used antibody is directed against epithelial cell adhesion molecule (EpCAM) (4-7).

CTC serve as important prognostic indicators. Various studies have concluded that CTC serve as independent prognostic markers in cancers of breast, prostate, lung and colorectum. The potential applications for CTC include isolation and identification of CTC (early diagnosis and prognosis), alteration in CTC levels to evaluate the response to new therapies (prognosis and prediction) and CTC phenotype and genotype (diagnosis, prognosis and direct therapy).

**MATERIAL and METHODS**

In the present case control study, we included 182 healthy controls, 108 cases of benign breast disease and 114 carcinoma breast cases. Healthy controls and diseased studied in the present work were of North Indian ethnicity and unrelated to each other. Patients were recruited (Dec 2010- Nov 2012) from the surgical oncology department; King George Medical University, Lucknow, India. Breast carcinoma patients included were those who had not received neoadjuvant chemotherapy yet. Controls were from healthy population and unrelated to diseased subjects. Informed consent in written was taken from all the study subjects. Approval from the institutional ethical committee was taken for the study protocols and the work done. The World Medical Association Declaration of Helsinki’s norms were followed by the authors. Controls included fulfilled the criteria: no chronic disease, no history of present/past malignancy or premalignant lesion. Cancer cases were frequency-matched to all the controls for characteristics like age, gender, and ethnicity.

Immunohistochemistry for estrogen receptor (ER), progesterone receptor (PR) and her-2 neu were performed on representative blocks of paraffin embedded tumor tissue. 4μm thick sections were taken on poly-L-lysine coated slides and submitted for immunohistochemistry. Antigen retrieval was done using citrate buffer at pH 2.5 for hormone receptors and pH 6 for her-2 neu. The normal breast ducts served as internal positive control for ER/PR. Breast carcinoma with known her-2 neu overexpression served as an external positive control for her-2 neu staining. ER or PR were considered positive when more than 1% of tumor cell nuclei were immunoreactive.

For interpretation of Her-2 neu staining the following method was used (8):

Score 0 (Negative): No staining is observed or membrane staining is observed in less than 10% of the tumor cells

Score 1+ (Negative): A faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells are only stained in part of their membrane

Score 2+ (Weakly positive): A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells

Score 3+ (Strongly positive): A strong complete membrane staining is observed in more than 30% (formerly 10%) of the tumor cells

Score 3+ was considered as positive immunostaining for Her-2 neu.

**Flow Cytometry**

This was performed on Beckton-Dickinson Fluorescence Activated Cell Sorter (FACS). The samples were immunostained with EpCAM peridinin chlorophyll protein complex, CD45 fluorescein isothiocyanate, and pan cytokeratin (CK – 8/18/19-phycoerythrin (PE) (all from BD Biosciences, San Jose, CA) for 30 minutes at 4°C. BD FACS lyse buffer (BD Biosciences) was added for 15 minutes after staining to lyse RBCs. A total of 500,000 events were collected for analysis on a 2-laser, 6-color BD FACS Canto device using BD FACS Diva software (both from BD Biosciences). The data were exported as FCS 3.0 files and analyzed using Flowjo (Tree Star, Ashland, OR) analysis software.

Genotypic frequency of miRNA polymorphisms was determined by PCR-RFLP assay. Details of genotyping and statistical analysis for miRNA’s have been given in our prior publication (9).

**Statistical Analysis**

The Statistical analysis was done by SPSS Software version 15.0 and graph pad prism version 5.01. We applied Chi-square and Fisher’s exact test wherever required.

**RESULTS**

**Characteristic Profile of Controls, Benign and Carcinoma Cases**

The present study included 404 study subjects, out of which 114 were breast carcinoma cases, 108 benign breast disease and 182 controls. Benign or malignant cases were biopsy/cytology-proven. Mean ages were 36, 33 and 64 years for controls, benign and malignant disease respectively. Most of the patients in control group (69%), benign breast disease group (62.28%) and breast carcinoma group (69.29%) were Hindus followed by Muslims. Premenopausal patients formed the majority in all study groups i.e. controls

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(88.46%), benign (100%) and carcinoma cases (62.28%). Details are shown in Table I.

**Clinico-Pathological Profile of Breast Cancer and CTC Positive Cases**

Eleven out of 114 breast cancer cases were positive for CTC (9.64%), with no CTC positive case in either control or benign group. In the <40 years age group, 3/36 (8.33%) patients were found to be CTC positive while 8/78 (10.25%) were CTC positive in > 40 years age group. Out of all CTC positive cases (11), 72.72% (8/11) were above 40 years of age (Figure 1). CTC positivity in premenopausal vs postmenopausal group was found to be 5.63% vs 16.27% respectively. Most of the CTC positive cases (63.69%; 7/11) were postmenopausal (Figure 2). Neither age of patients nor menopausal status was found to have any association with CTC positivity (Table II).

None of the CTC positive cases belonged to T1 group (tumor size <2 cm). In the T2 group (tumor size 2-5 cm), there were 2/61 (3.27%) cases while CTC positivity was very high (19.56%) in the tumors >5cm in size (T3). The difference was found to be statistically significant (p=0.0049). 9/11 (81.82%) CTC positive cases belonged to T3 group while 2/11 (18.18%) belonged to T2 group (Figure 3).

Regarding histologic type, the number of cases of invasive ductal carcinoma (IDC) with CTC positivity was 9/111 (8.1%) compared to 2/3 (66.67%) for invasive lobular carcinoma, which was statistically significant (p=0.0242). Out of all CTC positive cases, 81.82% belonged to IDC while 18.18% belonged to ILC (Figure 4, 5). CTC positivity when seen in relation to grade of tumor was highest for grade 3 (31.25%) followed by grade 2 (1.31%) and grade 1 (0) and the difference was found to be statistically significant (p=0.0001) (Figure 6).

Although CTC positivity was higher in node positive group (11.39%), we found 2 CTC positive cases (2/35; 5.71%) in node negative group as well but no association was found between CTC positivity and lymph node status. 81.82% of

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**Table I:** Characteristic profile of study subjects

<table>
<thead>
<tr>
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<th>Benign breast disease (n=108) distribution no. (%)</th>
<th>Control subjects (n=182) no. (%)</th>
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<td>0 (0)</td>
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<td>21 (11.53)</td>
</tr>
</tbody>
</table>

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**Figure 1:** Comparison of age distribution in CTC positive and negative cases.

**Figure 2:** Comparison of menopausal status in CTC positive and negative cases.
all CTC positive cases had lymph node metastasis (Figure 7). Positive CTC cases were 50% in patients with skin infiltration by the tumor compared to only 3.06% in those without skin infiltration, the difference being statistically significant (p < 0.0001). Among all CTC positive cases, 72.73% had skin infiltration by tumor (Figure 8).

**Table II: Clinicopathological profile of breast carcinoma cases and circulating tumor cells (CTC) positive cases**

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<tr>
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<tr>
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<td>&gt;40 years</td>
<td>78 (68.43)</td>
<td>8/11 (72.72)</td>
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<td>Religion</td>
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<td>8/11 (72.72)</td>
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<td>3/11 (27.28)</td>
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<td>0/11 (0)</td>
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<td>0/11 (0)</td>
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<td>4/11 (36.36)</td>
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<td>7/11 (63.64)</td>
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<td>61 (53.50)</td>
<td>2/11 (18.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;5 cm</td>
<td>46 (40.35)</td>
<td>9/11 (81.82)</td>
</tr>
<tr>
<td>5.</td>
<td>Tumor type</td>
<td>IDC</td>
<td>111 (97.36)</td>
<td>9/11 (81.82)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ILC</td>
<td>3 (2.64)</td>
<td>2/11 (18.18)</td>
</tr>
<tr>
<td>6.</td>
<td>In situ component</td>
<td>Absent</td>
<td>66 (57.89)</td>
<td>7/11 (63.64)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>48 (42.11)</td>
<td>4/11 (36.36)</td>
</tr>
<tr>
<td>7.</td>
<td>MRB grade</td>
<td>I</td>
<td>6 (5.26)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>76 (66.66)</td>
<td>1/11 (9.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>32 (28.08)</td>
<td>10/11 (90.91)</td>
</tr>
<tr>
<td>8.</td>
<td>Lymph node</td>
<td>Absent</td>
<td>35 (30.70)</td>
<td>2/11 (18.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>79 (69.3)</td>
<td>9/11 (81.82)</td>
</tr>
<tr>
<td>9.</td>
<td>Skin infiltration</td>
<td>Absent</td>
<td>98 (85.96)</td>
<td>3/11 (27.27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>16 (14.04)</td>
<td>8/11 (72.73)</td>
</tr>
<tr>
<td>10.</td>
<td>Metastasis</td>
<td>Absent</td>
<td>102 (89.47)</td>
<td>2/11 (18.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>12 (10.53)</td>
<td>9/11 (81.82)</td>
</tr>
<tr>
<td>11.</td>
<td>Intratumoral and peritumoral lymphocytes</td>
<td>Absent</td>
<td>61 (53.50)</td>
<td>6/11 (54.54)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>53 (46.50)</td>
<td>5/11 (45.46)</td>
</tr>
</tbody>
</table>

**Figure 3:** Comparison of tumor size in CTC positive and negative cases.

**Figure 4:** Distribution of tumor type among CTC positive and negative cases.
75% of CTC positive cases were observed in the metastatic breast cancer group while CTC positivity was 1.96% in the non metastatic group, which was statistically significant (p< 0.0001). 81.82% of all CTC positive cases had distant metastasis (Figure 9). There was not much difference in CTC positivity between breast cancer cases with and without intratumoral and peritumoral lymphocytes (9.43 vs 9.83) (Figure 10). Details are shown in Table III.
Table III: Distribution of breast cancer cases and CTC positive/negative cases

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Variables</th>
<th>Status</th>
<th>Breast cancer cases distribution, No. (%)</th>
<th>CTC positive cases distribution in relation to breast cancer cases distribution, No. (%)</th>
<th>CTC negative cases distribution in relation to breast cancer cases distribution, No. (%)</th>
<th>p value</th>
<th>Odds ratio (95% CI)</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
<td>&lt; or = 40 years</td>
<td>36 (31.57)</td>
<td>3/36 (8.33)</td>
<td>33/36 (91.67)</td>
<td>1</td>
<td>-</td>
<td>Fischer’s exact test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;40 years</td>
<td>78 (68.43)</td>
<td>8/78 (10.25)</td>
<td>70/78 (89.74)</td>
<td></td>
<td>0.053</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Menopausal status</td>
<td>Pre-menopausal</td>
<td>71 (62.28)</td>
<td>4/71 (5.63)</td>
<td>67/71 (94.37)</td>
<td>0.0988</td>
<td>-</td>
<td>Fischer’s exact test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-menopausal</td>
<td>43 (37.72)</td>
<td>7/43 (16.27)</td>
<td>36/43 (83.72)</td>
<td></td>
<td>0.0049*</td>
<td>Chi-square test for p-trend</td>
</tr>
<tr>
<td>3</td>
<td>Tumor size</td>
<td>&lt; or = 2 cm</td>
<td>7 (6.14)</td>
<td>0/7 (0)</td>
<td>7/7 (100)</td>
<td>0.0242*</td>
<td>0.04 (0.003-0.53)</td>
<td>Fischer’s exact test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;2-5 cm</td>
<td>61 (53.50)</td>
<td>2/61 (3.27)</td>
<td>59/61 (96.72)</td>
<td></td>
<td>0.0049*</td>
<td>Chi-square test for p-trend</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;5 cm</td>
<td>46 (40.35)</td>
<td>9/46 (19.56)</td>
<td>37/46 (80.43)</td>
<td></td>
<td>0.7582</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Tumor type</td>
<td>IDC</td>
<td>111 (97.36)</td>
<td>9/111 (8.1)</td>
<td>102/111 (91.89)</td>
<td>-</td>
<td>-</td>
<td>Fischer’s exact test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ILC</td>
<td>3 (2.64)</td>
<td>2/3 (66.66)</td>
<td>1/3 (33.33)</td>
<td></td>
<td>0.0242*</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>In situ component</td>
<td>Absent</td>
<td>66 (57.89)</td>
<td>7/66 (10.6)</td>
<td>59/66 (89.39)</td>
<td>-</td>
<td>0.7582</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>48 (42.11)</td>
<td>4/48 (8.33)</td>
<td>44/48 (91.67)</td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>MRB grade</td>
<td>I</td>
<td>6 (5.26)</td>
<td>0/6 (0)</td>
<td>6/6 (100)</td>
<td>-</td>
<td>0.4988</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>II</td>
<td>76 (66.66)</td>
<td>1/76 (1.31)</td>
<td>75/76 (98.68)</td>
<td></td>
<td>&lt;0.0001*</td>
<td>Chi-square test for trend</td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td>32 (28.08)</td>
<td>10/32 (31.25)</td>
<td>22/32 (68.75)</td>
<td></td>
<td>0.03158 (0.006 to-0.14)</td>
<td>Fischer’s exact test</td>
</tr>
<tr>
<td>7</td>
<td>Lymph node</td>
<td>Absent</td>
<td>35 (30.70)</td>
<td>2/35 (5.71)</td>
<td>33/35 (94.29)</td>
<td>-</td>
<td>0.006</td>
<td>Fischer’s exact test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>79 (69.3)</td>
<td>9/79 (11.39)</td>
<td>70/79 (88.61)</td>
<td></td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Skin infiltration</td>
<td>Absent</td>
<td>98 (85.96)</td>
<td>3/98 (3.06)</td>
<td>95/98 (96.94)</td>
<td>-</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>16 (14.04)</td>
<td>8/16 (50)</td>
<td>8/16 (50)</td>
<td></td>
<td>0.0001*</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Metastasis</td>
<td>Absent</td>
<td>102 (89.47)</td>
<td>2/102 (1.96)</td>
<td>100/102 (98.04)</td>
<td>-</td>
<td>0.0009 - 0.045</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>12 (10.53)</td>
<td>9/12 (75)</td>
<td>3/12 (25)</td>
<td></td>
<td>0.0001*</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Intratumoral and peritumoral lymphocytes</td>
<td>Absent</td>
<td>61 (53.50)</td>
<td>6/61 (9.83)</td>
<td>55/61 (90.16)</td>
<td>1</td>
<td>-</td>
<td>Fischer’s exact test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>53 (46.50)</td>
<td>5/53 (9.43)</td>
<td>48/53 (90.57)</td>
<td></td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

CTC: Circulating tumor cells), OR: Odds Ratio, CI: Confidence Interval, p-value<0.05 was considered significant. *refers to significant p-value given in bold. Sample size is too small and confidence intervals are therefore very wide.

Hormone Receptor Status of Breast Cancer and CTC Positive Cases

Out of all the CTC positive cases, 72.72% were ER negative, 54.54% were PR negative, 63.64% were HER-2 neu negative while 72.72% were triple negative as depicted in Table IV.

Most of the CTC positive breast cancer cases were estrogen receptor (ER) negative (16.32% vs. 4.61% in CTC positivity in ER negative vs ER positive groups). However, the difference was not statistically significant (p= 0.053). CTC positivity did not show much difference in progesterone receptor (PR) negative vs PR positive groups (9.83% vs. 9.43%) (Table V).

In the HER-2 neu positive group, CTC were detected in 4/55 patients (7.27%) compared to 7/59 (11.86%) in the HER-2 neu negative group. In the triple negative tumors (ER, PR, HER-2 neu negative), CTC positivity was observed in 8 out of 32 cases (25%). The details are depicted in Table V.
Table IV: Hormone receptor status of breast carcinoma cases and circulating tumor cells (CTC) positive cases

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Hormone receptor</th>
<th>Status</th>
<th>Breast cancer cases (114) distribution no. (%)</th>
<th>CTC positive cases (11) distribution in no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ER</td>
<td>Negative</td>
<td>49 (42.98)</td>
<td>8/11 (72.72)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>65 (57.02)</td>
<td>3/11 (27.28)</td>
</tr>
<tr>
<td>2</td>
<td>PR</td>
<td>Negative</td>
<td>61 (53.50)</td>
<td>6/11 (54.54)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>53 (46.50)</td>
<td>5/11 (45.46)</td>
</tr>
<tr>
<td>3</td>
<td>Her-2/Neu</td>
<td>Negative</td>
<td>59 (51.75)</td>
<td>7/11 (63.64)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Positive</td>
<td>55 (62.25)</td>
<td>4/11 (36.36)</td>
</tr>
<tr>
<td>4</td>
<td>ER/PR/Her-2</td>
<td>Triple negative</td>
<td>32 (28.07)</td>
<td>8/11 (72.72)</td>
</tr>
</tbody>
</table>

CTC: Circulating tumor cells.

Table V: Distribution of breast cancer cases and CTC positive/negative cases in relation to hormone receptor status

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Hormone receptor</th>
<th>Status</th>
<th>Breast cancer cases distribution no. (%)</th>
<th>CTC positive cases in relation to breast cancer cases distribution no. (%)</th>
<th>CTC negative cases in relation to breast cancer cases distribution no. (%)</th>
<th>p value</th>
<th>Statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ER</td>
<td>ER Negative</td>
<td>49 (42.98)</td>
<td>8/49 (16.32)</td>
<td>41/49 (83.67)</td>
<td>0.0532</td>
<td>Fischer's exact test</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ER Positive</td>
<td>65 (57.02)</td>
<td>3/65 (4.61)</td>
<td>62/65 (95.38)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PR</td>
<td>PR Negative</td>
<td>61 (53.50)</td>
<td>6/61 (9.83)</td>
<td>55/61 (90.16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PR Positive</td>
<td>53 (46.50)</td>
<td>5/53 (9.43)</td>
<td>48/53 (90.57)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Her-2/Neu</td>
<td>Her-2/Neu Negative</td>
<td>59 (51.75)</td>
<td>7/59 (11.86)</td>
<td>52/59 (88.14)</td>
<td>0.5309</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Her-2/Neu Positive</td>
<td>55 (62.25)</td>
<td>4/55 (7.27)</td>
<td>51/55 (92.73)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>ER/PR/Her-2</td>
<td>Triple negative</td>
<td>32 (28.07)</td>
<td>8/32 (25.0)</td>
<td>24/32 (75)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CTC: Circulating tumor cells, ER: Estrogen receptor, PR: Progesterone receptor, OR: Odds Ratio, CI: Confidence Interval, p-value<0.05 was considered significant. Sample size is too small and confidence intervals are therefore very wide.

In nutshell, CTC positivity was observed to be significantly associated with tumor size, histologic type, tumor grade, metastasis and skin infiltration.

Pre-miRNA Genetic Variations (miR-196a2 C/T (rs11614913), miR-146a G/C (rs2910164) and miR-499 T>C (rs3746444) Polymorphisms and Circulating Tumor Cell (CTC) Status

In the present study, we did not find any significant associations between pre-miRNA genetic variations miR-196a2 C/T (rs11614913), miR-146a G/C (rs2910164) and miR-499 T>C (rs3746444) polymorphisms and Circulating tumor cells (CTC) positivity in susceptibility to breast cancer (data not shown). Due to very low sample size, there were not significant cases in each group, so we were not able to analyze the association between the pre-miRNA genetic variations (miR-196a2 C/T (rs11614913), miR-146a G/C (rs2910164) and miR-499 T>C (rs3746444) polymorphisms and Circulating tumor cells (CTCs) unlike our previous work in which we could find associations between miR and breast cancer risk (9).

