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A PROSPECTIVE CLINICAL STUDY OF SERUM BIOMARKERS WITH TREATMENT OUTCOME OF EPITHELIAL OVARIAN CANCER CASES

Biochemistry

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ABSTRACT

Introduction: Ovarian cancer has worst prognosis and high mortality rate due to delayed onset of symptoms and lack of proper screening test that leads to late diagnosis in the advanced stages. Folate and vitamin B12 are involved in one carbon metabolism as cofactor and also has a role in conversion of homocysteine to methionine. Aberration in these processes may amplify carcinogenesis. **Objective:** To analyse the role of serum level of CA125, Folate, Vitamin B12 & Homocysteine as diagnostic tool in Epithelial Ovarian Cancer. **Methods & Materials:** 35 diagnosed cases of epithelial ovarian carcinoma were collected from Outpatient and Inpatient Department of Obstetrics & Gynaecology as per Eastern Cooperative Oncology Group performance status. Serum levels of CA125, Folate, Vitamin B12 and Homocysteine were assayed at the time of diagnosis, 3 months and 6 months post treatment. Comparison between pre and post treatment serum levels of parameters were analysed by Graph Pad Prism version 8.4.3. **Results:** Mean age of developing ovarian cancer is 51± 9 years. Pre-treatment Serum CA125 levels (mean 1021 ± 800.5 units/ml) & Serum Homocysteine levels (mean 11.23 ± 2.164 ng/ml) were significantly higher than 3 months & 6 months post-treatment serum levels. Pre-treatment Serum Folate levels (mean 3.231 ± 1.506 ng/ml) & Serum Vitamin B12 levels (mean 280.6 ± 140.8 ng/ml) were significantly lower than 3 months & 6 months post-treatment serum levels. **Conclusion:** The serum levels of selected parameters were significantly altered in cases of epithelial ovarian cancer & plays an important role in process of carcinogenesis.

KEYWORDS

Epithelial ovarian cancer, CA125, Folate, Vitamin B12, Homocysteine

INTRODUCTION

Throughout the life of a woman, the risk of developing ovarian cancer is 1 in 75 & 1 in 100 will be at risk of death with this fatal condition [1]. Morbidity & mortality rates higher than other gynaecological cancers. Early detection is difficult due to lack of recognizable symptoms & sensitive screening methods [2]. Thus, early detection of biomarkers with adequate sensitivity are in need to improve long-term survival of patients, to improve treatment outcomes of ovarian cancer & to obtain significant reduction of risk.

Most common ovarian cancer is epithelial ovarian cancer, which is difficult to detect at early stage and has high mortality rate [2]. Pelvic examination, ultrasonography for assessment of adnexal mass, serum level of CA125, ascitic fluid cytology (if ascitic fluid present) are done in symptomatic patients for screening and diagnosis of ovarian cancer [2]. Symptoms are non-specific at early stage but gradually more pronounced symptoms develop [3]. As per GLOBOCAN 2020 age standardised incidence rate of ovarian cancer in, World is 6.7% per 100,000 [4]. In Asia, India has the highest mortality rate [4]. The management of ovarian cancer consists of surgical staging with optimal cytoreduction followed by a platinum-based chemotherapy according to staging of cancer [5,6].

CA125 is widely used as tumour marker for ovarian cancer but has low sensitivity. It is detected at low level (< 35 U/ml) in normal healthy individuals and increases in about 80% of patients with advanced ovarian cancer. However, it may give false positive result, because level may get increased in pancreatic cancer, breast cancer, bladder cancer and liver cancer [7]. Also increases in non-malignant conditions like benign ovarian diseases, endometriosis, and some physiological conditions such as menstruation, pregnancy [7]. Skates and colleagues in their study of ovarian cancer patients has found progressive increase in level of serum CA125 with time, whereas, serum level remained unchanged in healthy women [8]. In a study by Sarojini et al. noted an increase in serum levels of CA125 in other types of cancer, endometriosis, during ovulation and pregnancy [9].

Micronutrients are essential for growth, development and disease prevention. However, the role of micronutrients in the development of ovarian cancer remains unclear [10]. Cancer has been thought to develop because of an excess of DNA damage or inappropriate expression and regulation of critical genes. Folate and vitamin B12 has essential role in DNA methylation, repair and nucleotide synthesis (fig-1). Aberration in these processes may alter the expression of tumour suppressor genes and proto-oncogenes [11]. 5, Methyltetrahydrofolate and cobalamin converts homocysteine to methionine which is further converted to s-adenosyl methionine, acts as methyl donor in several reactions in our body [12]. Hence, DNA hypomethylation, elevated serum homocysteine level and deficient in serum folate level may increase the risk for carcinogenesis [13].

We tried to find correlation between biomarkers like CA125, Folate, Vitamin B12, Homocysteine & Epithelial Ovarian Cancer. Till date the above-mentioned serum markers have not been simultaneously studied in relation to treatment of epithelial ovarian cancer.

