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Cytomorphological Changes in Patients Undergoing Taxanes Formation in Breast Cancer at Oncology and Cancer Research Center in Shendi Town, Sudan

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Abstract: ***Background***: Breast cancer is the most commonly diagnosed cancer in women worldwide and the most frequent cause of cancer death in women in low-income countries. Cytotoxic chemotherapy drugs called taxanes alter the cellular mechanisms that allow cancer cells to divide their microtubules. In a typical cell cycle, microtubules are formed by the cell at the start of division and disassembled after the cell stops dividing. Taxanes stabilize the microtubules, preventing them from breaking down normally. This causes the cancer cells to stop dividing, potentially slowing the growth of cancer or killing the cells. Taxane causes neutropenia and thrombocytopenia, as well as fatigue, nausea and vomiting, hair loss, diarrhea, mouth ulcers, and muscle pain. According to the literature, chemotherapy causes nuclear atypia and other cellular changes in buccal mucosa cells. There was no published data regarding the effect of taxane on buccal mucosa cells. This study aimed to detect cytomorphological changes among breast cancer patients receiving taxane chemotherapy. ***Materials and methods:*** This is a comparative cross-sectional study conducted during the period from December 2022 to February 2024, involved 50 buccal smears from breast cancer patients included as a case group versus 50 buccal smears from apparently healthy females. All smears were processed through conventional cytological methods. ***Results:*** Forty percent of patients have had a family history of breast cancer. We observed that in 80% of patients with advanced age (46 years old and older), our results indicated that there was a significant statistical difference between cases and controls regarding cellular changes in buccal mucosa. We also observed an increase in the significant correlation of cellular changes and the number of taxane doses, as the p value was less than 0.05. ***Conclusion:*** We concluded that taxane chemotherapy may cause buccal cellular changes as the incidence of nuclear atypia, infection, and degenerative changes is higher in cases than in controls.

Keywords: Chronic Renal Failure, *Secretors*, *Non-Secretors*, ABO Blood Group.

Research Paper

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INTRODUCTION

Breast cancer is the most commonly diagnosed cancer in women worldwide and the most frequent cause of cancer death in women in low-income countries. As breast cancer is considered to be a complex and heterogeneous disease, there are many prognostic clinicopathological characteristics, these include lymph node status, tumor size, and grade [1], age, and tumor histology [2], the presence or absence of hormone receptors (estrogen and progesterone), and other biomarkers, in particular human epidermal receptor type 2 (Her-2 ⁄ neu) expression [3]. Management of breast cancer is a multimodal approach including a combination of systemic therapy [computed tomography (CT) and/or anti-HER-2 therapy], surgery, and radiotherapy [4]. The choice of treatment depends on the patient's age, stage of the disease, patient's performance status, medical history, hormonal receptor (HR) status, and Her2 status [5]. According to the World Health Organization (WHO), based on histomorphology and growth patterns alone, 21 histological types of BC differ in risk factors, presentation, response to treatment, and outcomes [6]. A component that is always included in a pathology report

and has been a cornerstone in the determination of BC prognosis is histological classification and grade [7]. In situ vs invasive cancer is the first significant division. Invasive carcinoma is then broken down into multiple subtypes, including the most common infiltrating ductal carcinoma and invasive lobular carcinoma [8]. The determination of HR positivity through immunohistochemistry (IHC) is used in conjunction with histology as a starting point for determining therapeutic management. Tumors can then be classified molecularly for added prognostic value and therapeutic guidance [9]. Adjuvant chemotherapy improves survival in premenopausal and postmenopausal women with early breast cancer. Taxanes are highly active chemotherapy agents used in metastatic breast cancer [10]. Taxanes are cytotoxic chemotherapeutic drugs that alter the cellular architecture necessary for microtubule division in cancer cells. During a typical cell cycle, micro tubes are formed at the onset of cell division and disassembled when the cell ceases to divided. Taxanes stabilize the microtubules, preventing them from breaking down normally. This causes the cancer cells to stop dividing, potentially slowing the growth of cancer or killing the cells [10]. Two taxanes are commercially available paclitaxel and docetaxel. The two available taxanes have slightly different most prevalent side effects. Both agents cause neutropenia and thrombocytopenia, as well as fatigue, nausea and vomiting, hair loss, diarrhea, mouth ulcers (the oral cavity undergoes multiple changes during intensive cancer chemotherapy, which can cause moderate to severe oral mucositis.), and joint and muscle pain [10]. The purpose of this study is to examine cellular alterations in the buccal mucosa of breast cancer patients receiving adjuvant treatment that contains taxanes. Oral mucositis (OM) is a clinically significant complication of mycotoxin-based cancer therapy. The condition affects an estimated 5% to 40% of patients receiving standard-dose chemotherapy and >75% of patients receiving either high-dose chemotherapy with stem-cell transplantation or radiation therapy for head and neck cancer [11, 12]. Clinically significant OM, which involves both erythema and ulceration of the oral mucosa, can directly affect the clinical status of the patient and result in increased pain, difficulty swallowing, nutritional compromise, and an increased risk for infection [13, 14]. Taxane can cause oral mucositis, which may lead to an increased incidence of the infection. The impact of taxanes on buccal mucosa cells remains poorly understood. This study aims to detect cellular changes in buccal mucosa cells among breast cancer patients who take taxanes-containing adjuvant chemotherapy.