DISCUSSION

According to GLOBOCAN 2012 (WHO), breast cancer is the second most common cancer in the world and, by far, the most frequent cancer among women with an estimated 1.67 million new cancer cases diagnosed in 2012 (25% of all cancers). Breast cancer ranks as the fifth cause of death from cancer overall (522,000 deaths) and is the most frequent cause of cancer death in women in less developed regions (324,000 deaths, 14.3% of total). An estimated 70218 women died in India due to breast cancer, which is highest than any other country in the world (10). Thus early diagnosis by adequate screening of the breast lump...
is of prime importance to safeguard the health of women globally and particularly for our country.

Many patients continue to die of the disease especially in developing countries like India, including those diagnosed at an early stage despite advances in early detection and treatment. It is believed that after the completion of primary therapy, minimal residual disease ultimately leads to disease relapse and distant metastases. Circulating tumor cells are isolated epithelial cells with similar characteristics to the tumor cells of the primary site that have been identified in the peripheral blood of many solid cancers like breast, prostate and colon. The greatest challenge lies in the detection of these rare cells (1 in 10^6 to 1 in 10^7 of all nucleated cells) from among numerous hematopoietic cells (5,6).

A variety of methods have been developed for detection of CTC. To increase the chances of detecting these cells, we require techniques that utilize different methods to increase the concentration of CTC in blood, namely, differential centrifugation, Ficoll enrichment and cell separation by immunomagnetic technique. Another limitation is the loss of malignant cells on account of their fragility. The positive detection of CTC has been used in a number of techniques like immunohistochemistry, immunofluorescence, Fluorescent in situ hybridization (FISH), flow cytometry, southern blot, Northern Blot, Polymerase chain reaction (PCR), Real time PCR, etc (4-7).

Out of this exhaustive list, the Cell Search System is the most commonly utilized, commercially available technique, which is FDA approved. It is a semi quantitative device for detection of CTC based on expression of epithelial cell adhesion molecule (EpCAM) with antibody coated magnetic beads as an enrichment media. CTC are defined as cytokeratin +/-CD 45 – nucleated cells (4-7). Cristofanilli et al. (11) used cell search system to detect CTC and theirs’ was the first study to establish a threshold of 5 CTC per 7.5 ml blood for differentiating between patients with favourable and unfavourable prognosis.

Flow cytometry has also been applied for detection of CTC in patients with metastatic cancers by Riethdorf et al. (12), however, they found it to be less sensitive. Cruz et al. (13) comparatively evaluated different cytokeratin types (CK 7, CK 20, pan CK, CK8/CK18, CK 8 and CK 18) by flow cytometry for identification of best combination of DNA/ CK staining for detecting scarce circulating breast cancer cells. They observed that CK 18 was the brightest and more sensitive staining for breast cancer cells by flow cytometry. The advantage of this method is that a special machine is not required, so it can be of great utility in resource poor settings especially in developing countries like India.

Hristozova et al. (14) described a sensitive and reliable multicolor flow cytometry protocol for CTC detection by using an electronic threshold during data acquisition.

There is an ongoing debate as to which is better: morphologic or molecular detection of CTC. Slide based counting has the advantage of being highly specific, but many authors believe that this method has low sensitivity compared to quantitative mRNA techniques.

The importance of detecting CTC is more if it can be done in early stage cancers when metastasis has not taken place so that appropriate therapeutic remedy can be provided to patient. Lucci et al. (15) studied the prognostic value of CTC in early stage breast cancer: 73 patients had ≥ 1 CTC, 29 patients had ≥ 2 CTC while 16 had ≥ 3 CTC per 7.5 ml blood. They did not observe any correlation between primary tumor features and CTC detection. However, presence of CTC was associated with significant short progression free survival. On the contrary, in the present study, CTC positivity significantly correlated with tumor size, histologic type, tumor grade, metastasis and skin infiltration. 66.66% (2/3) of ILC cases as compared to 8.1% (9/111) cases of IDC were positive for CTC, this could be due to absence of E-Cadherin which leads to early dissemination of cancer cells into the blood stream.

Molecular methods in which mRNA of tumor cells is amplified can also be used to detect CTC and have greater sensitivity. Multimarker assay can be used instead of single probe assay to further improve sensitivity. Disadvantages of amplification-based tests are the false positivity, heterogeneity in expression levels of particular target transcripts as well as false negative (6).

Pukazhendhi and Glück (4) reviewed 81 manuscripts on CTC in breast cancer and categorized them into those in discovery datasets, prognostic factor in metastatic breast cancer, predicting clinical utility in early breast cancer. Based on this, they commented that the current diagnostic modalities for CTC mainly focus on epithelial markers, however measurement of circulating DNA is the best approach.

Giordano et al. (16) studied the clinical impact of CTC in various molecular subtypes of breast cancer. Baseline CTC detection had good prognostic value in all breast cancer subtypes except Her 2 neu positive cancer. Guiliano et al. (17) observed the effect of different first line systemic
treatment on the prognostic value of CTC in 492 advanced breast cancer patients. A pre treatment level more than or equal to 5 CTC/7.5 ml blood was associated with an increased baseline number of metastatic sites compared to those with less than 5 CTC/7.5 ml (p=0.0077). They had 4 different treatment groups, out of which groups with endocrine treatment and CT alone, high CTC was associated with worse prognosis while the groups receiving either her 2 neu targeted treatment or biological agent, did not maintain the negative prognostic value of high CTC at baseline.

Krishnamurthy et al. (18) evaluated the presence of CTC in peripheral blood and its correlation between various clinicopathological characteristics and hormone receptor profile. CTC were found in 13 out of 43 T1 tumors while in T2 tumors 12/38 were CTC positive. There was no correlation between detection of CTC and standard prognostic factors contrary to our findings of significant association of CTC with tumor size, histologic type, tumor grade, metastasis and skin infiltration. In our study, 31.25% of grade 3 tumors were positive for CTC, followed by 1.31% of grade 2 tumors, signifying that higher the grade, more the positivity for CTC.

Turker et al. (19) determined the effectiveness of CTC in 22 metastatic and 12 Early stage breast cancer cases for prediction of progression free survival (PFS) and overall survival (OS) as an adjunct to standard treatment care in breast cancer management. CTC was positive in 3 (13.6%) patients before chemotherapy (CT) and 6 (27.3%) patients during CT in the metastatic subgroup whereas positive in only one patient in early stage subgroup before and during CT. CTC positivity was confirmed as a prospective marker in this study even with small patient group.

Franken et al. (20) undertook a study to explore whether the presence of CTC at the time of diagnosis was associated with recurrence free survival (RFS) and breast cancer related death (BRD) in 404 breast cancer patients. Patients were stratified into unfavorable (CTC ≥ 1) and favorable (CTC =0 in 30 ml peripheral blood). They concluded that CTC in breast cancer patients before undergoing surgery with curative intent is associated with an increased risk of BRD.

Peeters et al. (21) explored potential differences in the detection and prognostic significance of CTCs in MBC according to immunohistochemical subtypes of breast cancer. They did not observe any significant differences in the absolute CTC counts (P=0.120) or in CTC positivity rates according to ≥1 and ≥5 CTCs per 7.5 ml blood detection thresholds (P=0.165 and P=0.651, respectively) between immunohistochemical subtypes. Very high CTC counts, defined as ≥80 CTCs per 7.5 ml, were observed more frequently in patients with Luminal A and triple negative (TN) breast cancer (P=0.024). In the total study population, the presence of ≥5 CTCs was the single most significant prognostic factor for both PFS and OS in multivariate analysis (P<0.001).

Rack et al. (22) analyzed CTC in 2026 patients with early breast cancer before adjuvant chemotherapy and in 1492 patients after CT using Cell Search System. Before CT, CTC were detected in 21.5% of patients (438/2026), out of which node negative versus node positive patients with CTC were 19.6% vs. 22.4% (p<0.001), similar to the current study where the node status significantly correlated with CTC positivity. However, no association was found with tumor size, tumor grade or hormone receptor status which is contrary to our results as we found a significant association with tumor size and tumor grade.

This study had many limitations. The sample size is too low and confidence intervals are very wide, and the power of the study is too low to reach to any significant conclusion. The study strongly needs to be validated and replicated in a bigger sample size.

In conclusion, the flow cytometry protocol for detection and molecular characterization of CTCs is a time and cost-effective technique, suitable for routine clinical use. However, more elaborate studies are needed to establish the role of flow cytometry in detection of circulating tumor cells as a prognostic marker. One added advantage of flow cytometric immunophenotyping is that panels can be expanded to get additional information. Estrogen and Progesterone receptors and Her2neu status in metastatic breast carcinomas or BRAF mutation status (using of mutation-specific antibodies) can be very useful in the current approach towards personalized treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


Expression of Neutrophil Gelatinase-Associated Lipocalin and Kidney Injury Molecule-1 in Wilms Tumor

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ABSTRACT

Objective: Neutrophil gelatinase-associated lipocalin (NGAL) and Kidney injury molecule-1 (KIM-1) play important roles in both immunity and cell proliferation. It was reported previously that they are overexpressed in various human cancers. The present study was undertaken to examine the expressions of NGAL and KIM-1 in Wilms Tumors.

Material and Method: Tissue samples of 50 Wilms Tumors were evaluated and underwent immunhistochemical staining for NGAL and KIM-1 protein expressions. The correlations between them, and some clinical prognostic factors such as tumor weight, stage and histological features were also evaluated.

Results: Twenty-three (46%) of the cases were male while 27 (54%) were female. The mean age was found to be 3.26±2 years. The average tumor size was 9.16 ± 2.9 cm in diameter and the average weight of the kidney was 478±312 gr. Thirteen (26%) cases were stage I, 18 (36%) cases were stage II, 7 (14%) cases were stage III, and 6 (12%) cases were stage IV. Thirty-nine cases were alive (78%), while 11 cases (22%) were deceased. Mean overall survival time was 68.2±39.5 (2-148) months. NGAL expression was negative in all tumors except the neutrophils within the tumors. KIM-1 expression was positive in 37 tumors (74%), while it was absent in 13 tumors (26%). Using Mann-Whitney U Analysis, KIM-1 expression was found to be associated with the stage of the tumor (p=0.027).

Conclusion: The preliminary data indicates that KIM-1 expression may be associated with stage in Wilms Tumor. However, further studies are needed to validate these pilot observations and to clarify the functional and mechanistic significance of this relevance.

Key Words: Wilms Tumor, NGAL protein, KIM-1 protein

INTRODUCTION

Wilms Tumor (WT) is the most common malignant renal tumor of children, accounting for approximately 14% of pediatric cancers (1). Although survival rates in WT have been improved in the past decades due to a multidisciplinary therapeutic approach, a certain population of the patients continue to experience poor survival and increased rates of relapse (2). Mutations and abnormal expressions of the 6 WT genes basically contribute to tumorigenesis of WT but other genes also participate in its development. Recent studies have revealed that several genetic abnormalities are associated with a worse prognosis in WT, even in those with localized stage and favorable histology (3, 4).

Neutrophil gelatinase-associated lipocalin (NGAL), a member of the lipocalin superfamily, was first isolated as a 25 kDA glycoprotein covalently bound to matrix metalloproteinase 9 (MMP9) in human neutrophils (5). Although initially in neutrophils, it was later found to be expressed in most epithelial cells and to participate in the diverse processes of growth, development, differentiation and tumorigenesis of many tissues (6, 7).

Kidney injury molecule-1 (KIM-1) was first reported as a sensitive and specific biomarker in detecting injury of the proximal tubules in 1998 by Ichimura (8). KIM-1 is a type 1 membrane protein that contains a novel six-cysteine immunoglobulin-like domain and a mucin domain. Structurally, KIM-1 is a member of the immunoglobulin gene superfamily most reminiscent of mucosal addressin cell adhesion molecule 1 (MAdCAM-1). Human KIM-1 is also homologous to the monkey hepatitis A virus cell receptor 1 (HAVcr-1) (9). KIM-1 is expressed at a low level in the normal kidney but is increased dramatically in the post-ischemic kidney (8-10).

Hitherto, many parameters have been suggested as relevant markers for assessing the proliferative activity and tumor cell dynamics of WT (11-13). However, the presence

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of NGAL and KIM-1 expressions in WT has not been investigated widely (10). The aim of this study was to explore the importance of these two markers in Wilms tumor and also to investigate the correlations between them, and some clinical prognostic factors such as tumor weight, stage and histological features.

MATERIAL and METHODS

WT resection specimens of 50 cases diagnosed and treated in Dr. Behçet Uz Children's Education and Research Hospital between 1999 and 2014 were included in this study. The study was approved by the Local Ethics Committee of Tepecik Education and Research Hospital. The staging system developed by the National Wilms Tumor Study Group (NWTS) was used to describe the extent of spread of these tumors (14, 15).

For immunohistochemistry (IHC), hematoxylin and eosin (H&E) staining was used to select appropriate paraffin blocks and to identify the viable tumor areas. IHC was performed by the streptavidin biotin peroxidase method (Invitrogen, Camarillo, 85-9043). Serial 5-µm sections were obtained and these slides were baked over-night at 60°C, dewaxed in xylene, and hydrated with distilled water through decreasing concentrations of alcohol. All slides were treated with heat-induced epitope retrieval in the microwave (in 10mM/L citrate buffer, pH 6.0, for 20 minutes, followed by cooling at room temperature for 20 minutes) and blocked for endogenous peroxidase and biotin. An affinity purified monoclonal mouse antibodies against NGAL (Novus Biologicals, Littleton, USA, NDP1-90331) and KIM-1 (Bioss, Philadelphia, USA, HAVCR1) were used at a dilution of 1: 300. Renal tissue with acute tubular necrosis was used as positive control for KIM-1 and splenic tissue for NGAL (Figure 1). The evaluation was blinded to any of the clinical features and staining patterns were classified according to the severity of staining. For KIM-1, cytoplasmic staining similar to the proximal tubules in control tissues was considered as positive (Figure 2). Focal staining occupying less than 5 % of the field or diffuse weak staining were considered as negative. In previous studies, it was reported that NGAL showed both cytoplasmic and membranous expression in most tissues. Contrary to the other studies, there was no expression of NGAL in tumor cells. We counted the neutrophils that infiltrated the tumors. If there were up to five cells in every high power fields, we evaluated this as negative for NGAL. Spearman Correlation analysis, Mann-Whitney U test, Chi square test and Kaplan-Meier survival analyses were performed for statistical analysis with SPSS 15.0. P values less than 0.05 was considered to be statistically significant.

RESULTS

Surgery, chemotherapy and radiotherapy were the treatment modalities that were applied alone or in combination to the total of 50 patients according to their individual features. We used the NWTS protocol with surgery approach first for patients with unilateral tumor, but pre-operative chemotherapy was added and the combination of drugs was changed for patients with bilateral tumors. In addition, unfavorable histology required radiation therapy, even in some localized diseases. Therefore we classified the histology of all tumors as favorable or unfavorable. Thirty-nine (78%) cases had triphasic tumors, while 11 (22%) were biphasic and the blastemal component was predominant.

Figure 1: Cytoplasmic NGAL expression in red pulp of spleen (NGAL; x100).

Figure 2: Cytoplasmic KIM-1 expression in proximal tubules of post-ischemic kidney (KIM-1; x200).
These latter 11 cases were evaluated as showing unfavorable histology. In the whole series, 11 patients died at follow-up; 3 of these died because of bilateral tumor, and 4 from conditions apparently unrelated to WT such as pneumonia, sepsis, hepatic insufficiency and veno-occlusive disease.

Twenty-three (46%) of the cases were male while 27 (54%) were female. The mean age was found to be 3.26±2 years (ranging from 5 months to 8 years). The tumor was right-sided in 25 (50%) cases, left-sided in 19 (38%) cases and 6 (12%) cases had bilateral tumors (stage V). The average tumor size was 9.16 ± 2.9 cm in diameter and the average weight of the kidney was 478±312 gr (15). Thirteen (26%) cases were stage I, 18 (36%) cases were stage II, 7 (14%) cases were stage III, 6 (12%) cases were stage IV. Thirty-nine cases were alive (78%), while 11 cases (22%) were deceased. Mean overall survival time was 68.2±39.5 (3-148) months.

The frequency of KIM-1 expression varied between different components in the same tumor. KIM-1 was negative in 13 (26%) cases. Expression was limited in the epithelial component in 19 (38%) cases (Figure 3), while it was limited in the blastemal in 7 (14%) cases (Figure 4) and in mesenchymal areas in 3 (6%) cases. In 8 cases (16%), diffuse KIM-1 expressions were determined (Figure 5). NGAL expressions were determined in only NGAL-positive inflammatory cells within the WTs (Figure 6). In most tumors, less than 5 NGAL-positive neutrophils per high-power field were determined. Therefore NGAL was considered as negative in all WTs.

Figure 3: Cytoplasmic KIM-1 expression in epithelial component in WT (KIM-1; x200).

Figure 4: Cytoplasmic KIM-1 expression in blastemal component in WT (KIM-1; x100).

Figure 5: Diffuse KIM-1 expression in a triphasic WT (KIM-1; x200).

Figure 6: Note the NGAL-positive inflammatory cell within a WT (NGAL; x200).
Most prognostic parameters such as kidney weight (p=0.127), tumor diameter (p=0.271), patient age (p=0.340) and therapy response (p=0.407) were not found to be associated with KIM-1 expression using the Mann-Whitney U and Chi-square Analyses. The overall survival was 61.5±11.7 months in patients with KIM-1 positive tumors while it was 70.8±6.3 months in KIM-1 negative tumors. There were no relationship between the KIM-1 expression and the survival (Log Rank, p=0.932) by Kaplan-Meier Survival Analysis (Figure 7).

KIM-1 expression was positive in all stage I tumors and most stage II and III tumors. In contrast, KIM-1 was determined as negative in most stage IV tumors (Figure 8). While 67.5% of KIM-1 positive tumors were in the early-stage, 46.2% of KIM-1 negative tumors were in the early stage, excluding the bilateral tumors. The Chi square test revealed a relationship between KIM-1 expression and stage that was statistically significant (p = 0.027).

**DISCUSSION**

NGAL was initially defined as a useful bacteriostatic agent, and was found to be over-expressed in many types of cancers including breast, pancreatic and ovarian cancers (5, 6). The reported effects of NGAL in tumors are contradictory. For example, it was shown to have protumoral effects in breast (16), stomach (17) and esophageal cancers (18). In contrast, some studies showed that NGAL demonstrates antitumor and antimetastatic effect in anaplastic thyroid carcinoma cells (19), prostate cancer (20) and cholangiocarcinoma (21). Recently Wang et al. (22) reported that both NGAL gene and NGAL expression in tumor tissue was down-regulated in head and neck squamous cell carcinoma (HNSCC) and this down-regulation may correlate with tumorigenesis in HNSCC. It was also reported that NGAL could form a complex with MMP-9 to prevent its degradation and increase MMP-9 activity. Moreover, NGAL is bound to siderophores and participates in iron metabolism in mammalians. Thus, iron homeostasis was speculated to be involved with NGAL in promoting cancer. Based on these studies, NGAL was speculated to be a new kind of metastasis biomarker. However, the detailed mechanism has not been totally understood yet (16-22). In the present study, we evaluated the NGAL expression in WT. However, we did not determine NGAL expression in tumor cells or renal tissue. Therefore, we think that NGAL may not have any role in tumorigenesis in WTs.

Clinical investigations have revealed that the prognosis of WT correlates with stage and favorable histology, which is characterized by the presence of all three histological elements and the absence of diffuse anaplasia (12,14,15). These three histological components of WT have different proliferation potentials and different responses to therapy. Hitherto, many studies have revealed these differences. In most reports, the lowest proliferative capacity was determined in the mesenchymal component and this component generally survived after chemotherapy (14,15). In the present study, KIM-1 expression was determined in the early stage tumors. In addition, we determined KIM-1 expressions confined to the epithelial and blastema components in the most cases. Our results have two important implications. Firstly, the relationship between the KIM-1 expression and stage suggests that KIM-1
may be used an important indicator of localized disease. Secondly, KIM-1 expression is potentially relevant in WT differentiation. However, further research is required to define how KIM-1 expression status can be used to clinical advantage in WT.

As in several body fluids, the urine is a rich reservoir of various substances and extracellular vesicles, directly originating from cells facing the urinary lumen. These substances are secreted by all types of cells under both physiological and pathological conditions. Some of them are accepted as markers of glomerular and tubular damage, as well as of renal regeneration. In addition, some substances appear to be involved in the cell-to-cell communication along the nephron and to emerge as potential amplifying or limiting factors in renal damage. Substances secreted from injured cells may favor the demonstration of fibrosis or disease progression. KIM-1 is one of these substances in the urine and it has been identified as representing an incredible source of information for diagnostic purposes (23). Several studies revealed that KIM-1 is expressed in both proliferating and dedifferentiated epithelial cells in regenerating proximal tubules. In addition, it is an epithelial cell adhesion molecule up-regulated in the cells, which are dedifferentiated and undergoing replication. KIM-1 may play an important role in the restoration of the morphological integrity and function to the post-ischemic kidney. KIM-1 is a sensitive and specific biomarker in detecting injury of proximal tubules in humans and other animals (10,23,24). Recent studies indicate that KIM-1 may play an important role in the tumorigenesis of renal cell carcinomas (10,24). Kidney development is a complex process regulated by transcription factors, proto-oncogenes, and several growth factors that act as signaling molecules and their receptors. WT can be considered as a failure of this transition (11). In this study, we determined KIM-1 expression in most WTs. This finding indicates that urinary KIM-1 may also be a marker in WTs. KIM-1, a marker of tubular damage, may possibly be useful to gain information about tissue damage, regeneration and even tumorigenesis in WTs.

In summary, the preliminary data indicates that KIM-1 is frequently expressed in WT and this expression is negatively associated with stage in WTs. However, further studies are needed to validate these pilot observations and to clarify the functional and physiopathologic significance of this relevance. Contrary to the other studies, we did not determine any association with NGAL protein and tumorigenesis in WT.

CONFICT OF INTEREST

The authors declared no conflict of interest

REFERENCES


Comparison of Microvessel Density with Prognostic Factors in Invasive Ductal Carcinomas of the Breast

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ABSTRACT

Objective: Angiogenesis plays a key role in tumor growth and metastasis. Determination of microvessel density is the most common technique used to evaluate the amount of the intratumoral angiogenesis in breast cancer. We have aimed to investigate the relationship with tumor angiogenesis and prognostic parameters in breast invasive ductal carcinomas.

Material and Method: In this study, a total of 100 invasive ductal carcinoma patients, who were diagnosed at the Department of Pathology, Ataturk University Faculty of Medicine between the years 2003-2008, were re-evaluated. Patient characteristics and clinicopathological findings were obtained from archival records. In the present study, microvessel density was determined by immunohistochemical staining by using anti-CD34 monoclonal antibody in the paraffin blocks. First, the most vascular area was selected in the tumor under a low magnification (40x) by a light microscope and then microvessels were counted under a higher magnification (200x). Patients were classified as low and high microvessel density depending on their microvessel counts. Chi-square test and multivariate linear regression analysis were used for statistical analysis (p≤0.05).

Results: We have determined that microvessel density increases as tumor size increases (p=0.001). Microvessel density was higher in patients with at least 10 lymph node metastases compared to those with no metastasis (p=0.05). However, there was no statistically significant difference between microvessel density and other prognostic factors such as histological grade, nuclear grade, patient age, vascular invasion, estrogen, progesterone receptor status, HER2/neu expression.

Conclusion: In our study, we have found that microvessel density is associated with tumor size and lymph node metastasis in patients with invasive ductal carcinoma.

Key Words: Breast cancer, Invasive ductal carcinoma, Angiogenesis

INTRODUCTION

Breast cancer is the most common malignant tumor among women in Turkey and the world. It accounts for approximately 30% of all cancers in women. According to the 2013 data of Ministry of Health, breast cancer constitutes 24.6% of all cancers among women in our country and the most common type is invasive ductal carcinoma (1). Therefore, the etiologic and prognostic studies on breast cancer are still important.

Angiogenesis is the process of new capillary vessel formation and it is observed in physiological events such as embryonic development, wound healing and organ hypertrophy. However, uncontrolled angiogenesis is held responsible for the progression and etiopathogenesis of many neoplastic formations, especially growth and metastasis of solid tumors (2). The numerical value of tumor angiogenesis is defined as microvessel density (MVD). MVD is measured by counting small and tortuous vessels in the tumor tissue by immunohistochemical staining using antibodies such as CD31, CD34, CD105 and Von-Willebrand factor (Factor VIII) that are specific for vessel endothelium. In the earlier studies, MVD is reported to be associated with advanced pathologic stage and poor prognosis of disease in breast, lung, colon, stomach, prostate and bladder cancers, and malignant melanoma (3-5).

In this study, we aimed to determine the angiogenesis in invasive ductal carcinoma, which is the most common breast cancer type, by using microvessel counting and the relationship between MVD and known prognostic parameters such as patient’s age, tumor size, lymph node metastasis, vascular invasion, estrogen-progesterone receptor status, human epidermal growth factor (HER2/neu) expression.

MATERIAL and METHOD

In this study, a total of 113 patients, who were diagnosed with invasive ductal carcinoma, not otherwise specified (NOS) in the Department of Pathology, Atatürk University School of Medicine, were included. The demographic characteristics and clinicopathological findings were obtained from archival records.
Medicine between the years 2003-2008 and did not receive any neo-adjuvant treatment, underwent modified radical mastectomy and axillary lymph node dissection were re-evaluated. However, 13 patients were excluded from the study since we could not access their paraffin blocks from the pathology department archive. Hematoxylin and eosin (H&E) preparations of these patients were re-evaluated and the best formalin fixed-paraffin embedded (FFPE) block representing the tumor for each patient was selected. MVD was determined by immunohistochemical staining with this tissue. Results were compared with clinicopathologic parameters such as patient’s age, tumor size, histological grade, lymph node involvement, the presence of vascular invasion, estrogen-progesterone receptor status, human epidermal growth factor (HER2/neu) expression. The data about clinicopathologic features were obtained from the pathology reports. These features were also used to evaluate mastectomy materials during routine pathology practice. The study was approved by the Ethics Committee of Erzurum Ataturk University, School of Medicine.

The Nottingham modification of Bloom-Richardson system was used for histological grading (6). When 1% and higher nuclear staining was present in the tumor cells at any density, hormone receptor status was accepted as positive. According to immunostaining results, HER2/neu expression was considered as negative (0, 1+), equivocal (2+) and positive (3+) (7). Tumor diameter and lymph node status were grouped according to the TNM system.

Immunohistochemistry

The 5μ thick samples taken from FFPE blocks of each patient were put on poly-L-lysine coated microscope slides. These samples were washed in phosphate buffered saline (PBS) after deparaffinization with xylene and rehydration process with alcohol. In order to eliminate the endogenous peroxidase activity, they were incubated in 3% hydrogen peroxide solution for 15 minutes. They were washed again in PBS. Then, anti-CD34 primer antibody (Monoclonal Mouse Anti-human CD 34 class II Clone QBend-10) (Dako code No. M 7165), which was diluted at a ratio of 1:50, was dropped onto tissues and waited for 60 minutes. Tissues were re-washed in PBS. Biotinylated-link was treated for 30 minutes as the secondary antibody. It was re-washed in PBS. Treated with streptavidin peroxidase for 30 minutes and washed in PBS. Tissues were incubated for 6 minutes after dropping chromogenic DAB (3,3’-Diaminobenzidine tetrahydrochloride). The samples washed with distilled water were counterstained with Mayer’s hematoxylin and then closed by immunohistochemistry sealing solution after being washed off with distilled water again.

Microvessel Density (MVD) Calculation

MVD was evaluated by counting anti-CD34 positive microvessels and calculated by the counting method developed by Weidner using a light microscope (8). Accordingly, after scanning the whole tumoral section with a light microscope under a low magnification (x40), the area with highest number of microvessels was identified as ‘hot-spot’ (Figure 1) and microvessels were counted under a higher magnification (200x) in this area. Any brown-stained single endothelial cell or endothelial cell clusters separated from surrounding tumor cells and connective tissue elements were considered to be a microvessel regardless of whether they had a lumen or not. No erythrocyte was necessarily required in the lumen. Branching vessel structures were counted as a single vessel. Vascularity was not considered in the areas of necrosis within the tumor. After determining microvessel counts of all patients, the average MVD was found as 89.3 (SD:±28.74). This value was regarded as the cut-off value. Patients with microvessel counts below this cut-off value were classified as 'low MVD' (Figure 2), and patients with microvessel counts above this cut-off value were classified as 'high MVD' (Figure 3) (9, 10). Olympus BX51 (Tokyo, Japan) light microscope was used for counting microvessels. Microscopic photographs were captured by Olympus DP70 (Tokyo, Japan) camera.

Statistical Method

SPSS 20.0 for Windows (SPSS Inc. Chicago. IL. USA) software package was used to investigate whether there is a significant relationship between all the findings.

![Figure 1: Immunohistochemical staining of an invasive ductal carcinoma, NOS with anti-CD34 antibody. The hot-spot with higher density of microvessels was identified and microvessels were counted in this area (CD34; x100).](image-url)
Chi-square test was used to examine the relationship between MVD and other prognostic parameters. Those with a p-value smaller than 0.25 (tumor size, lymph node involvement, progesterone receptor status, lymphovascular invasion) in the univariate analysis were re-examined using the multivariate linear regression analysis model.

The error value was set as 0.05. p-values either higher or equal to 0.05 were considered to be statistically significant.

RESULTS

All of the patients included in the study were women with an average age of 51.8 (SD:±11.9 years; age range, 26-80). Tumor size ranged from 0.7 cm to 10 cm and the average tumor size was 4.15 (SD:±2.03) cm. No lymph node metastasis was observed in 28% of the patients, while 1-4 lymph node metastases were observed in 34% of the patients, 4-9 lymph node metastases were observed in 24% of the patients and ≥10 lymph node metastases was observed in 14% of the patients, respectively. 2% of the patients were graded as grade 1, 75% of them were graded as grade 2, and the remaining 23% were graded as grade 3, respectively.

When MVD was calculated by anti-CD34 antibody, at least 31 and up to 185 microvessels were counted. High MVD was observed in 48% of the patients and low MVD was observed in the remaining 52%, respectively. In our study, MVD was found to increase as tumor diameter increases (p<0.001). MVD was higher in patients with at least 10 lymph node metastases compared to those with no metastasis (p=0.05). The relationship between lymph node status and MVD is shown in Figure 4.
No significant relationship was found between MVD and clinicopathologic parameters such as patient’s age, histological grade, the presence of vascular invasion, estrogen-progesterone receptor status, human epidermal growth factor (HER2/neu) overexpression. The relationship between MVD and clinicopathological parameters is summarized in Table I.

The clinicopathological parameters with a p-value smaller than 0.25 in the univariate analysis were examined using the multivariate linear regression analysis model. Similar to the univariate analysis, the multivariate linear regression analysis showed a statistically significant relationship between MVD and tumor size and lymph node involvement. The results are summarised in Table II.

**Table I:** The relationship between clinicopathological parameters and MVD in patients with breast cancer

<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>AMC ± SD</th>
<th>MVD</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High (%)</td>
<td>Low (%)</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50 years of age</td>
<td>45</td>
<td>86.33 ± 25.74</td>
<td>23 (51.1)</td>
<td>22 (48.9)</td>
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<tr>
<td>&gt;50 years of age</td>
<td>55</td>
<td>88.49 ± 31.12</td>
<td>25 (45.5)</td>
<td>30 (54.5)</td>
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<tr>
<td><strong>Tumor size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2 cm</td>
<td>15</td>
<td>71 ± 16.56</td>
<td>2 (13.3)</td>
<td>13 (86.7)</td>
</tr>
<tr>
<td>2–5 cm</td>
<td>60</td>
<td>85.25 ± 27.84</td>
<td>28 (46.7)</td>
<td>32 (53.3)</td>
</tr>
<tr>
<td>5 cm</td>
<td>25</td>
<td>102.88 ± 30.06</td>
<td>18 (72)</td>
<td>7 (28)</td>
</tr>
<tr>
<td><strong>Lymph node involvement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No lymph node involvement</td>
<td>28</td>
<td>80.64 ± 24.64</td>
<td>11 (39.3)</td>
<td>17 (60.7)</td>
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<tr>
<td>≥10 lymph node involvement</td>
<td>14</td>
<td>106.36 ± 33.08</td>
<td>10 (71.4)</td>
<td>4 (28.6)</td>
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<tr>
<td><strong>Estrogen</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Positive</td>
<td>46</td>
<td>87.41 ± 32.21</td>
<td>23 (50)</td>
<td>23 (50)</td>
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<tr>
<td>Negative</td>
<td>54</td>
<td>87.61 ± 25.66</td>
<td>25 (46.2)</td>
<td>29 (53.8)</td>
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<tr>
<td><strong>Progesterone</strong></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Positive</td>
<td>58</td>
<td>83.29 ± 26.53</td>
<td>25 (43.1)</td>
<td>33 (56.9)</td>
</tr>
<tr>
<td>Negative</td>
<td>42</td>
<td>93.36 ± 30.84</td>
<td>23 (54.8)</td>
<td>19 (45.2)</td>
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<tr>
<td><strong>HER2/neu</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Negative</td>
<td>47</td>
<td>85.04 ± 25.84</td>
<td>20 (42.6)</td>
<td>27 (57.4)</td>
</tr>
<tr>
<td>Equivocal</td>
<td>15</td>
<td>92.80 ± 35.43</td>
<td>9 (60)</td>
<td>6 (40)</td>
</tr>
<tr>
<td>Positive</td>
<td>38</td>
<td>88.50 ± 29.66</td>
<td>19 (50)</td>
<td>19 (50)</td>
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<tr>
<td><strong>Lymphovascular invasion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>82</td>
<td>88.34 ± 28.73</td>
<td>42 (51.2)</td>
<td>40 (48.8)</td>
</tr>
<tr>
<td>No</td>
<td>18</td>
<td>83.78 ± 29.09</td>
<td>6 (66.7)</td>
<td>12 (33.3)</td>
</tr>
</tbody>
</table>

AMC: Average number of microvessel count, SD: Standard deviation. MVD: Microvessel density

**Table II:** The relationship between clinicopathological parameters and MVD by multivariate linear regression analysis

<table>
<thead>
<tr>
<th>Model</th>
<th>B</th>
<th>SD</th>
<th>Beta</th>
<th>t</th>
<th>p</th>
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<tr>
<td>Constant</td>
<td>46.738</td>
<td>11.352</td>
<td>4.117</td>
<td>0.000</td>
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<tr>
<td>Tumor size</td>
<td>15.195</td>
<td>4.145</td>
<td>0.332</td>
<td>3.666</td>
<td>0.000</td>
</tr>
<tr>
<td>Lymph node involvement</td>
<td>8.91</td>
<td>2.739</td>
<td>0.315</td>
<td>3.253</td>
<td>0.002</td>
</tr>
<tr>
<td>Progesterone</td>
<td>-7.477</td>
<td>5.227</td>
<td>-0.129</td>
<td>-1.43</td>
<td>0.156</td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td>-8.333</td>
<td>7.057</td>
<td>-0.114</td>
<td>-1.181</td>
<td>0.241</td>
</tr>
</tbody>
</table>

R: 0.484, R²: 0.234, F: 7.269
DISCUSSION

The most important clinicopathologic factors influencing the biological behavior and treatment of the disease of breast cancer are patient’s age, tumor size, tumor type, axillary lymph node involvement, the presence of vascular invasion, estrogen-progesterone receptor status and human epidermal growth factor (Her2/neu) overexpression (11-13). Prognostic factors can be useful to identify poor clinical outcomes and select patients who will receive adjuvant therapy (14).

As known, angiogenesis plays a key role in tumor growth, invasion and metastasis. In recent years, the growing importance of targeted approaches in treating cancer highlights the target treatment options that inhibit angiogenesis in cancer treatment (15-17). Angiogenesis inhibitors slow and inhibit tumor growth and metastasis via different mechanisms. For example, anti-angiogenic drugs target directly pro-angiogenic molecules, while some of them inhibit angiogenic receptors, signal pathways or angiogenic external factors. Using anti-angiogenic drugs together or combining them with chemotherapeutic agents are more effective in treating breast cancer (18).

In this study, we have determined angiogenesis in invasive ductal carcinoma by counting microvessels using anti-CD34 antibody and compared MVD that we have obtained from each patient with prognostic factors. We have found a statistically significant relationship between increasing tumor size and MVD (p=0.001). There are consistent studies (19-21) with our results in the literature as well as some studies (16, 22) found no significant relationship between MVD and tumor size and some studies found an inverse correlation between them (23).

When we compared MVD with lymph node involvement we have found that MVD was higher in patients with at least 10 lymph node metastasis compared to those with no metastasis (p=0.05). Similar to our results, there some studies found that high MVD is correlated with axillary lymph node metastases (21, 24, 25). However, there are also some other studies found no relationship between MVD and axillary lymph node metastases (16, 26).

In our study, we have found no relationship between MVD and prognostic parameters such as patient’s age, tumor size, histological grade, vascular invasion, estrogen-progesterone receptor status, human epidermal growth factor (HER2/neu) overexpression. In the literature, some studies have reported that there is no relationship between MVD and prognostic parameters such as patient’s age (25, 27), histological grade (28), lymphovascular invasion (9), estrogen and progesterone receptor status (27), HER2/neu overexpression (27). On the other hand, there are also some other studies that found a significant relationship between high MVD and patient’s age (29), high histologic grade (16, 25, 27), presence of lymphovascular invasion (30), estrogen (16, 22) and progesterone receptor negativity (22) and HER2/neu overexpression (31).