MATERIALS AND METHODS

Study Design:

The study was a comparative cross-sectional study to detect the effect of taxane chemotherapy on oral cells in patients suffering from breast cancer.

Study Area:

The study was conducted on breast cancer at the Oncology and Cancer Research Center in Shendi Town. Shendi Town is a small city in northern Sudan, situated on the northeast bank of the Nile River, 150km southwest of Khartoum, and also about 45km southwest of the ancient city of Meroe.

Study Duration: This study was performed during the period from December 2022 to February 2024.

Study Populations: The populations involved in this study were females with breast cancer who received Taxan chemotherapy.

Inclusion Criteria:

All case groups recruited in this study were females suffering from breast cancer undergoing Taxan chemotherapy of all ages, while the control group included healthy females. Females in the case and control groups shared the same sociodemographic and age data.

Exclusion Criteria:

Females with other cancer types or other diseases and breast cancer patients not receiving taxane chemotherapy were excluded from this study.

Study Sample: Buccal smear samples were taken from each participant to detect oral cytomorphological patterns.

Sample Size:

One hundred buccal smears were taken from the participants (fifty of them from the cases and the other fifty from the controls).

Tools of Data Collection and Study Variables:

Questionnaire sheets were used to record all participants and results. Cytomorphological changes in buccal mucosa cells were detected by using the conversational cytology method.

Sample Collection and Processing:

At first, each participant was asked to wash his mouse to avoid contamination. A sterile disposable teethbrush was used to scrape buccal cells from patients with care and adequate safety precautions to avoid contamination during collection. The sample was collected from each patient after taking chemotherapy, smeared, and fixed immediately in 95% ethanol for at least 15 minutes. After fixation, each slide was stained through the Papanicolaou staining method.

Papanicolaou Staining Method:

Each fixed smear was rehydrated in 90%, 70%, and distilled water for 2 minutes in each. After rehydration, each slide was stained in Harris's hematoxylins for 3 minutes, then the smear was differentiated in 1% acid alcohol, blued in running tap

water, then the smear was rinsed in 95% ethanol, then the smear was stained in orange G6 for 2 minutes, then the smear was washed in 95% ethanol, eosin azure 50 stain was applied for 3 minutes, then the slide was dehydrated in 95% and absolute ethanol consecutively, each slide was cleared in xylene and finally mounted in Disterene A plasticizer and Xylene (DPX). The smear was examined under the microscope using 10 x and 40 x, and the results were reported independently by the researcher and the supervisor.

Interpretation of Results:

Identification of cellular changes achieved by the presence of the following conditions; the presence of primary criteria of malignancy (irregular chromatin pattern, chromatin strands of unequal size and shape, condensation of large chromatin clumps at nucleus border unevenly leaving empty center) to indicate cancer cells, presence of dyskaryotic cells (malignant chromatin with a normal amount of cytoplasm), presence of secondary criteria of malignancy to indicate nuclear atypia (hyperchromasia, increase amount of chromatin, enlarged cells and nuclei, multinucleation, irregular nuclear border, presence of mitotic figures, abnormal enlarged and multinucleoli), cellular changes also identified by the presence of metaplastic cells, vacuolated cytoplasm and prenuclear hallo in buccal smear, presence of keratosis (para and hyperkeratosis). Inflammatory change is identified by the presence of neutrophilia, lymphocytosis, macrophages, and degenerated cells (pyknotic, karyorrhexis, and karyolitic cells).

Quality Control:

Sterile disposable toothpaste was used to collect the samples; the buccal sample was smeared directly upon the frosted end microscopic glass slide to avoid air-drying artifacts. Each smear was already labeled with the participant number using a soft pencil. Each smear was immediately fixed in 95% ethanol for the immediate killing of chromatin. All staining solutions are filtered before being used. All dishes and coplanars are washed before and after use. The quality of staining solutions is checked before use. During work, all dishes and coplanar were closed well by a screw top cover to avoid evaporation and contamination. Contamination is also avoided during mounting and cover-slipping.

Data Analysis and Presentation:

The data was computed and analyzed using the Statistical Package for Social Sciences software program. The means were obtained, other variables, frequencies, and percentages were calculated and presented in the form of figures and tables, and the p-value was used to assess the significance of the results.

Ethical Consideration:

The study was approved by the department of histopathology and cytology in the College of Medical Laboratory Sciences at Shendi University. The study matched the ethical review committee board. Sample collection was done after agreement with the participants. Permission for this study was obtained from the hospital administration. The aims and benefits of this study were explained with the assurance of confidentiality. All protocols in this study were done according to the Declaration of Helsinki (1964).