As it can be seen, there are different results in the literature regarding the relationship between MVD and prognostic parameters. One of the reason of this may be using different antibodies such as CD34, CD31, Factor VIII and CD105 to highlight the microvessels (24, 32, 33). In the literature, some studies reported that the anti-CD34 monoclonal antibody is more sensitive than the anti-CD31 antibody and anti-factor VIII-related antigens in the calculation of MVD in breast cancer (31, 32). Therefore, we used the anti-CD34 monoclonal antibody to calculate the MVD. Since we did not use any other antibodies in the calculation of MVD in breast cancer, we do not know whether our results were affected by this selection.

One another reason for having different results may be the calculation method of MVD. Weidner et al. have identified the hot-spot area with the largest number of microvessels at low magnification (x40 and x100) to determine MVD and counted microvessels in this area under a magnification of x200 (34). This method used by Weidner is used in many studies conducted on microvessel count (16, 28, 35). Some authors have counted a single area under x200 or x250 magnification, while some other counted a single area under x400 magnification (36-38). In this study, we have used the microvessel counting method used by Weidner.

In the tumoral area, heterogeneity of microvessel distribution may be another reason for the different results (39). Bosari et al. have shown that the number of microvessels counted in a single area is 20% more than the average number of microvessels counted in three areas (9). Heterogeneity of MVD is thought to be reduced with increasing number of areas counted (23). Examining each tumor tissue blocks and applying immunohistochemistry for all tumor tissue blocks may be useful in order to overcome the problem of heterogeneity. However, this is an expensive and time consuming process and it is difficult to maintain its sustainability in routine practice. In our study, after scanning the whole tumor sections, we have counted the microvessels in appropriate tumor tissue by immunostaining with anti-CD34 antibody. And also, all tissue samples that we have used to count microvessels were resected materials. Due to tumor heterogeneity, MVD should be determined in the resection materials and it should be avoided to determine MVD from biopsy specimens.
Another reason for the different results between studies may be different cut-off values used to classify patients depending on their MVD. Some studies identify the cut-off value as the average number of microvessels (9, 27), but in other studies, the cut-off value is the median number of microvessels (10, 40). There are also some other studies accepted absolute values as the cut-off value (36, 41, 42). In this study, we have accepted the average number of microvessels as the cut-off value. All these different cut-off values may be the main cause of different results by affecting the p value. However, we found similar results when we re-analyzed the data by accepting the median value as the cut-off value.

In conclusion, we have found MVD in invasive ductal carcinomas associated with tumor size and lymph node metastasis. However, there are different results regarding the relationship between MVD and prognostic parameters in the literature. These differences may be due to different microvessel counting methods and antibodies used to count microvessels. Since the exact identification of MVD may be helpful in estimating the impact of the anti-angiogenic drugs used in the treatment of breast cancer and the selection of high-risk patients who will receive adjuvant therapy, the microvessel counting method and antibodies used to count the microvessels should be standardized.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**REFERENCES**


INTRODUCTION

The “quality system” terminology originates from ISO 9000 Quality Standards that have been used in business life and industry. A Quality system comprises organizational structure, liabilities, procedures, operations and sources that are required for quality management. This system has also been modified for medical sciences (1). Laboratory medicine specialists emphasized the quality control model in daily operations such as instrument calibration and validation, reagent performance, linearity measurements, and result output. Total quality management including policies, written documents, organization, personnel, equipment and safety has been applied in pathology laboratories worldwide (2). Studies concerning quality management improvement and standardization have also taken place in the medical literature in Turkey (3,4).

Six Sigma have been used in industrial sciences for regulating validity according to statistical analyses and improving quality and minimizing errors in operation processes. Six Sigma was first used by a Japanese company in the 70s for decreasing the error rate. The five main principles of Six Sigma are: 1. Defining, 2. Measuring, 3. Analysis, 4. Improving, and 5. Control.

It is suggested that Six Sigma can have positive impacts on efficiency of laboratory safety (2,5). Six Sigma approach in laboratory medicine was first tested in pathology, and the data of Q-Probes Program created by College of American Pathologists are present in literature (6-8).

Six Sigma is a procedure of detecting errors used for the purpose of improvement under the roof of total quality management. Six Sigma is a methodology targeting zero error (3.4 errors per million events). This method has also been used as a statistical term demonstrating a process' degree of deviation from excellence. Six Sigma enables the determination of the number of defects per million events via monitoring the processes. The error risk per million events is called the "process Sigma level". The process Sigma level demonstrates the quantity of value that the
process has deceived. There is a close relation between the Sigma level and characteristics like error, cost of quality loss (repetitions, time loss, wrong therapy, morbidity and mortality, etc.) and efficiency per each test result. Elements of the processes are analyzed according to the process Sigma levels, and the area of improvement (AOI) is evaluated (9).

Total quality management application in surgical pathology laboratories is rapidly increasing. One of the main principles in quality management is the analysis and prevention of errors.

Errors in the pathology unit are classified as pre-analytic, analytic and post-analytic. Pre-analytic errors include errors during the process from entry to macroscopical analysis, and analytic errors include errors from macroscopic analysis to the reporting phase. Post-analytic errors include reporting/diagnosis errors and the errors that occur after tissue processing procedures, such as the tissue disposal process, archiving, delivery of the reports, communication errors, laboratory information system errors, comprehensibility of the reports or misinterpretation errors. Most of the errors in routine pathology processes are easily recognized before sign out and are revocable; however, some are unidentified irrevocable errors harmful to the patient. Reporting errors in quality management is significant in terms of reducing the repetition of an error (10).

Several measurement and classification systems for errors are introduced in surgical pathology. A system that focuses on the clinical impact to the patient has been described by Raab et al. (11). In this system, errors were separated into two categories; major and minor errors. Major errors were subclassified in 4 categories causing no harm, near miss, harm and unknown in measure of clinical severity. Minimal harm was described as being associated with unnecessary, further noninvasive testing, or a delay in diagnosis or therapy of <6 months. The second category, mild harm, represented unnecessary but invasive further testing, a delay in diagnosis or therapy over 6 months, or minor morbidity due to this delay. The third category, moderate harm, included situations where moderate morbidity due to a delay in therapy or unnecessary therapy occurred due to the unjustified diagnosis. The last category, severe harm, included loss of life, limb, or other body part, and any long-lasting morbidity of over 6 months (11,12).

In our study, we aimed to examine all of major and minor errors that had been encountered in our department in a 1-year period and to assess the effect of Six Sigma implementation in error reduction and process improvement.

**MATERIALS and METHODS**

In our pathology laboratory, the ISO 9001 program is used as the standard program for quality management and 26,000 cases (16,000 biopsies and 10,000 cytology samples) are assessed in a year. Errors concerning both biopsy and cytology samples encountered between April 2014 and April 2015 were recorded.

Pathology personnel (specimen registry personnel, laboratory technical personnel, or pathologist) determined the error, and recorded the characteristics of the error in a follow-up form including his/her own errors. The standard form is shown in Figure 1.

Error follow-up forms were examined by the quality control supervisor, administrative supervisor and the head of the department. The causes of errors were investigated and revocable errors were corrected. Six Sigma principles were applied to the evaluation of problems in our department for 6 months.

In the defining phase, the causes and characteristics of problems and the damages they caused were investigated. The distribution of errors at pre-analytic, analytic and post-analytic phases was examined in the measuring phase. In the analysis phase, problem-solving activities were applied regarding the prevention of the occurrence of errors. Finally, the implementations for reducing all these errors were initiated in the improvement phase. An example of Six Sigma is presented in Figure 2.

Regarding these errors, intradepartmental meetings were held in monthly periods and error analyses were carried out. Extradepartmental meetings with the responsible clinical staff were organized in order to optimize sample delivery procedures. In the intradepartmental meetings, where the employees could express their problems and which were based on the possible legal dimensions of the errors, activities aiming to increase efficiency and to decrease errors were performed. In these meetings, the employees were given the opportunity to work in fields where they felt most productive and solution offers about the problems of employees were prioritized. With this purpose, not only was a quality file created which all employees of the pathology unit could access via their computers, but also a platform was formed where errors, critical-diagnosis, biopsy-cytology correlations, examination requests and the number of denied samples were handled.

Additionally, a double-checking control system was initiated in the phases of recording and macroscopy, macroscopy and embedding, embedding and sectioning,
PATHOLOGY LABORATORY ERROR FOLLOW-UP FORM

THE PERSONNEL WHO CAME ACROSS THE ERROR

<table>
<thead>
<tr>
<th>Patient:</th>
<th>Name-Surname:</th>
<th>Duty:</th>
<th>Department:</th>
<th>Adjustment date:</th>
<th>CONFIRMED BY:</th>
<th>Name-Surname:</th>
<th>Duty/Department:</th>
<th>Confirmation date:</th>
</tr>
</thead>
</table>

ERROR PLACE AND TIME

<table>
<thead>
<tr>
<th>Patient admission-Record</th>
<th>Specimen delivery-Clinic</th>
<th>Transport services</th>
<th>Information systems</th>
<th>Other</th>
<th>Date/Time:</th>
</tr>
</thead>
</table>

DEFINITION OF THE ERROR

**PREANALYTIC**

- Recording Errors (Identity information)
  - Name-Surname errors
  - Age-gender errors
  - Erroneous identity number

- Sampling errors
  - Inadequate sample
  - Cross labeling

- Incorrect sample admission
  - Errors due to incorrect storage/preservation
  - Transfer delay

- Incorrect specimen record
  - Erroneous report as a result of incorrect sampling
  - Admission errors

- Erroneous report as a result of incorrect report
  - Infectious disease not notified

- Errors associated with software
  - Transfer errors

- Record delay

- Cancellation of the record

Accept / Rejected according to specimen rejection criteria

**ANALYTIC**

- Errors concerning technical devices
  - Technical failure
  - Calibration errors

- Histotechnical-immunohistochemistry errors
  - Quality of slides
  - Histology turn around time (TAT)
  - Block/slide labeling
  - Extraneous tissues

- Errors concerning personnel
  - Deficiency of quality management
  - Technical maintenance
  - Accessioning errors
  - Disobeyance of the procedures
  - Erroneous reporting as a result of personnel mistakes

**POSTANALYTIC**

- Verification errors
- Transcription errors
- Report delivery errors
- Incomplete reports
- Frozen-permanent biopsy result correlation
- Cytology-biopsy correlation defect

**OTHER**

INCIDENT / CORRECTING ACTIVITIES:

PAT-F-06       Rev.01       Rev.Date:23/09/2013

Figure 1: Pathology laboratory error follow-up form.
and the other operations after sectioning and the delivery of the slides. In this system, the technical personnel working in successive steps, such as reception staff and macroscopy staff, checked each other. For example, all of the recorded specimens were listed and checked by two staff members (delivery and reception staff). After recording, the list was also double-checked by the reception and macroscopy staff. In any problematic circumstance, the staff member in charge called the quality control supervisor. The quality control supervisor checked the materials and forms and communicated with the corresponding specialists of the clinical departments if needed. Hence all of the sub-units were double-checked and connected to the quality control unit (Figure 3).

The quality control supervisor also checked the quality of processing, sectioning and staining by checking 10 random slides prepared from different tissues every day. The present immunohistochemistry staining machine was replaced with a new and more automated device. In the control phase, units with high error rates were checked among employees.

RESULTS
Fifty-six (52.4%) of 107 recorded errors in total were at the pre-analytic phase. Forty-five errors (42%) were recorded as analytical and 6 errors (5.6%) as post-analytical. Distribution of the errors with standard error classification codes (12,13) in preanalytical, analytical and postanalytical phases are shown in Table I. The number of errors differed between each month and these are shown in Figure 4. The highest number of errors (n=41) was detected in May 2014, while the lowest number was detected in August 2014, December 2014 and February 2015 (n=2). Eighty-nine errors were detected in the first semiannual period, while 18 were detected in the second semiannual period.

The overall error rate of our laboratory was 0.041% in 4.1 per million cases (107 errors per 26,000 cases) in one year. The error rate was 6.8 per million in the first half of the year and 1.3 per million in the second half.

Pre-analytic errors were subclassified into intradepartmental and extradepartmental errors. The intradepartmental error ratio was 58.3%, while the extradepartmental ratio was 31.7%. No errors regarding the cytology interpretation-biopsy correlation were recorded.
Of the recorded 107 errors, 2 errors were major errors. One of the major errors was a pre-analytic phase error. A kidney core biopsy in two fragments was delivered to the pathology laboratory in frozen state in 10% formalin. Both routine examination and direct immunofluorescein application failed due to erroneous fixation and preservation. The patient underwent an additional biopsy procedure. The other major error was the discharge of a tonsillectomy specimen before the completion of the reporting process. This was noticed when the relevant pathologist wanted to receive additional pieces from the specimen for further evaluation. The specimen had accidentally been discharged by the macroscopy assistant. Further evaluation was available by application of an immunohistochemistry panel to the present two blocks of the specimen in order to exclude lymphoma. The final diagnosis of the case was chronic tonsillitis. The clinician of the patient was informed about the failure and follow-up of the patient was recommended. All of the remaining errors were minor errors and were corrected before the pathology report finalization.

The initial immunohistochemistry device required 2 hours of manual handling procedures. Technical problems were also encountered regarding the software system, and technical support was needed 4-5 times per month. The workload of technical personnel was reduced by 50% by moving to a more automated system that required 1 hour of manual handling procedures.

When the first semiannual and second semiannual periods were compared, the number of errors was 89 in the first semiannual period while this number was 18 in the second semiannual period, decreasing by 79.77%.

### Table I: The distribution of the errors in pre-analytical, analytical and postanalytical phases

<table>
<thead>
<tr>
<th>Pre-analytical phase n: 56</th>
<th>Analytical phase n: 45</th>
<th>Post-analytical phase n: 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intradepartmental n: 35</td>
<td>Frequency and causes of repeat stains n: 19</td>
<td>Frozen section-permanent section correlation error n: 2</td>
</tr>
<tr>
<td>Nondepartmental n: 21</td>
<td>Histology turn around time n: 8</td>
<td></td>
</tr>
<tr>
<td>Specimen and patient identification n: 30</td>
<td>Specimen fixation n: 12</td>
<td></td>
</tr>
<tr>
<td>Accessioning errors n: 5</td>
<td>Quality of histologic sections n: 5</td>
<td>Verification of errors during electronic signout or report finalization n: 3</td>
</tr>
<tr>
<td>Specimen and patient identification n: 8</td>
<td>Extraneous tissue n: 4</td>
<td></td>
</tr>
<tr>
<td>Specimen delivery n: 1</td>
<td>Block labeling n: 7</td>
<td>Specimen discharging during the routine examination process n: 1</td>
</tr>
<tr>
<td></td>
<td>Work environment problems (refrigerator fault after power failure, water deluge) n: 2</td>
<td></td>
</tr>
</tbody>
</table>

### Figure 4: The distribution of the number of errors in one year.
DISCUSSION

The Association of Directors of Anatomical and Surgical Pathology (ADASP) recommends the usage of Quality Assurance and Improvement plans and monitors in the pre-analytic, analytic and post-analytic phases in order to enhance patient safety, minimize error rates, ensure timely delivery of reports and monitor physician competence (13). A formal root cause analysis is recommended for incidents in which there is significant patient harm (14). Dhir et al described a multiple-step algorithm of a formal root cause in an incident in which there was an error in specimen accessioning. After the identification of errors, changes in procedures were implemented based on “one-by-one” accessioning (15).

In our institute we initiated the Six-Sigma trial as a formal root cause after the erroneous discharge of the tonsillectomy specimen that is stated as a major error in our study. Identification (“Defining”) of the probable causes of errors provided the construction of the one by one accessioning double-checking system in the laboratory. Thanks to the implementation of this system, the error rates decreased by 79.77%.

Organization of the intradepartmental and extradepartmental meetings provided training for the personnel on the main principles of pathology laboratory processing. Pereira et al. compared two 9-month periods during which a monthly anatomic pathology quality and safety conference was held and noticed a marked improvement in the second time period with fewer cases of incomplete examination, incorrect tumor classification, incorrect tumor staging and clinically significant incorrect diagnosis (16).

We observed the highest error rate (52.4%) in the pre-analytic phase. Pre-analytic component constitutes up to 80% of recorded cases based on a study revealing errors in a ISO 9002:1994 certified clinical laboratory (17). When the cause of higher rate of pre-analytic errors was investigated, the main reasons of errors in pathology unit were considered to be the carelessness of recording personnel as well as psychological burden due to being face to face with the patient constantly.

In our study, we predominantly tried the Six Sigma methodology in order to reduce the rate of errors in pre-analytic and analytic phases because of the high number of errors assessed in these phases. This is a limitation of our study because this system could have also been applied for post-analytic errors including interpretation errors. Another limitation is the lack of the examination of the second review considerations of the consulted cases that were initially reported in our institute. Hence the interobserver variability and the error rate of interpretation results (if present) could be detected. Unfortunately we were unable to reach the majority of the second review results because of the lack of guidelines regarding the interdepartmental consultation protocols.

All of the mentioned errors in this study were recorded errors and it should be considered that unrecorded errors might also be present. This situation may depend on overlooking the errors or trying to solve the problems with direct relations without recording. However, checking the errors by recording through a transparent procedure should be the target for a pathology laboratory with a lower error rate. Finally, training and further studies regarding the implementation of this system in Turkey will provide more efficient pathology units with low error rates.

ACKNOWLEDGMENTS

The authors want to thank Yücel Duduoğlu for her time and technical support about the Six Sigma methodology in industry.

REFERENCES

Comparison Between HER2, Estrogen Receptors and Progesterone Receptors in Primary Breast Carcinomas and Matched Lymph Node Metastases

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ABSTRACT

Objective: In the current work, we compared HER2 by fluorescence in situ hybridization and estrogen and progesterone receptors by immunohistochemistry in matched primary breast carcinomas and their lymph node metastases.

Material and Method: Thirty-nine cases of primary and lymph node metastases were assessed for HER2. Primary tumors of the cases selected were known to be HER2 negative. Also, immunohistochemistry for estrogen and progesterone receptors was performed on 36 cases from the same cohort to assess any discrepancy between the primary tumor and the lymph node metastases.

Results: Out of 39 cases, one case was HER2 amplified in lymph node metastasis compared to non-amplified primary tumor. Approximately eight percent of cases (3/36) were estrogen receptor-negative in LN metastasis and 5.55% (2/36) were less strongly positive compared to the positive primary tumors. Nineteen percent (7/36) were progesterone receptor-negative in lymph node metastasis in contrast to the matched positive primary tumors, and 5.55% (2/36) were progesterone receptor-positive in lymph node as compared to their corresponding negative primary tumors.

Conclusion: While most matched primary breast tumors and lymph node metastases show concordance in HER2, estrogen and progesterone receptor status, we confirmed the multiple reports that identified discordant results in a subset of cases. These results support the newly adopted guidelines that require testing for HER2 on metastatic lesions.

Key Words: Estrogen receptor, Progesterone receptor, HER2, Breast cancer, Lymph node metastasis

INTRODUCTION

Estrogen (ER) and progesterone receptors (PR) are considered to be predictive markers for the patient response to hormonal therapy in breast cancer (1). In addition to its prognostic value, Human epidermal growth factor receptor (HER2) is an important predictive marker to predict the patient’s response to Trastuzumab in mammary carcinomas. Trastuzumab is a humanized monoclonal antibody used, in combination with other drugs, in the treatment of HER2 positive breast carcinomas (2, 3). The American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) outlined guidelines for testing of HER2, ER and PR with continuous review and updates (1, 4, 5). Currently these guidelines require HER2 testing on metastatic and recurrent breast carcinomas (6, 7).

It has been published that approximately 20% of breast cancers are HER2 positive for gene amplification or show protein overexpression by immunohistochemistry (IHC) (8, 9). Therefore, determination of HER2 status is critical for patient care and for prediction of response to Trastuzumab. IHC and Fluorescence in situ hybridization (FISH) are the most commonly used methods for testing the HER2 status (5).

FISH analysis may be considered by some superior to that of IHC in predicting response to trastuzumab in patients with mammary carcinoma. This may be related to the strict criteria used as cut off in FISH analysis compared to the subjective analysis with personal variations in evaluating IHC results (10). However, others have reported that IHC is as effective as FISH in predicting the response to treatment (11). The concordance has been found to be high among IHC and FISH in negative (0 and 1+) and positive (3+) cases.

Steroid hormone receptors (ER and PR) are prognostic markers that determine to great extent the response to adjuvant hormonal therapy. It is the standard of care to test ER and PR in all cases of invasive breast carcinomas and to test ER in ductal carcinoma in situ (DCIS) (5, 12).
The data show that the expression of ER and PR is highly correlated between the primary tumors and their matched lymph node (LN) metastasis (13-16). Different scoring systems exist for evaluation of hormone receptor status (17). ASCO/CAP guidelines recommended a cut off as low as 1% to be considered positive for both markers by IHC (1).

The introduction of tamoxifen and trastuzumab has significantly altered the clinical outcomes of mammary carcinoma. Phenotypic inconsistency in ER, PR, and HER2 expression between the primary and metastatic site exists which leads to multiple clinical considerations. Testing the markers in the nodal or distant metastatic site in addition to testing in the primary tumor remains elusive. A meta-analysis addressing ER, PR, and HER2 expression in LN and distant metastases and in local recurrence showed multiple combination of inconsistency (18). These findings support the guidelines of evaluating the markers in metastasis and local recurrence.

Since breast cancer is a heterogeneous disease, it may be important to determine HER2, ER and PR status in LN metastases (19). Therefore, in this study, we compared HER2, ER and PR status between paired primary breast tumors and axillary LN metastases at our institution.

MATERIALS and METHODS

Breast Specimens: Specimens were obtained from 39 archived, formalin fixed, paraffin embedded tissue sections of LN metastasis from the Pathology Department at the Medical University of South Carolina, Charleston, SC. Selection was based on cases with known un-amplified HER2 on the primary tumor. This study was approved by the Medical University of South Carolina Internal Review Board.

Fluorescence in situ Hybridization: FISH was performed on the 39 blocks for the assessment of HER2 status. Slides were placed in xylene for 3x5 min, and dehydrated twice in two separate 100% ethanol baths for 5 minutes. Slides were then placed in a solution of 2 M HCl at room temperature for 20 minutes, rinsed for 1 minute in distilled water, and placed in the pre-treatment reagent (1M NaSCN) at 80°C for 30 minutes, and rinsed with distilled water for 3 minutes. After pre-treatment, slides were placed in a 37°C solution of 0.2M HCl/4 mg/ml protease (Paraffin pre-treatment kit: Vysis, Inc.) for 10-20 minutes, and rinsed with distilled water for 3 minutes. After controlling digestion, slides were placed in 10% neutral buffered formalin for 10 minutes and rinsed with distilled water. Dehydration was performed through graded alcohol (70% ethanol, 85% ethanol, and 100% ethanol). Slides were then heated to 73°C on a hot plate with a 10µl probe for 5 minutes (Vysis multicolor-probe Topo IIα Spectrum Green, HER2 Spectrum Orange and CEP17 Spectrum Aqua). Slides were cover-slipped, sealed with rubber cement, and placed in a humid environment at 37°C for 16 hours. Coverslips were then removed by immersing slides in SSC/0.3% Nonidet P-40 at 23°C for 2 minutes. Slides were then placed in another change of SSC/0.3% Nonidet P-40 at 73°C for 2 minutes, dried without light, and counterstained with 10µl of 0.2µM 4,6-diamidino-2-phenylindole (DAPI) in anti-fade solution (Vectorshield: Vector Laboratories, Inc). Scoring is performed according to the CAP/ASCO 2010 guidelines (5) which were the guidelines at the time of performing the assay and before the new guidelines (4) have been released. In summary, amplified HER2 by FISH is considered with a ratio of HER2 to CEP17 of > 2.2 or average HER2 gene copy number of > 6 signals/nucleus, equivocal result is defined as HER2/CEP17 ratio of 1.8-2.2 or average HER2 gene copy number of 4-6 HER2 signals/nucleus and non-amplified is defined as HER2/CEP17 ratio of < 1.8 or average HER2 gene copy number of < 4 (no Indeterminate case is encountered in the current cohort) (5). The same guidelines were followed at the time of assay for interpreting Her2 results by IHC in which positive results (3+) is defined as uniform intense membrane staining in > 30% of cells, equivocal (2+) is defined as circumferential incomplete or weak staining in > 10% of cells or complete, circumferential staining in ≤ 10% of the cells and negative result if no staining (0) or weak incomplete membrane staining in any proportion of cells or weak, complete membrane staining in < 10% (5).

Immunohistochemistry (IHC): ER and PR IHC analysis was performed on serial tissue sections for only 36 of the cases from the same case cohort (the paraffin blocks were exhausted with no residual tissue left for the other 3 cases), the results of which were compared to that of the primary tumors. Paraffin slides were deparaffinized in two changes of xylene for 10 minutes each, and hydrated through graded alcohol and distilled water (2 changes of 100% ethanol, 2 changes of 95% ethanol, 2 changes of distilled water). Heat induced epitope retrieval with citrate buffer was performed. Slides were then cooled and rinsed with distilled water, rinsed in tris buffered saline with tween for 5 minutes. Slides were then rinsed with 3% hydrogen peroxide, followed by rinse with wash buffer. Slides were then rinsed with wash buffer and covered with 300µl of protein block for 5 minutes. Following protein block, slides were treated with monoclonal anti-rabbit ER and PR Abs (NeoMarkers, Fremont, CA) used in 1:100 and 1:200 dilutions, respectively. Slides were then rinsed with wash buffer, and the secondary reagent Dako Envision labeled polymer HRP anti-Rabbit was applied. After the secondary reagent, DAB was applied for 10 minutes, and the slides were rinsed with distilled water. Counterstaining was done...
with hematoxylin for 3 minutes, and slides were washed in tap water. Slides were then blued in ammonia water, rinsed in tap water, dehydrated in graded alcohol (95% ethanol, 100% ethanol), cleared in xylene (two changes), and coverslipped for microscopic examination.

With appropriate internal and external controls, positive ER or PR is considered if ≥ 1% of tumor cell nuclei are immunoreactive according to the guidelines (1). Allred scoring system is used in the current study. The score is assigned based on the summation of the proportion of positive cells (0: no positive cells; 1=1/100; 2=1/10; 3; 1/3; 4=2/3 and 5=all cells) and intensity of staining (0=negative; 1=weak; 2=intermediate and 3=strong) (17).

RESULTS

HER2 status in primary breast cancer compared to metastatic lymph node: One of the 39 cases was HER2 amplified in a nodal metastasis compared to the negative status in the primary tumor by FISH (Figure 1A-F). IHC was negative (1+) in both primary tumor and the nodal metastasis (Figure 1A-F) (Table I).

ER status in primary breast cancer compared to metastatic lymph node: Most cases, 31 of 36, show concordant ER status detected by IHC in LN metastasis as well as primary tumors, respectively (Figure 2A-F) (Table I). Three of 36 cases were ER negative in LN metastasis in contrast to the matched positive primary tumors (Figure 3A-F). Two of 36 the cases were positive in primary breast tumors (Allred score of >2) while the matched LN metastasis were ER-less strongly positive (Allred score of 1) (Figure 4A-F) (Table I).

PR status in primary breast cancer compared to metastatic lymph node: Most cases, 27 out of 36, show concordant PR status detected by IHC in LN metastasis as well as primary tumors, respectively (Figure 2A-F) (Table I).

Seven of the 36 cases were PR negative in LN metastasis in contrast to the matched positive primary tumors (Figure 3A-F) (Table I). Two of the 36 cases were PR-negative in primary breast tumors while the matched LN metastasis were positive (Figure 4A-F) (Table I).

Figure 1: Case with amplified HER2 in lymph node metastasis compared to the primary tumor. Upper panel is the primary tumor and the lower panel is lymph node metastasis. A,D) (H&E; x100), B,E) (IHC; x HER2) and C,F) (FISH; x HER2).
Figure 2: Case with concordant ER&PR IHC in both primary tumor and lymph node metastasis. Upper panel is the primary tumor and the lower panel is LN metastasis. A,D) (H&E; x100), B,E) (IHC; xER) and C,F) (IHC; xPR).

Table I: ER, PR and HER2 status in primary tumors and matched lymph node metastasis

<table>
<thead>
<tr>
<th></th>
<th>ER IHC</th>
<th>PR IHC</th>
<th>HER2 FISH</th>
</tr>
</thead>
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<tr>
<td></td>
<td>LN MET</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER IHC</td>
<td></td>
<td></td>
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</tr>
<tr>
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<td>0</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Weak Positive</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>PR IHC</td>
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<td>7</td>
<td>12</td>
</tr>
<tr>
<td>HER2 FISH</td>
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<td></td>
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</tr>
<tr>
<td>Amplified</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Non amplified</td>
<td>0</td>
<td>38</td>
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</tr>
</tbody>
</table>

IHC: Immunohistochemistry, ER: Estrogen receptor, PR: Progesterone receptor, LN: Lymph node, Met: Metastasis, FISH: Fluorescence in situ hybridization

DISCUSSION

In the present study, we evaluated the HER2, ER, and PR status of primary breast tumors with their matched LN metastases using FISH and IHC techniques. Most published studies have evaluated the HER2 status in primary tumors only. However, others studied HER2 in primary and metastatic sites with inconsistent data. This
inconsistency may be attributed to tumor heterogeneity in primary versus metastatic sites (20-23).

In the current study, only one of the 39 cases with HER2 non-amplified primary breast carcinoma showed amplified HER2 in the nodal metastasis. Likewise, a high concordance rate between primary breast tumors and matched LN metastasis have been reported by others (24, 25).

Niehans et al. compared HER2 expression in primary breast tumors in comparison to metastatic sites in autopsy samples from 30 decedents with known history of metastatic breast disease (26). This study documented 8 of these decedents was HER2 positive and, among those, there was a single case of discordant results. The authors concluded that HER2 expression is usually concordant between primary and metastatic sites. In agreement with our findings in the current report, Shimizu et al. evaluated HER2 protein, by IHC, in primary and metastatic breast cancer from 21 patients. The authors found no significant differences in the HER2 expression between the primary tumors and the nodal metastases (20). Masood et al. evaluated HER2 expression in 56 patients by IHC with 11 cases had distant site metastases. The score of the HER2 expression is identical in the primary tumors and the metastases with heterogeneity present in only one case (23).

Tanner et al. analyzed HER2 amplification in 46 primary mammary tumors and their matched metastases, using IHC and FISH techniques (22). The authors documented complete concordance regarding HER2 status between the primary tumors and the metastases. Ganberg et al. studied HER2 status in the primary and corresponding metastatic lesions and documented a high level of concordance (94% and 93% when analyzed by IHC or FISH, respectively) (27). All discordant cases showed an increase in the staining intensity in the metastatic site. Among the discordant cases by FISH assay, 3 had HER2 gene amplified in the metastatic site, and the reverse (HER2 gene amplification in the primary tumor) in 2 cases.

Numerous studies have been done to evaluate the ER expression in both primary and metastatic carcinomas (28-30). One study incorporated regional nodal and distant

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**Figure 3:** Case with positive ER and PR in primary tumor compared to negative LN metastasis. Upper panel is the primary tumor and the lower panel is lymph node metastasis. A,D) (H&E; x100); B,E) (IHC; xER) and C,F) (IHC; xPR).
In conclusion, while most matched primary breast tumors and LN metastases show concordance in HER2, ER, PR status, we found discordance in a minority of cases. These results support the newly adopted guidelines of ASCO/CAP to do HER2 studies in metastatic and recurrent breast tumors to guide further treatment options and predict prognosis in breast cancer patients.

**CONFLICT OF INTEREST**

The authors have no conflict of interest to declare.

**REFERENCES**


Management and Outcome of Uveal Melanoma in a Single Tertiary Cancer Center in Jordan

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Departments of 1Ophthalmology, 2Radiotherapy, 3Pathology and 4Medical Oncology, King Hussein Cancer Centre (KHCC), AMMAN, JORDAN

ABSTRACT

Objective: The aim of this study was evaluate the features and outcome of management of uveal melanoma in King Hussein Cancer Center as an example of a referral tertiary cancer center in the Middle East.

Material and Method: This was a retrospective, observational case series of 46 eyes of 46 patients with uveal melanoma. Data collection required access to medical records, radiology and pathology reports, and laboratory results. The main outcome measures included age at diagnosis, tumor location and dimensions, TNM stage, treatment modality, visual outcome, metastasis, and mortality.

Results: There was slight female preference, and the median age at diagnosis was 45 years. Eighteen (39%) eyes were treated by primary enucleation, and 28 (61%) eyes were treated by I-125 radioactive plaque. The melanoma was in the choroid in 40 (87%) eyes and in the ciliary body in 6 (13%) eyes, with no single tumor in the iris. According to the 7th edition of the American Joint Committee on Cancer staging system (UICC/AJCC); 8 (17%) were T1, 17 (36%) were T2, 16 (35%) were T3, and 5 (11%) were T4. One (2%) patient showed lymph node metastasis (N1), and 6 (13%) patients showed distant metastasis (M1). Pathologically, 2 (10%) of the enucleated eyes were spindle cell type, 4 (20%) were epithelioid cell type, and 14 (70%) were mixed type. Extrascleral extension was seen in three (15%) eyes, and optic nerve invasion in two (10%) eyes. After brachytherapy, 26 (93%) eyes were salvaged, and 2 eyes were consecutively enucleated; one for tumor recurrence, and one for uncontrolled painful neovascular glaucoma. The eye salvage rate post plaque was 93% (26/28), and the visual acuity for the salvaged eyes was equal or better than 0.5 in 11 (42%) eyes, 0.1-0.4 in 5 (19%) eyes, and less than 0.1 in 10 (38%) eyes.

Conclusion: The incidence of uveal melanomas in our region is low compared to that in the West with a younger age at presentation. Candidate tumors for radioactive plaque therapy were successfully controlled in 93% of cases

Key Words: Choroid, Enucleation, Melanoma, Radioactive plaque therapy

INTRODUCTION

Uveal melanoma is the most common primary intraocular malignancy in adults and accounts for 5% of all melanomas (1). It is seen more frequently in Caucasians in comparison with Hispanics, Asians and Africans. For the Whites in the United States, uveal melanoma has an incidence of 0.69 and 0.54 per 100,000 person-year for males and females consecutively with a mean age of 60 (1).

Uveal melanoma mostly appears in the choroid (85-91% of cases), and it is localized to the ciliary body or the iris in 9-15% of cases (2). Iris melanomas are associated with the earliest detection and overall best prognosis while ciliary body melanomas are associated with the worst prognosis (3,4). Around 50% of patients diagnosed with uveal melanoma will develop metastasis, despite treatment, with survival time after metastasis averaging 6-12 months (5,6).

The Collaborative Ocular Melanoma Study (COMS) concluded that there was no significant difference between brachytherapy and enucleation in terms of prevention of metastasis and mortality for medium sized melanomas. Globe and vision-preserving radiation therapy is therefore currently the primary treatment of choice for most uveal melanomas in the developed world (1,7,8).

There is limited data about the features and outcome of management of uveal melanoma in the Middle East in general and in Jordan specifically. The aim of this study is to describe the features and outcome of uveal melanoma management in a single tertiary cancer center in Jordan (King Hussein Cancer Center (KHCC), Amman, Jordan) in a developing country in the Middle East.
MATERIAL and METHODS

This study was approved by the Institutional Review Board in KHCC. It was a retrospective case series of 46 eyes of 46 consecutive patients from July 2006 to April 2014 who had intraocular uveal melanoma. Selection required access to patients’ medical charts, pathologic records, radiology reports, and laboratory results.

Outcome measures included: patient’s age at diagnosis, gender, laterality, smoking, presenting symptoms and visual acuity at presentation. Evaluated tumor clinical characteristics included: tumor location, surface features, shape, thickness, largest basal diameter, size, pigmentation, presence of subretinal fluid, vitreous hemorrhage, cataract, neovascular glaucoma, rubeosis, MRI features, TNM staging, presence and site of metastasis. For tumors treated by brachytherapy, additional features included plaque size, apex dose, rate of radiation, distance between tumor’s edge and the optic nerve and the fovea, tumor thickness and visual acuity after treatment.

Inclusion and Exclusion Criteria: The eligibility criteria for inclusion were eyes with clinical and/or pathologic diagnosis of intraocular uveal melanoma treated either by radioactive plaques or by enucleation.

Pathological Characteristics and Definitions: In this study, the tumors were classified according to the Collaborative Ocular Melanoma Study (COMS) classification. The COMS divided uveal melanomas based on size into small, medium and large tumors (9). Small melanoma; 5-16 mm at the largest basal diameter (LBD) and 1-3 mm in apical height. Medium-sized melanoma; 16 mm or less at the LBD and had an apical height between 3 mm and 10 mm and uveal melanomas more than 16.0 mm at the LBD and more than 10 mm in height were defined as large tumors. Pathologically; and according to the Callender Classification, mixed tumors have been defined as tumors that had less than 50% of cells as epithelioid in type, while epithelioid tumors are those with more than 50% of cells as epithelioid in type (10,11). TNM staging was according to the 7th edition of the American Joint Committee on Cancer (AJCC) staging system (12).

Reviewed data from the medical records regarding treatment included the following: complications of brachytherapy, and histopathological features of enucleated eyes. The histopathology was further reviewed regarding extraocular extension of the tumor such as extracocular and/or optic nerve involvement at the time of enucleation.

Follow-up of these patients was documented including period, evidence of metastasis and patient status during the period of the follow-up.

In our center, indications for enucleation included a tumor involving or touching the optic nerve, large-sized tumor, recurrence of tumor after brachytherapy, associated total retinal detachment and secondary neovascular glaucoma (NVG).