RESULTS

Table 1: Occurrence of cellular changes among study groups

Study group	Cellular change		Percentage	*P. value*
	Present	Absent		
Case	50	00	50%	0.000
Control	26	24	50%	
Total	**76**	**24**	**100%**	

Table 2: Presence of nuclear atypia among study groups

Study group	Nuclear Atypia		Percentage	*P. value*
	Present	Absent		
Case	32	18	50%	0.000
Control	14	36	50%	
Total	**46**	**54**	**100%**	

Table 3: Comparison between cellular change and number of doses

Number of dose	Cellular change		Percentage	*P. value*
	Present	Absent		
1 and 2	12	00	25%	0.000
3 and 4	18	00	25%	
5 and 6	18	00	25%	
7 and 8	02	00	25%	
Total	**50**	**00**	**100%**	

Table 4: Presence of micronuclei among study group

Study group	Micronuclei		Percentage	*P. value*
	Present	Absent		
Case	04	46	50%	0.695
Control	03	47	50%	
Total	**06**	**94**	**100%**	

Table 5: Prevalence of infection among study groups

Study group	Infection		Percentage	P. value
	Present	Absent		
Case	27	23	50%	0.001
Control	11	39	50%	
Total	**38**	**62**	**100%**	

Table 6: Incidence of degenerative cells among study groups

Study group	Derivative cell		Percentage	P. value
	Present	Absence		
Case	18	32	50%	0.000
Control	0	50	50%	
Total	**18**	**82**	**100%**	

DISCUSSION

This is a comparative cross-sectional study performed among breast cancer patients at the Oncology and Cancer Research Center in Shendi Town during the period from December 2022 to February 2024. This study included fifty females with breast cancer receiving taxanes chemotherapy and fifty apparently healthy females as the control group. Regarding to the age of patients with breast cancer, our results showed that the commonest observed age was among elderly females (80% of patients aged 46 and above), and this is due to the fact that breast cancer incidence increases with age. This result is similar to that obtained by the contractor in 2008 Our result shows that women who have never been pregnant and women who did not breastfeed have an increased risk; starting your periods at a very young age and starting menopause at a late age may also increase your risk, a high-fat diet may also be a contributing factor, although the greatest risk is after age 40 [15]. Another study performed by Kelsey and Bernstein in 1996 shows that the potential risk factors include the age of the patient, family and personal history, proliferative breast disease, alcohol consumption, early menarche, lack of exercise, late age at first pregnancy, late menopause, obesity, exposure to ionizing radiation, and long-term use of hormone replacement therapy. As detailed below, age is a major risk factor for breast cancer [16]. Another study performed by Joerger M *et al.*, in 2016 shows that about 19% of breast cancer cases are diagnosed in women aged 30 to 49 years, and 44% occur among women aged 65 years or older [17]. Another study performed by Leong *et al.*, in 2010 found that in Africa and Asia, breast cancer incidence peaks among women in their 40s and 50s, whereas in the United States and Europe, it peaks among women in their 60s and 70s [18]. Concerning the marital status of patients with breast cancer among the study populations, our result shows that; the majority of females were married and 10% of females were unmarried. Many studies have correlated the incidence of breast cancer with marital status, pregnancy, and the number of children. A study conducted by Williams in 2010 concluded that; unmarried women had a higher risk of developing breast cancer than married women [19]. Regarding the correlation of family history of breast cancer with the development of breast cancer, our results show that nearly half of patients have had a family history of breast cancer. This result is consistent with the study performed by Evans *et al.*, (2008), *who* concluded that the potential risk factors include the age of the patient, family, and/or personal history. The development of breast cancer is considered to result from multiple hereditary factors and is increased by inherited mutations in the BRCA1/BRCA2 gene [20]. Concerning to the presence of cellular changes (nuclear atypia, candida infection and histiocytes, micronuclei, binucleation, polymorphism and degenerative cellular changes), our results reveals that; there is statistical significant difference between cases and controls groups regarding to the incidence of candida infection and histiocytes, presence of binucleated cells, occurrence of cellular polymorphism, candida and histiocyte as the p. value was less than 0.05, these finding agree with one study on effect of chemotherapy on tissues conducted by Hussain Gadelkarim Ahmed and Dalia AI Elemirri at 2009 who summarizes that; Cytological atypia, viral infections, and inflammatory infiltrates were detected after exposure to radiotherapy and/or chemotherapy [21]. Another study performed by Kennedy in 1990 and Briffod in 1993 summarizes that the changes described include enlarged nuclei, nuclear vacuolization, and foamy cytoplasm, which change more often after an almost complete response. We also observed enlargement of nuclei and nucleoli after chemotherapy [22, 23].

CONCLUSIONS

Breast cancer incidence increases with age among Sudanese females attending the Oncology and Cancer Research Center in Shendi. Family history is a risk factor for breast cancer development among Sudanese females. Taxanes can cause cellular changes such as nuclear atypia, degeneration of cells, candida infection and histiocytosis, micronuclei, binucleated cells, and cellular polymorphism.

RECOMMENDATIONS

1. A well-developed documentation system should be carried out in histopathology laboratories.
2. Further similar studies with large sample sizes should be done to correlate the effect of taxane chemotherapeutic drugs on cancer types, stages, and grades.
3. Future studies should compare the effects of taxanes and other chemotherapeutic drugs on buccal mucosa cells.

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