RESULTS

Seventy-six eyes were diagnosed with uveal melanoma in King Hussein Cancer Center (KHCC) between July 2006 and April 2014. Thirty patients were excluded from the data analysis because of inadequate data and/or because the patients refused treatment and were lost for follow up.

Demographics and clinical features: 46 eyes with uveal melanoma from 46 patients were studied. The mean age at diagnosis was 46 years (median 45 years, range; 1.5-75 years.). There were 21(45%) males and all (100%) patients had single tumor. Eighteen (39%) eyes were treated by primary enucleation, and 28 (61%) eyes were treated by I-125 radioactive plaque therapy. Two of the eyes treated by plaque therapy were consecutively enucleated. Demographics are shown in Table I.

Tumor features: The melanoma was in the choroid in 40 (87%) eyes, in the ciliary body in 6 (13%) eyes, and no single patient had iris melanoma in this series. According to the 7th edition of the American Joint Committee on Cancer staging system (UICC/AJCC); 8 (17%) were T1, 17(36%) were T2, 16 (35%) were T3, and 5 (11%) were T4. One (2%) patient had lymph node metastasis (N1), and 5 (11%) patients showed distant metastasis (M1). One patient already had metastasis at time of diagnosis while the others were discovered to have metastasis later after the diagnosis by an average interval of 26 months. Details of tumor features in both groups (enucleation and plaque group) are shown in Table II.

Pathologic features of enucleated eyes: A definitive diagnosis of uveal melanoma was confirmed by histopathology in 20(43%) eyes after enucleation. On histopathological examination, 2 (10%) tumors were spindle cell type uveal melanoma, 4 (20%) tumors were epithelioid cell type, and 14 (70%) tumors were of mixed type uveal melanoma (Figure 1A,B). Extradural extension was seen in 3 (15%) eyes (Figure 1C), and optic nerve invasion was seen in 2 (10%) eyes.

Plaque features: The radioactive plaques used had a median size of 16 mm with a range between 12 mm and 20 mm. The total radiation apex dose was 85 Gy in all patients (median radiation rate = 7.25, range: 4.5 to 13). The main complications included 5 cases of cataract, 7 cases of NVG,
Table I: Demographics and clinical features of the patients

<table>
<thead>
<tr>
<th>Number</th>
<th>Enucleated Group</th>
<th>Plaque Group</th>
<th>Total Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>20*</td>
<td>28</td>
<td>46</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>1.5-67</td>
<td>21-75</td>
<td>1.5-75</td>
</tr>
<tr>
<td>Gender**</td>
<td>6 M 14 F</td>
<td>16 12</td>
<td>21 25</td>
</tr>
<tr>
<td>Side</td>
<td>6 Right 14 Left</td>
<td>11 17</td>
<td>19 27</td>
</tr>
<tr>
<td>Smoking</td>
<td>7 Yes 13 No</td>
<td>8 20</td>
<td>14 32</td>
</tr>
</tbody>
</table>
| Presenting symptom | 18 Impaired vision 20 | 36 87 | Others*** **
| Visual acuity at presentation | >=0.5 18 | 14 50 | 16 35 |
|              | 0.1-0.4 5       | 9 32         | 13 28       |
|              | <0.1 13         | 5 18         | 17 37       |

* 18 eyes were primarily enucleated and 2 eyes were enucleated after failure of salvage by radioactive plaque. ** M= Male, F= Female.
*** Others included: floaters, wondering eyes

Figure 1: Histopathologic appearance of choroid melanoma. A) Spindle cell melanoma type B showing pigmented spindle cells with vesicular oval large nuclei and prominent nucleoli with prominent mitotic activity (H&E; x200), B) HMB-45 positivity in case of epithelioid melanoma (HMB-45; x200), C) Mixed type choroid malignant melanoma with perivascular extrascleral extension (H&E; x40).
Outcome and follow up: Twenty eyes were enucleated; 17 (85%) eyes were large (more than 16.0 mm at the LBD and more than 10 mm in height), 17 (85%) eyes had RD, 1 (5%) eye had tumor recurrence after plaque therapy, 2 (10%) eyes had neovascular glaucoma, and 2 (10%) tumors were touching the optic nerve. Three (15%) patients required additional external beam radiation post enucleation due to extraocular tumor extension confirmed pathologically. At a median follow up of 24 months in the plaque group, 26 eyes were salvaged while 2 eyes have been enucleated.

Five patients out of 46 patients (11%) included in our series had metastasis (5 had liver metastasis, 1 had lung

1 case of recurrence, 1 case of radiation optic neuropathy, and 5 cases of radiation retinopathy. At last follow up after therapy, tumor thickness was < 5 mm in 13 (46%) eyes, and 5-10 mm in 13 (46%) eyes. The median tumor thickness after therapy was 4.5 mm (range: 2-8 mm), and the decrease in tumor thickness was variable between the treated eyes (Table III). Two eyes were consecutively enucleated; one for tumor recurrence, and one for uncontrolled painful NVG. After therapy, visual acuity was equal or better than 0.5 in 11 (42%) eyes, 0.1-0.4 in 5 (19%) eyes, and less than 0.1 in 10 (38%) eyes. 2 (7%) eyes had better vision while 15 (54%) eyes had worse vision after treatment.

Table II: The characteristics of the tumors

<table>
<thead>
<tr>
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<th>Enucleated Group</th>
<th>Plaque Group</th>
<th>Total Group</th>
</tr>
</thead>
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<td>Number</td>
<td>20*</td>
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<tr>
<td>Number</td>
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<td>%</td>
<td>%</td>
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<tr>
<td>Site</td>
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<td>Ciliary body</td>
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<td>Iris</td>
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<tr>
<td>Shape</td>
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<td></td>
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<td>Thickness at diagnosis</td>
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<td>8</td>
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<tr>
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<td>N1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M-stage</td>
<td>M0</td>
<td>18</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>M1</td>
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<td>10</td>
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<td>Associated features**</td>
<td>Subretinal fluid</td>
<td>17</td>
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<td>Vitreous hemorrhage</td>
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<tr>
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<td>Cataract</td>
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</tr>
<tr>
<td></td>
<td>Orange Pigments</td>
<td>17</td>
<td>85</td>
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</tbody>
</table>

*2 Eyes were enucleated after failure of salvage by radioactive plaque. ** Some patients had multiple associated features.
metastasis, 1 had lymph nodes metastasis and 1 had bone metastasis). 3 patients had plaque therapy and the other 2 underwent enucleation (one found to have epithelioid cell type and the other mixed cell type). 2 patients had a large-sized tumor, all tumors located in the choroid, and 3 patients were dead from the metastasis at the last date of follow up after an average of 30 months from time of diagnosis. Details of outcome in enucleation and plaque groups are shown in Table III.

### DISCUSSION

Well-developed data about uveal melanoma in the Middle East is missing except in the form of small local case series and case reports. Our series showed 77 cases of uveal melanoma documented between 2006 and 2014 in a tertiary cancer center in Jordan, and this low number provides the impression that uveal melanoma occurs with a low frequency in our region. In Saudi Arabia, one study showed only 40 cases of uveal melanoma diagnosed between 1983 and 2005, only 28 of them were of Saudi Arabian ancestry (13). Similarly, another report from the Shanghai Eye, Ear, Nose and Throat Hospital in China showed only 103 cases of uveal melanoma diagnosed between 1955 and 1979 (14). On the other hand, 688 cases of uveal melanoma were diagnosed among New York State residents between 1975 and 1986 (15), and similarly, 2997 patients had been registered to have uveal melanomas in Sweden during the period from 1960 to 1998 (16). Even statistics about the incidence of uveal melanoma in the Middle East and most of the developing countries are missed, it seems that the incidence in the Middle East and in Asia is less than the incidence of uveal melanoma in USA and Europe.

This wide variation in incidence can be attributed to the light skin color in USA and Europe residents which is one of the risk factors for developing this tumor.

The average age at diagnosis in this series was 46 years, while the average age at diagnosis in the COMS study was 60 years (17), which is 14 years older than the age at diagnosis in our series and 10 years more than in patients participating in a study performed in Saudi Arabia reporting an average age at 50 years (13). The reasons for younger age of incidence in our patients are not known. There was a slight, statistically insignificant, predominance of females in our retrospective study, in contrary to most reported studies that showed male predominance (18-21). This difference may be due to the low number of patients in our series. However, no sex predilection was found in the COMS randomized prospective study (6).

In our review, a significant percentage (65%) of affected eyes had a visual acuity of less than 0.1, which is worse than the visual acuity for of patients studied in the COMS study where only 33% had visual acuity of less than 0.1 (9).
after the onset of ocular complaints in developing poor countries where health care could be unachievable because of the high cost or of far distance to travel.

Most (70%) of our patients who underwent enucleation had the mixed cell type melanoma, 20% had the epithelioid cell type, and 10% had the spindle cell type, which is almost similar to COMS findings where 86% were of the mixed cell type (22). This indicates that melanoma in our community is pathologically similar to the west.

In the COMS study, the estimated melanoma-related mortality was 1% at 5 years and was 4% at 8 years for patients with small melanomas (23). 5-year melanoma-related mortality, based on histopathologically confirmed metastasis, increased to 10% for patients with medium-sized tumors, and to 28% for patients with large tumors (24). The delay in presentation to our institution likely played a major role in finding a significant number of patients (about 98%) with medium and large uveal melanomas. Due to the short follow-up period available (median of 24 months), it was difficult to determine the survival outcome among our patient population. Of the 5 patients who had metastasis in this series, 3 patients had medium sized tumors and 2 patients had large sized tumors. In the COMS, the liver was the predominant site of metastasis, which was reported in 89% of metastatic patients (25). Our study showed metastasis in only 11% of patients and in all the liver was involved.

It can be concluded that the incidence of uveal melanomas in our region is low compared to that in the West. A significant number of Arabic patients, unfortunately, present to ocular oncology clinics at a time where the tumor reaches a large size or is associated with complications that make it non-amenable for brachytherapy and end up with enucleation. Therefore, awareness must be increased and early detection improved with prompt referral by the general ophthalmologist to save more eyes and to enhance survival of affected patients. This study was retrospective, so the follow-up was limited after treatment. It is recommended to perform larger, multicenter and longer term follow-up studies with more emphasis on accurate and detailed gathering of information from the patients before and after treatment in addition to comprehensive clinical and investigational exams to determine the true incidence and predisposing risk factors in addition to improving statistical data on uveal melanomas in our region.

ACKNOWLEDGEMENTS

We acknowledge the support of the Eye Cancer Foundation Inc. (New York, NY USA, http://eyecancerfoundation.net) for Dr. Zewar for the Ocular Oncology Fellowship.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

FUNDING SOURCE

The authors declared no funding source was involved in the creation of this manuscript.

REFERENCES


Pulmonary Benign Metastasizing Leiomyoma: An Extremely Rare Case

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Presented at the 6th Thoracic Surgery Congress, April 28 - May 1, 2011 in Antalya, Turkey

ABSTRACT

Benign metastasizing leiomyoma is typically seen in young premenopausal women after a mean period of 15 years following uterine leiomyoma or hysterectomy surgery. They are usually incidentally seen on chest x-rays and are nodular lesions that appear as bilateral nodules with a benign appearance and consist of smooth muscle proliferation. A 44-year-old female presented at her healthcare institution for backache for the last 9 months. Multiple nodules (largest 15 mm) scattered in both lungs and consistent with metastases were detected on computed tomography. The PET-CT results revealed multiple nodular densities with increased metabolic activity (SUVmax: 1.92) in both lungs, with the largest one measuring approximately 15 mm and located in the lower lobe superior segment of the right lung. A benign metastasizing leiomyoma was diagnosed with open wedge biopsy of the lung. We present this case due to its interesting clinical presentation and rarity and emphasize the pathogenesis.

Key Words: Leiomyoma, Uterus, Lung, Metastasis, Lymphatic

INTRODUCTION

Benign metastasizing leiomyoma (BML) is a rare condition with interesting clinical characteristics. This terminology was first reported in English literature in 1939 by Steiner (1). BML generally occurs in women during their reproductive years. BML is usually associated with uterine leiomyoma and may develop years after a hysterectomy or myomectomy. They are incidentally noticed on chest radiography as bilateral benign lesions proliferating from smooth muscles. Although most commonly seen in the lungs, other sites of involvement have been reported in the literature including lymph nodes, omentum, pelvis, abdomen, mediastinum, vertebra, cranium, skeletal muscle, skin, vena cava inferior, right atrium, breast, trachea, esophagus, liver and adrenal glands (2,3). Patients with pulmonary BML are almost always asymptomatic, though some patients present with cough or breathlessness not affecting pulmonary function. There is no standardized treatment available; treatment options include hormonal manipulation with bilateral oophorectomy or hormonal therapy together with pulmonary nodule resection (4, 5).

CASE REPORT

A 44-year-old woman with a backache for 9 months was referred to our center for further evaluation and treatment of a suspected diagnosis of pulmonary metastasis. Her computed tomography scan revealed multiple nodules (largest 15mm) scattered in both lungs (Figure 1A,B) and the tru-cut transthoracic biopsy was non-diagnostic. The patient had undergone a myomectomy in 1994 and a total abdominal hysterectomy (adnexal preservation) in 1996 for fibroids. A pelvic USG revealed a 31x18 mm hypoechocic fusiform mass in the right lateral abdominal wall. PET-CT results, correlated with CT findings, revealed a large nodule in the right lower lobe superior segment with increased activity (SUVmax: 1.92) and another 31x18 mm soft tissue lesion in the right pelvic region with increased metabolic activity (SUVmax: 4.60). In October 2010, a total of 5 nodules, the largest measuring 1.5cm, were excised from the middle and lower lobe via right thoracotomy and later, in November 2010, a total of 6 nodules, the largest measuring 1.2 cm, were excised via left thoracotomy (Figure 2).

On microscopic examination, a well-circumscribed lesion with compressed adjacent lung parenchyma and interlacing bundles of spindle cells was seen (Figure 3). Anaplasia, mitosis, necrosis, or hemorrhage was not seen. Strong positivity for vimentin, smooth muscle actin, and desmin were detected by immunohistochemical methods. Immunoreactivity for progesterone and estrogen receptors was strongly positive (Figure 4). The proliferative index with Ki-67 was 3%. According to these findings, benign metastasizing leiomyoma was diagnosed. Consequently, in 2011, bilateral
salpingo-oophorectomy was performed to complete the treatment. The previous lesion noticed in the right pelvic region was reported to be leiomyoma.

**DISCUSSION**

Benign metastasizing leiomyoma (BML) is a very rare condition that is predominantly seen in women during their reproductive years. BMLs are usually associated with uterine leiomyoma and may develop years after a hysterectomy or myomectomy (approximately 14.9 years). The average number of nodules is 6 with an average size of 1.8 cm. Pulmonary lesions are bilateral in 70%, unilateral in 17% and solitary in 13% of cases. The average survival after pulmonary resection is 94 months (1, 6). In our case, the patient had undergone a myomectomy 16 years earlier and total abdominal hysterectomy 14 years before the recurrence. A total of 5 nodules, the largest measuring 1.5 cm, were excised from the right lung and a total of 6 nodules, the largest being 1.2 cm were excised from the left lung. This finding is similar to reported literature. We could not obtain paraffin blocks of previous specimens and hence were unable to recheck them.

The pathogenesis of BMLs is controversial. Various hypotheses on the histogenesis of these lesions have been suggested, which include: (i) Metastases of low grade and undetected leiomyosarcomas of the uterus; (ii) Smooth muscle proliferation in several organs, such as the lung and uterus resulting from an abnormal sexual hormone status; (iii) The last, and most accepted hypothesis, is the spread by lymphovascular dissemination of benign uterine leiomyoma cells. The theory of lymphovascular dissemination of uterine leiomyomas is based upon reports of spontaneous

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**Figure 1A,B**: Chest CT showing bilateral separate nodules.

**Figure 2**: Macroscopic view of the excised pulmonary nodule.

**Figure 3**: The specimen showed a well-circumscribed nodule with compressed adjacent lung parenchyma having interlacing bundles of spindle cells (H&E x200).

**Figure 4**: Wide nuclear ER expression in the smooth muscle cells (ER x400).
regression of pulmonary leiomyoma during pregnancy, absence of evidence of necrosis, and lack of mitotic activity (1,7). Studies of the pulmonary and/or extra uterine nodules have revealed similar immunohistochemical, genetic and molecular characteristics to uterine leiomyomas (8-11). Vimentin, smooth muscle actin and desmin expressions, together with positive ER and PR is present in 80% of cases. There is no positive ER expression in extra uterine leiomyoma. There is 13% weak focal positive ER in LMS cases. In our case, vimentin, smooth muscle actin, desmin, ER and PR immunohistochemical expression were present. The Ki-67 proliferative index is low in BML cases. In two different studies the ratios were reported to be 2.3% and 2.9%. In these studies, the Ki-67 expression in LMS cases was 28.6% and 11% (1,9). In our study, the Ki-67 proliferation index was approximately 3%. In a 3 case literature report, 2 cases showed monoclonality in the uterine and pulmonary tumors; while the third was non-informative. In the same study, the length of the telomere in the uterine and pulmonary tumors was found to be long, yet a longer telomere length was noticed in the other case. Meanwhile, deletion in the longer arm of chromosomes 19 and 22 is frequently noticed (10). With in situ hybridization techniques using mir-221 micro-RNA analysis 13 out of the 15 LMS cases showed expression, while there was no expression in the 10 BML and 8 leiomyoma cases. The study pointed out the use of these features in the differential diagnosis between BML and LMS (11). Usually diagnosis of pulmonary BML is made by open lung biopsy. In a 7 case literature report, diagnosis with cytology and transbronchial biopsy was inconclusive, while in 1 case diagnosis was made with tru-cut biopsy (12). In our case, transthoracic tru-cut was non diagnostic and hence open lung biopsy was used. There is no standardized treatment, thus medical (anastrozole, tamoxifen, raloxifene, progesterone and GnRH agonists) or surgical (bilateral oophorectomy) and hormonal manipulation together with general excision of the pulmonary nodules may be conducted (1,4,5). In our case, bilateral salpingo-oophorectomy was used to block the estrogen release. The lesion in the paratubal region was diagnosed as leiomyoma. Metastases, sarcoidosis, inflammatory diseases, hamartoma, rheumatoid nodules, Wegener’s granulomatosis and amyloidosis should be considered in radiological and clinical differential diagnosis (13). In our case, the thorax CT, radiological and clinical findings suggested metastases. The course of the disease depends on the state of the estrogen receptor (ER). Decrements in the pulmonary nodule size have been noticed after menopause, during pregnancy and hormonal contraception (14). With GnRH agonist, there has been a 50% decrement in the size of pulmonary nodules (15). In our case, after the nodule excision, there was no recurrence on the CT taken 12 months postoperatively.

REFERENCES

Nasal Polyp – An Incidental Paraganglioma

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ABSTRACT

The nose is an uncommon site for head and neck paraganglioma. The diagnosis is seldom established pre-operatively; its rarity, infrequent functionality and often benign biologic outcome underlie this fact. We present one such case in a 60-year-old man who presented with right nasal obstruction and episodic epistaxis. Rhinoscopy revealed a fleshy polypoid mass arising from the anterior cartilaginous nasal septum. Imaging studies excluded extra-nasal extension. The tumor was highly vascular showing numerous variable sized, mostly thin walled branching blood vessels akin to stag-horn shape simulating a vascular neoplasm. There were large areas of hyalinization. The typical tumor morphology was discernible only in focal areas. Immuno-histochemistry confirmed the diagnosis. The tumor cells expressed neuron specific enolase; S-100 stain demonstrated a vague zell-ballen pattern. Paraganglioma is a rare histologic diagnosis in nasal polypectomy specimen. We discuss the approach to exclude its morphologic mimics including vascular tumors.

Key Words: Nose, Paraganglioma, Epistaxis, Extra-adrenal, Nasal polyp

INTRODUCTION

Tumors of paraganglia arising outside the adrenal medulla are called paraganglioma (PG) (1). They may come to clinical attention due to their potentially curable symptoms associated with hyper-function. Although considered the extra-adrenal counterpart of pheochromocytoma, they differ from the latter in many aspects. The head and neck (HN) region is the most common extra-adrenal site for these tumors, however nasal location of such tumors is rare (2-5). We share our experience with this unexpected occurrence and discuss the diagnostic approach to exclude its morphologic mimics.

CASE REPORT

A 60-year-old male presented to a tertiary care hospital in Delhi with complaints of right nasal mass of 8-10 weeks duration causing obstruction and intermittent epistaxis. There were no complaints of rhinorrhea, allergy, headache, palpitation, tinnitus or cranial nerve palsies. He had undergone an appendectomy 18 years ago. General physical examination did not reveal any abnormalities of pulse or blood pressure. Rhinoscopy revealed a fleshy, polypoid mass arising from the anterior cartilaginous nasal septum and distending the right nostril. It bled on touch during the examination. Pre-operative investigations including imaging studies were unremarkable. With a clinical diagnosis of an inflammatory nasal polyp, right nasal polypectomy was performed under general anesthesia. The sessile polyp was excised along with an adjacent sleeve of mucoperichondrium and anterior nasal packing done for 48 hrs. His intra-operative period and post-operative course were uneventful. Subsequent radiologic investigations were unremarkable. The patient had been asymptomatic in his 18 months follow up.

The specimen consisted of a single mucosa covered polypoid soft tissue mass measuring 1.5 x 1.0 x 0.4 cm. It was formalin fixed and paraffin embedded in toto. Sections revealed a highly vascular well circumscribed tumor. The vascular channels’ caliber varied from small to ectatic; few were branching and stag-horn shaped. Majority of vessels were thin walled, few larger ones had thicker walls. There were large intervening areas of hyalinization especially prominent in the perivascular location (Figure 1). The cellular areas were few and showed large cells arranged in sheets and nests. The cells had abundant clear to pale eosinophilic granular cytoplasm (Figure 2). Few showed cytoplasmic vacuoles. Nuclei were centrally located and contained inconspicuous nucleoli in many cells. Mitoses were occasional; there were no areas of necrosis. The tumor was reaching up-to the overlying epithelium, which showed squamous metaplasia and focal ulceration. Although PG was suspected, definite diagnosis was deferred to immuno-histochemistry (IHC). Polygonal cells with abundant pale cytoplasm in a highly vascular hyalinized background raised other possibilities- malignant melanoma, metastatic carcinoma, PEComa, chordoma, glomus tumor and epithelioid hemangioendothelioma.
The high vascularity and hyalinization warranted exclusion of sino-nasal type hemangiopericytoma (SNTHP) and solitary fibrous tumor (SFT).

A panel of IHC comprising chromogranin A, neuron specific enolase (NSE), S-100, HMB-45, CD34, CD31, cytokeratin, epithelial membrane antigen (EMA), smooth muscle antigen (SMA) and desmin was performed using appropriate positive and negative controls. Tumor cells strongly expressed chromogranin A and NSE (Figure 3). S-100 expression was scarce and outlined the occasional sustentacular cells and zellballen pattern. CD31 and CD34 expression was limited to endothelial cells (Figure 4). Results of other immuno-stains were negative/ non-contributory. Hence the diagnosis of PG of nose was confirmed.

**DISCUSSION**

PGs, the neural crest origin tumors arise from paraganglia, structures lying adjacent to autonomic ganglia (1). HN region is the most common site of PG followed by abdominal and thoracic (2). Almost half of HN PGs are located at carotid artery bifurcation. Others in descending frequency in this region are jugulotympanic tumors and glomus vagale (2, 3). Rare sites of HN PG include nose, orbit, larynx and thyroid (1, 3, 4). Almost all HN PGs are suspected pre-operatively either due to their peculiar location and/or typical imaging characteristics. Only 1.2% of HN PGs are true incidentilomas, i.e. require pathological examination of an indeterminate mass to confirm the diagnosis, as happened in our case (2).

HN PGs differ from abdominal PGs in many aspects (2,3). They are associated with parasympathetic nervous system, while abdominal PGs show evidence of sympathetic activity. Despite ultra-structural evidence of neuro-endocrine differentiation in HN PGs, less than 10% produce catecholamines in amounts sufficient to result in headaches, palpitations and perspiration. This is in contrast to almost one-forth of abdominal PGs patients having such symptoms. The frequency of hypertension in HN PGs (42%) is also reported to be lower than abdominal ones (64%) (2). Endocrine silence of HN PGs usually makes them symptomatic either as space occupying lesion related to their anatomic site or with symptoms secondary to nerve compression such as tinnitus and cranial nerve palsies (2, 3). HN PGs are distinctly smaller and less likely to be malignant than their abdominal counterparts (2, 5). In a retrospective analysis, Flines et al. did not find any
difference in survival between their cohort (mean follow up = 26.4 years) in comparison to the general population. The deaths in their study population were attributed to surgical complications of carotid body tumors (5). Up-to 30% of HN PGs may be associated with mutations of genes encoding various subunits of succinyl dehydrogenase (SDH) enzyme. Familial PGs are commonly multiple, bilateral and present at an earlier age than sporadic tumors. Molecular tests are indicated only in the setting of family history, previous pheochromocytoma, multiple tumors and age < 40 years (6).

The nose is an exceptional site of PG. Till date, less than 50 nasal PGs have been described, a testimony to its rarity (1, 3-5, 7-10). Nasal PGs have been reported in a wide age range, 8-72 years (3, 7). They often present with nasal obstruction and/or epistaxis as happened in our case (3, 4, 7). The highly vascular nature of the tumor and trauma attendant to its peculiar location may explain the epistaxis. It may be said that most nasal PGs are non-functional, although occasional reports of Cushing’s syndrome secondary to ACTH production are on record (8). Distinction of de novo nasal PGs arising from nasal mucosa from extension of jugulo-tympanic or vagal tumors is essential to decide surgical aspects and is based on radiologic features (9). As for HN PGs, most reported nasal tumors have had a benign course, although occasional cases with late recurrence have also been reported (10).

The typical morphology of PGs composed of chief cells and surrounding sustentacular cells may not be obvious in all cases. Extensive secondary changes like hyalinization, ectatic blood vessels, sclerosis and others may render their recognition difficult as happened in our case. NSE expression is almost invariable in PGs. The sustentacular cell network is outlined by S-100 and GFAP immuno-stains. Demonstration of sustentacular cells may be difficult when tumor cells are present in sheets or large nests, especially in small or fragmented sections (1). Neither atypical histological features nor infiltration are considered indicative of malignancy. Metastasis to organs normally devoid of chromaffin cells remains the only evidence of malignancy (1,10).

Cellular areas of PGs may resemble malignant melanoma, metastatic carcinoma, chordoma, perivascular epithelioid cell tumor (PEComa), glomus tumor and EH, all tumors rare to the nose (11-17). They share cytologic feature of voluminous clear to eosinophilic cytoplasm of the tumor cells. Malignant melanomas usually have prominent nucleoli; they invariably show diffuse positivity for S-100 and HMB-45 (11). Appropriate clinical setting and expression of epithelial markers will help establishing diagnosis of metastatic carcinoma (12). Physalliferous cells are characteristic of chordoma, but may be few. Chordomas are decorated by cytokeratin, EMA and S-100 (13). In the present case thorough search neither revealed cells with prominent nucleoli nor with spidery cytoplasm. PGs do not stain with epithelial markers or HMB-45 and S-100 staining is limited to sustentacular cells. Radial arrangement of cells around blood vessels and low grade nuclei are seen in PEComa and glomus tumor. Although highly vascular, our case showed large areas of perivascular hyalinization rather than cellularity typical of these tumors. PEComas and glomus tumors typically stain with HMB-45 and SMA respectively (1, 14, 15). Expression of desmin is variable in both. PGs are typically negative for HMB-45, SMA and desmin. Conversely PEComas and glomus tumors do not express NSE. EH, an angiocentric tumor may show clusters of large cells resembling PG. Intracytoplasmic vacuoles often containing erythrocytes indicate its vascular origin. Endothelial cell markers like CD31 and CD34 are expressed consistently in EH (1,16). The cells in the present case did have vacuolated appearing cytoplasm but did not have the typical blistered look of EH; none contained erythrocytes. IHC for CD31 and CD34 outlined the endothelial cells lining the ecstatic vessels; tumor cells did not take the stain. Highly vascular tumors with staghorn-like branching vessels and prominent hyalinization in this region, albeit rare include SNTHP and SFT (17,18). SNTHP is a low to intermediate grade tumor of perivascular myoid phenotype containing HP like staghorn vessels. Its cells are bland, spindle shaped and arranged in fascicular and/or whorled pattern. Their immuno-phenotype resembles glomus
tumor rather than HP. Most react with vimentin (98%), SMA (92%), and factor XIIIa (78%); expression of CD34 is variable (17). The cells in our lesion were polygonal rather than spindle and their immuno-phenotype was typical of neuro-endocrine nature. SFT, a fibroblastic mesenchymal tumor also has prominent HP like branching vasculature. Its cells are generally arranged in fascicular fashion unlike the nesting pattern of polygonal cells of PGs. Its cells stain with CD34, bcl-2 and CD99 (18). CD34 stain was consistently absent in tumor cells in our case.

To conclude, we have shared our experience with an unsuspected nasal PG. This rare neoplasm of the nose is likely to be a histologic surprise in the rather common nasal polypectomy specimens. Identification of its typical morphology is essential for correct diagnosis and proper management. Immuno-staining for NSE and chromogranin A is likely to be useful in cases obscured by extensive secondary changes.

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ABSTRACT

A congenital pulmonary airway malformation is a rare disorder of the pulmonary airway and a hamartomatous mass of disorganized lung tissues with various degrees of cystic change. A 20-year-old pregnant woman who did not have previous clinical follow-up during her pregnancy visited the gynecology department for her first check on the 19th week of gestation. The sonogram showed severe hydrops fetalis. Laboratory findings were consistent with non-immune hydrops fetalis. Medical abortion was performed and the fetus was sent to our department for a complete fetal autopsy. Macroscopically, whole parts of the fetus had striking oedema. Massive pleural and peritoneal effusions were seen on dissection. The left lung filled the whole thoracic cavity. The heart was displaced to the right and the right lung was compressed. Microscopically, the left lung mass showed dilated bronchiole-like structures (1-20 mm) that were lined with ciliated columnar cells without any intervening mucinous cells. The subepithelial stroma contained thin, interrupted smooth muscle fibers and elastic connective tissue without cartilage plates. Our case is a very good example of non-immune hydrops fetalis associated with congenital pulmonary airway malformation type 2. Prenatal clinical and ultrasonographic follow-ups during pregnancy are very important for early diagnosis of congenital malformations.

Key Words: Cystic adenomatoid malformation of lung, Congenital, Lung diseases, Hydrops fetalis

INTRODUCTION

Congenital pulmonary airway malformation (CPAM) of the pulmonary airway is an unusual lesion, combining features of hamartoma, malformation or dysplastic proliferation. CPAM was first described using the past terminology of congenital cystic adenomatoid malformation by Ch’in and Tang in 1949 (1-3). Stocker (4) suggested an expanded classification renaming this group of malformations as congenital pulmonary airway malformation (CPAM). There are five types. Only one of the types (type 3) is adenomatoid and only three (types 1, 2 and 4) are cystic. CPAM is quite rare and the etiology and incidence are unknown (5). However CPAM represents about 25% of all congenital lung lesions. In the literature, male and female populations are affected equally. Lower lobe lesions predominate with 44% of all cases while the rest of the cases are scattered in other lobes and is primarily unilateral, but may also occur bilaterally (6). Associated findings in CPAMs are polyhydramnios, pleural effusion and rarely non-immune fetal hydrops (7).

CPAM is characterized by the lack of normal alveoli, an excessive proliferation and cystic dilatation of terminal respiratory bronchioles with various types of epithelial lining. Microscopically the cyst linings are composed of ciliated, cuboidal or columnar cells and those cysts have lack of normal architecture and are frequently devoid of cartilage (8). Type 2 CPAM that is associated with other congenital anomalies is seen more frequently than other types (2). We report a fetal autopsy case of type 2 CPAM with an unusual combination of accompanying extra pulmonary abnormalities with prenatal diagnosis at the 19th week of gestation.

CASE REPORT

A 20-year-old woman who did not have previous clinical follow-up during her pregnancy was referred to the gynecology department for her first control at the 19th week of gestation. The ultrasonogram (USG) showed severe hydrops fetalis with other sonographic abnormalities including a large mediastinal mass filling the left thoracic cavity, causing the mediastinal shift, displacing the heart to the right side, and compressing the right lung, vena cava superior and vena cava inferior. There was dilatation in the lateral ventricle of the brain and the choroid plexus was depressed. There was no relationship between mass and
vascular system in Doppler USG. Medical abortion was performed at 30th week of gestation and the fetus was sent to the pathology laboratory for a complete medical autopsy. Macroscopically whole body parts of the male fetus had a striking oedema. There were massive pleural and peritoneal effusions on dissection of peritoneal and pleural cavities. The left and right main bronchus and their relationships with mass were evaluated. The left lung was enlarged with dimensions of 12x11x7 cm, filling the entire thoracic cavity. It had cystic dilated structures of various size (Figure 1). The heart was displaced to the right side and the right lung was compressed and atrophied with dimensions of 1.5x1.3x1 cm. Massive fetal oedema was present due to compression of both vena cava inferior and superior by the huge mass of the left lung. Thus, hydrops fetalis was caused by a non-immune reason, which was later also supported by the absence of maternal antibodies.

Microscopically, the left lung mass showed dilated bronchiole-like structures that were lined by ciliated columnar cells without any intervening mucinous epithelial cells. The sub-epithelial stroma between the cystic spaces contained thin, interrupted bands of smooth muscle fibers and elastic connective tissue without any cartilage plates (Figure 2A,B). Immunohistochemically dilated cystic structures showed positive staining for CK7 and TTF1 and subepithelial stroma revealed SMA positive smooth muscle fibers (Figure 3). Other fetal tissue and organs showed massive oedema and immaturity development concordant with the gestational age. The diagnosis was CPAM, type II, according to the modified Stocker's classification (3).

DISCUSSION

Cystic malformation of the lung is an unusual congenital lesion characterized by cystic spaces of various sizes composed of airway or alveolar-like structures (6). CPAM was first described by Ch’in and Tang in 1949 (1). CPAM is generally a unilateral lesion of one lobe and represents about 25% of all congenital lung lesions (5). The lesion consists of cysts and solid airless tissue with no cartilage in the wall. It may affect the pulmonary lobes partially or entirely (9).

Congenital pulmonary airway malformation is a hamartomatous, dysplastic developmental abnormality of the lung. It shows hamartoma, dysplasia or tumorous features. There is a putative differentiation from proximal to distal with type 0 originating from the trachea and bronchi and type 4 is originating from the acinus. It is apparent that narrowing or obliteration of the bronchial lumen is a common pathological feature most of the time. Bronchial atresia may especially cause this morphology. The primary bronchial atresia, bronchial segmental disability, pause in the development of the fetal lung, parenchymal differential disability, and dysplastic bronchopulmonary tissue are generally seen at 5-7th weeks of gestation (9). Abnormal Hoxb-5 regulation causes specific alterations in airway branching. Normal lung tissue does not express significant levels of Hoxb-5 protein, while the adjacent CCAM with abnormal and immature airway express the high levels of Hoxb-5. The abnormal expression of this Hox gene could be associated with the development of aberrant branching patterns in BPS and CCAM (10).

Congenital pulmonary airway malformation classification scheme has been revised in 2002 by Stocker and categorized them as: type 0, trachea-bronchial; type 1, bronchial/
bronchiolar; type 2, bronchiolar; type 3, bronchiolar/alveolar duct; and type 4, distal acinar (16) (Table 1).

The prenatal rate of detection of lung cysts at the routine 18–20th week scan is almost 100% and may be the most common example of actual presentation. Late pregnancy diagnosis of CPAM is less sensitive. Once a cystic lung lesion is detected on ultrasound, the location, volume, size, macrocystic or microcystic classification, and blood supply should be evaluated (11).

Serial prenatal sonographic examinations are important for helping to determine the prognosis and necessity for possible intrauterine treatment in patients with CPAM. The prognosis is highly variable and depends on the presence of fetal hydrops and the size of the mass (12).

Hung-wen Chen et al. (13) proposed an algorithm for practical management of patients with CPAM. They suggested that if prenatal ultrasonographic screening reveals a suspicious fetal lung lesion, a series of ultrasonographic examinations should be planned to evaluate the size, content (microcystic, macrocystic or solid) and distribution of the lesion. Fetal therapies such as needle aspiration, catheter shunt placement and fetal surgical resection can be applied. The majority of these lesions will regress or become normal echoic in late pregnancy.

The two major factors affect the management after the birth: the timing of respiratory decompensation and the presence of associated complications. Most cystic lesions can be resected with thoracic surgery (at the age of 3–6) (14).

Sometimes recurrent pulmonary infections with severe respiratory decompensation can develop in an asymptomatic one month-old patient. Although small asymptomatic lesions can regress, there has been an increasing number of reports of malignancy associated with CPAM over the last decade, which cannot be ignored. These associated neoplasms consist mainly of pleuropulmonary blastoma in infants and young children, and bronchoalveolar carcinoma in older children and adults. Type 4 CPAM is accepted by most authors as type 1 pulmonary blastoma (15). Other cystic or pseudocystic lung lesions include post-infarction peripheral cysts resulting from intrauterine pulmonary artery thrombosis. The cysts have also been noted in Down’s syndrome. Air-filled cysts within the interstitium are features of acute and persistent interstitial pulmonary emphysema, and limited to the interlobular septa. Fluid-filled cysts of congenital pulmonary lymphangiectasia

**Figure 2:** Microscopic appearance of congenital pulmonary airway malformation. A) Dilated bronchiole-like structures of various sizes were seen (H&E x50), B) Dilated bronchiole-like structures were lined with ciliated columnar cells without any intervening mucinous epithelial cells (H&E x400).

**Figure 3:** Immunohistochemically, columnar cells of dilated cystic structures showed positive staining for TTF1 (A) and SMA (B) positive smooth muscle fibers in the subepithelial stroma.
**Table 1: Congenital pulmonary airway malformation types**

<table>
<thead>
<tr>
<th>CPAM types</th>
<th>Synonym</th>
<th>Incidence</th>
<th>Macroscopic features</th>
<th>Microscopic features</th>
<th>Prognosis</th>
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| Type 0     | Congenital acinar dysplasia/ aplasia | 1-3%       | Solid appearance, small and firm lungs | Bronchial-type airways with cartilage, smooth muscle and glands separated by abundant mesenchymal tissue. | - Neonates  
- Poor prognosis |
| Type 1     | Bronchial | 60-70%    | One or more large cysts measuring 2-10 cm in diameter | - Larger cysts are often accompanied by smaller cysts, and their walls contain muscle, elastic, or fibrous tissue, cartilaginous plates (12%).  
- Cysts are frequently lined by pseudostratified columnar epithelial cells.  
- Often interspersed with rows of mucous cells, focal mucous cell | - Neonates and infants  
- Resectable  
- Good prognosis  
- Possible carcinomatous change) |
| Type 2     | Bronchial/Bronchiolar | 10-15%    | Sponge-like appearance, multiple small cysts (0.5 to 2 cm) | - Small, relatively uniform cysts resembling bronchioles separated by normal alveoli.  
- Cysts are lined by cuboid-to-columnar epithelium and have a thin fibromuscular wall. | - Neonates  
- Other malformation  
- Poor prognosis |
| Type 3     | Bronchiolar | 5%        | Solid appearance | - Excess of bronchiolar structure separated by small air spaces, with cuboidal lining.  
- Resembling late fetal lung, grossly a solid mass without obvious cyst formation, microscopic adenomatoid cysts. | - Neonates  
- Poor prognosis |
| Type 4     | Peripheral | 28%       | Large cysts (up to 10 cm), | - Cysts lined by a flattened epithelium (type 1 and 2 pneumocytes) resting on loose mesenchymal tissue.  
- Focal stromal hypercellularity (50%), focal immature cartilage,  
- Associated pleuropulmonary blastoma (bilateral type 4 CCAM with stromal cellularity) (14%). | - Neonates and infants  
- Good prognosis |
are present within the interlobular septa, and extend laterally from the septa beneath the pleura. Congenital pulmonary lymphangiectasia is also frequently associated with congenital malformations of the heart. Bronchogenic cysts are rarely seen in infants, and are solid lesions usually separate from the lung. Extralobar sequestrations are also un aerated lesions separate from the lung and occasionally found within or beneath the diaphragm. Intralobar sequestrations are usually acquired lesions (through infection), and may display air- or fluid-filled cysts, representing re-epithelialized post-infectious abscesses. The infantile lobar emphysema, one of the most common pulmonary lesions in infants and children, is not cystic but simply the over inflation of a segment of lung (16).

Congenital pulmonary airway malformation is a quite rare malformation of fetus. In our case, fetal left lung was larger than that of a stillborn term fetus. The compression of large vessels and the heart was the cause of hydrops fetalis. With these clinical and laboratory findings, the present case is a very good example of non-immune hydrops fetalis associated with CPAM type 2. Thus prenatal clinical and ultrasonographic follow-up during pregnancy is very important for the early diagnosis of such congenital abnormalities.

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Sclerosing Angiomatoid Nodular Transformation of the Spleen: A New Entity or a New Name?

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ABSTRACT

Sclerosing angiomatoid nodular transformation of the splenic red pulp has been described quite recently; many of the lesions previously diagnosed as splenic exuberant granulation tissue, multinodular hemangioma, and inflammatory pseudotumor could actually belong to this category. The lesion has been well reported intermittently in the past, but new cases with still newer associations keep appearing from time to time. There are no known risk factors and no inciting triggers have been proven. We report two such cases- one of which has extensive extramedullary haematopoiesis; a feature that has never been reported earlier. Clinico-morphological and radiological features along with pathogenesis are discussed in detail.

Key Words: Spleen, Extramedullary haematopoiesis, Hemangioma

INTRODUCTION

Vascular tumors are common in spleen. Recently, a distinct subset of these tumors with characteristic morphology and typical immunoprofile has been designated as "Sclerosing Angiomatoid Nodular Transformation" (SANT). Herein we describe two cases of SANT with characteristic histomorphology and immunoprofile. One of our cases was associated with extensive extra-medullary haematopoiesis in the spleen - a finding that has never been reported in literature.

CASE REPORTS

Case 1

A 35-year-old gentleman presented to the hospital with left upper quadrant discomfort since 6 months and gradually increasing lump in upper abdomen since 4 months. History of fever, weight loss or night sweats was absent. Per-abdomen examination revealed splenomegaly 6 cm below coastal margin. Haematological parameters and liver function tests were unremarkable except for mildly elevated serum lactate dehydrogenase levels (124 U/L). Ultrasound abdomen revealed splenomegaly 6 cm below costal margin. Haematological parameters and liver function tests were unremarkable except for mildly elevated serum lactate dehydrogenase levels (124 U/L). Ultrasound abdomen revealed splenomegaly with nodular mass lesion in the lower part of spleen. On computed tomography (CT) scan, there was splenomegaly with a large isodense mass (Hounsfield scale = 40-55 HU) measuring 8.5 X 7.3 cm at the lower medial pole of spleen. Contrast enhanced CT (CECT) showed minimal heterogeneous enhancement with calcifications (Figure 1A-D). Few sub-centimetre lymph nodes at portal, porto-caval and para-aortic regions were noted. High resolution CT scan of the chest was normal. The bone marrow evaluation showed normoblastic erythropoiesis, normal and orderly myelopoiesis, and morphologically unremarkable megakaryopoiesis. However, the overall cellularity for age was reduced. Rest of the haematological work up was unremarkable. In view of the solitary nature of the mass, the patient underwent splenectomy.

Grossly the spleen measured 13 X 7 X 7 cm and weighed 260 grams. The external surface was congested with discrete nodularity at lower pole. Cut surface showed well circumscribed mass measuring 6 cm in diameter, with a bulging cut surface and fibrous septa traversing throughout, thus dividing it into yet discrete complete or incomplete nodules (Figure 2A and inset). Light microscopy recapitulated the nodular appearance seen at gross. Within the nodules, there was slit like arrangement of capillary sized vessels lined by plump endothelial cells. Few of these vessels were slit like with sclerosed and hyalinised walls. The nodules were separated by fibro-sclerotic stroma with hemosideophages, fibroblasts and lymphomononuclear cells. Striking feature was the presence of extensive extramedullary haematopoiesis composed of mainly myeloid, few megakaryocytic and erythroid precursors. Histochemical stains- reticulin, Masson’s trichrome, Periodic Acid Schiff (PAS) and Perls’ stains were used to highlight the collagen rings surrounding the nodules, hemosiderin deposits and hematologic precursors.
A diagnosis of sclerosing angiomatoid nodular transformation with extensive extramedullary haematopoiesis was given.

Immunohistochemistry (IHC) was performed with CD31, CD34, CD68, smooth muscle actin (SMA) and myeloperoxidase (MPO). The vasculature within nodules was a variable admixture of CD31 and CD34 positive vessels (Figure 3A-F) indicating their derivation from sinusoidal, capillary like and vein like elements.

Case 2

A 12-year-old girl presented to this hospital in 2005 with upper quadrant discomfort since 6 months. Ultrasound revealed splenomegaly. CECT abdomen showed a hypodense soft tissue mass without contrast enhancement at the upper pole. $^{99m}$Tc-sulphur colloid single photon emission tomography computed tomography (SPECT/CT) scan revealed normal tracer activity within the liver and spleen with no active uptake within the lesion. Other investigations including colour Doppler, bone marrow evaluation, electro-cardiogram and blood investigations were unremarkable. The patient was taken up for splenectomy. Grossly, the mass had a nodular, firm to hard, gray white cut surface (Figure 4A). Microscopy revealed nodular appearance of variable sized vascular channels lined by plump endothelial cells. The nodules were surrounded by bands of sclerosis (Figure 4B). At that time, it had been designated as multinodular hemangioma. However in view of the recent concept of SANT, review of H&E stained sections along with relevant immunohistochemistry was done. There was nodular arrangement of vascular channels with a characteristic immunoprofile as described for case 1. The diagnosis has been revised as SANT.

Figure 1: CT scan image of abdomen in arterial phase (A) Plain and (B-D) after contrast administration showing enlarged spleen extending well below the kidney. There is a definite mass lesion, isointense to the normal splenic parenchyma, with heterogenous contrast enhancement.

(Figure 2B-F).
Both the patients were given standard pre and post splenectomy precautions. They are symptom-free and on follow up (Case 1-11 months, Case 2-5 years). The follow up bone marrow evaluation of case 1 was similar to the previous one without any symptoms thereof.

**DISCUSSION**

Vascular neoplasms in spleen are common and may exhibit a variety of biological behaviour. These include those with a benign course (littoral cell angioma, hemangioendothelioma and hemangiopericytoma) and those that are frankly malignant (angiosarcoma) (1).

The characteristic morphology of multiple angiomatoid nodules forming a space-occupying lesion in spleen is currently called as sclerosing angiomatoid nodular transformation (SANT). Though known to exist as early as 1978, many of these cases have been previously diagnosed as splenic exuberant granulation tissue, hamartomas, multinodular hemangiomas or even as inflammatory pseudotumors (2-4).

Herein we describe two cases of SANT, extramedullary hematopoiesis in one of our cases was an association never earlier reported. The characteristic gross features of this case prompted us to revise the diagnosis of the earlier case.

SANT as a distinct entity was initially described by Martel in 25 patients and recently by Diebold in 16 splenectomy specimens (4,5). The female: male ratio reported is 2:1 and mean age is 48 years (range, 22–74 years). The size ranges from 3 to 17 cm in diameter (5).

Clinically, the patients are usually asymptomatic. They may have variety of unrelated coexisting conditions. Associated haematological conditions that have been reported include leucocytosis, polyclonal gammopathy, increased erythrocyte sedimentation rate and myelodysplastic syndrome (5,6). Possibly the transient bone marrow suppression leading to extra-medullary hematopoiesis could have triggered red pulp transformation in this case. Apart from this explanation, the patient did not have any haematological abnormality that could offer explanation for other associations as mentioned above.
between individual tumours. This is well reflected in their pattern of immunostaining for CD34, CD31 and CD8. Angiomatoid nodules of SANT are composed of vessels or vascular spaces lined by cells showing either of the three immunotypes 1) CD34+/CD31+/CD8- indicating capillary derivation 2) CD34-/CD31+/CD8+ indicative of splenic sinusoidal lining cells and 3) CD34-/CD31+/CD8- indicating small veins. The splenic red pulp also has similar pattern of expression; thus indicating that nodules of SANT recapitulate the normal splenic red pulp. The microscopic features and immunoprofile of other close differentials have been well described in literature (2).

The mass is usually detected during radiological work-up for other unrelated conditions. Grossly, the mass shows multiple individual and confluent variable sized nodules with diameter ranging from 3 to 17 cm. This classic appearance was a clue to diagnosis in this present case and diagnosis was suspected on gross examination.

Microscopically, the nodules show a variable admixture and sieve like arrangement of vascular spaces that represent an admixture of cells lining splenic sinusoids, capillaries and veins (4). The degree of circumscription of nodules by collagen and the quality of the intervening stroma (whether fibromyxoid, sclerotic or hyaline) may vary

Figure 3: Immunohistochemistry with (A) CD31 positivity in few slit like vessels within the lesion (x100) (B) CD34 positivity in small calibre vessels (x100) (C) Myeloperoxidase (MPO) marking the hematopoietic cells (x400) (D) CD68 highlighting few scattered macrophages (x400) and (E) Smooth muscle actin (SMA) positive musculature surrounding nodules surrounded by bland fibrosis (x400).
CD68 positivity in SANT favours non-neoplastic origin of this entity and may be indicative of active phagocytosis due to increased splenic proliferative activity (7). Radiological features of SANT have now been well described (6,8). Plain and contrast enhanced CT features in our cases correlated with those already reported. On CT scanning, the lesions are usually isodense or hypodense when compared with splenic parenchyma. Magnetic resonance imaging with contrast and 99mTc-sulfur colloid scanning in case 1 would have helped us in pre-operative assessment of extensive extra-medullary hematopoesis, but it was not done. This is a common scenario in a developing country like ours where this investigation is not routinely available.

The combined effect of a stagnant splenic circulation (due to passive congestion) and local metabolic effects as anoxia are triggers for formation of angiomatoid nodules. Damaged endothelial cells when coupled with myofibroblast and neoangiopillary proliferation, lead to fibrin deposition and granulation tissue formation akin to wound repair. The end result is an exuberant (and to a little extent organised) proliferation and transformation of the red pulp to form SANT.

The differential diagnosis of SANT includes hemangioma, littoral cell angioma, splenic hemangioendothelioma, inflammatory myofibroblastic tumor, hamartoma and nodular transformation of splenic red pulp in response to metastasis. Hemangiomas in the spleen are usually smaller than 2 cm and of the cavernous type. Littoral cell angioma is a tumor of the littoral cells, which exhibit both endothelial and histiocytic phenotype. The cells are negative for CD34 unlike the mixed immune-expression of SANT. Unlike other body sites, hemangioendothelioma in the spleen is a controversial entity. In addition to the presence of characteristic intracytoplasmic red blood cells, cells of hemangioendothelioma are variably positive for CD34. Many previously described splenic multinodular hemangiomas and hamartomas could in the present times be categorised as SANT. The former lesions are benign, whereas the outcome of SANT is intermediate between benign tumors and malignant sarcomas. With classic gross and histopathological features as in this case, we consider the name ‘SANT’ more appropriate as it better conveys the intermediate prognostic significance.

Extensive extramedullary haematopoiesis is unreported in these cases. Probably our first case had transient bone marrow suppression which could have caused the spleen to take up haematopoiesis, though this feature is not well characterised in adults. It is unclear as to whether extra-medullary haematopoiesis in this case could have incited the excessive organised red pulp transformation. Further reported cases will help better demographic and clinical characterisation of SANT.

REFERENCES


Adrenokortikal Onkositik Karsinom: Olgu Sunumu ve Histopatolojik Tanı Kriterlerinin Gözden Geçirilmesi

Adrenocortical Oncocytic Carcinoma: A Case Report and Review of the Histopathologic Diagnostic Criteria

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ABSTRACT
Oncocytic tumors are rare in the adrenal gland. The histopathological diagnosis of adrenocortical carcinoma is difficult due to the lack of precise diagnostic criteria for malignancy. A 44-year-old man was admitted to our hospital with left flank pain. Radiologically an adrenal mass was detected. After the excision and histopathologic evaluation of the mass, a diagnosis of adrenocortical oncocytic carcinoma was made. At least one of the features of more than 5 mitoses in 50 high power fields, atypical mitotic figures or venous invasion is required for the diagnosis of malignancy in adrenocortical tumors. It has been suggested that tumors that have more than one of the minor criteria of large size (>10 cm or >200 gr), necrosis, capsular or sinusoidal invasion, should be evaluated as having uncertain malignant potential.

Key Words: Adrenal gland neoplasms, Adrenocortical carcinoma, Oxyphil cells

ÖZ

Anahtar Sözcükler: Adrenal gland tümörleri, Adrenokortikal karsinom, Oksifilik hücreler

GİRİŞ
Adrenokortikal karsinomlar insidansi 1 milyonda bir olan nadir tümörlerdir. Böyle çok nadir durumlar görülür. Görülme yaşarı erken çocukluk döneminde ve 60’lı yıllarda olmak üzere iki pik yapar. Tanı sırasında tümörün uzak metastaz yapmış olma olması olup, ortalama yaşam süresi 3 yılda azdır (1).

Adrenokortikal karsinomlarında malignite tanısı en çok kabul gören kriter tümörün ağırlığı ve boyutlarıdır. Büyük boyut ve ağırlık 50 gram ve 6,5 cm sınır değerleri malign davranışa sırasıyla %91 ve %100 sensitivite ve spesifiteye sahiptir. Son dönemde kullanılan tanisal parametrelerle一直到いずれ de malign davranan karsinomlarda malign davranış saptanmış olduğu belirtmektedir (2). Weiss kriterlerinden sadece bazılarının (mitotik aktivite, nekroz, vasküler ve kapsüler invazyon) onkositik tümörlerde değerlendirilmesi önerilmektektir (1). Weiss kriterleri ile ilgili}

(Turk Patoloji Derg 2016, 32:211-215)
Received : 18.01.2012 Accepted : 29.09.2012

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son çalışmada majör kriterlerden (>5/50 BBA mitoz, atipik mitoz ve venöz invazyon) sadece birinin varlığı malignite için yeterli kabul edilmektedir. Eğer sadece minör kriterlerden (>10 cm boyut, >200 gram ağırlık, nekroz, kapsül veya sinüzoidal invazyon) varsası tümör malignite potansiyeli belirsiz olarak değerlendirilir (4).

OLGU SUNUMU

TARTIŞMA

Şekil 1: Onkositik sitoplazmalı hücrelerden oluşan tümör dokusu gözlenmektedir (H&E; x200).

Şekil 2: Tümörde tripolar atipik mitotik figür izlenmektedir (H&E; x400).

Proliferasyon indeksi ve yüksek p53 ekspresyonu adrenokortikal tümörlerde potansiyel prognostik parametre olarak ileri sürülmuştur (12,13). Bisceglia’nın sonuçlarına göre onkositik karsinomlar ve konvansiyonel adrenal kortikal karsinomların Ki-67 proliferasyon indeksleri uyumluştur. Ancak literatürde p53 eksprese eden sadece

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<td>Atipik mitoz</td>
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<td>2- Atipik mitotik figür</td>
<td>2- Nekroz</td>
</tr>
<tr>
<td>3- Venöz invazyon</td>
<td>3- Kapsüler/sinüzoidal invazyon</td>
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- Bir majör kriter maligniteyi gösterir
- Majör kriter yoksa, bir veya daha fazla minör kriter varsa malignite potansiyeli belirir.
- Majör veya minör kriterleri taşıyorsa benign olarak değerlendirilir.

Şekil 3: Adrenal parankim içerisinde tümöral hücre grupları mevcuttur (H&E; x200).

Şekil 4: Tümöral hücreler vasküler lümende ve duvara tutunmuş olarak gözlenmektedir (H&E; x400).

Şekil 5: Inhibin ile fokal olarak tümör hücrelerinde sitoplazmik membran boyanması izlenmektedir (Inhibin; x200).

Şekil 6: Tümörde yaygın kalretinin ekspresyonu mevcuttur (Kalretinin; x100).
bir onkositik karsinom olgusu raporlanmıştır (7). Bununla birlikte son dönemde Ki-67 proliferasyon indeksi ve p53 ekspresyonunun adrenal onkositik tümörlerde biyolojik davranışı belirlemeye önemli olduğundaki yayınlar öne çıkmaktadır (6-8,11). Olgumuzda Ki-67 proliferasyon indeksi çok yüksek değildi (%5).


**KAYNAKLAR**