AIM

To analyse the role of serum level of CA125, Folate, Vitamin B12 & Homocysteine as diagnostic tools in Epithelial Ovarian Cancer.

OBJECTIVES

1. To assess the ability of serum level of CA125, Folate, Vitamin B12 & Homocysteine in aiding the clinical diagnosis of newly detected cases of Epithelial Ovarian Cancer
2. To assess the prognostic ability of above-mentioned serum biomarkers after 3 months & 6 months of initiation of the treatment of clinically diagnosed cases

MATERIALS & METHODS

Study Design & Setting

The prospective clinical study conducted in Department of Biochemistry in collaboration of Department of Obstetrics & Gynaecology, R.G. Kar Medical College & Hospital. After obtaining the ethical clearance the study was started from 1st JAN 2020 and continued till 30th JUNE 2021 for a period of 18 months. The ethical committee approval no. is RKC/62.

Study Population

35 diagnosed cases of epithelial ovarian cancer from Outpatient & Inpatient Department of Obstetrics & Gynaecology were recruited after obtaining voluntary informed consent. Clinically and radiologically cases were diagnosed and confirmed by histopa-

thological examination of the tissue obtained during reductive surgery. Cases were included on the basis of ECOG (Eastern Cooperative Oncology Group) performance status: Stage 0 to stage II.[14]

Exclusion Criteria

- Partially treated ovarian cancer cases.
- History of addiction to alcoholism, tobacco.
- Non ambulatory and comatose patient.
- Pregnant and lactating mother.
- Chronic debilitating disease—Chronic Kidney Disease, Chronic Lung Disease, Uncontrolled Diabetes, Tuberculosis.

Sample Size: 35 cases of epithelial ovarian cancer were selected depending on total patients attended in the desired period and estimated number of samples was calculated by the below mentioned formula:

- Where, Zα= 1.96 at 95% CI, p= prevalence (5.8%) [15],
- q=1-p and L=absolute precision (8%).
-

Power of the study: Power analysis was done by using G*power 3.1.9.7. The power of the study achieved is 85%.

METHODOLOGY

5 ml whole blood collected from cases under aseptic precautions at the time of diagnosis and after 3 months and 6 months post treatment. Serum was separated and CA125, Folate, Vitamin B12 and Homocysteine levels were assayed by Chemiluminescence technique.
Normal CA125 level:< 35 units/ml [16].
Normal folate level in adult female: 9.5-39.0 ng/ml [16].
Normal vitamin B12 level in adult female: 188-908 ng/ml [16].
Normal Homocysteine level in adult females: 3.7- 10.9 µmol/ L [16].

Statistical Analysis

Data were analysed by Graph Pad Prism version 8.4.3. D'Agostino & Pearson omnibus normality test was done to test the normality. Statistical significance was evaluated by 'P' value ≤0.05. Comparison between pre and post treatment serum levels of parameters in cases of epithelial ovarian cancer was analysed by paired t test or Wilcoxon signed rank test on the basis of Gaussian distribution or not.

RESULTS

The demographic data of selected cases are: Mean age of developing ovarian cancer is 51± 9 years. Mean age of menarche & menopause are 13 years & 48 years. Most cases are multiparous. 37% cases have used oral contraceptive pills & 63% cases have not taken any contraceptive pills. 91.43% cases have exclusively breastfed their babies.

In our study pre-treatment Serum CA125 levels (mean 1021 ± 800.5 units/ml) in cases were significantly higher (p value <0.0001) than 3 months post-treatment serum CA125 levels (Table-1; mean 385.9 ± 392 units/ml) and 6 months post-treatment serum CA125 levels (Table-2; mean 116 ± 132.8 units/ml). But after treatment, the serum levels of CA125 were found to be decreased significantly after 3 months and 6 months (Fig- 2 & Fig-3).

Pre-treatment Serum Folate levels in cases (mean 3.231 ± 1.506 ng/ml) were significantly lower (p value <0.0001) than 3 months post-treatment serum Folate levels (Table-3; mean 5.012 ± 1.824 ng/ml) and 6 months post-treatment serum Folate levels (Table-4; mean 10.08 ± 3.010 ng/ml). But after treatment, the serum levels of Folate were found to be increased significantly after 3 months and 6 months (Fig- 2 & Fig- 3).

Pre-treatment Serum Vitamin B12 levels in cases (mean 280.6 ± 140.8 ng/ml) were significantly lower (p value 0.0005) than 3 months post-treatment serum Vitamin B12 levels (Table-5; mean 407.3 ± 137.5 ng/ml) and also lower (p value <0.0001) than 6 months post-treatment serum vitamin B12 levels (Table-6; mean 669.2 ± 131.3 ng/ml). But after treatment, the serum levels of vitamin B12 were found to be increased significantly after 3 months and 6 months (Fig-2 & Fig-3).

Pre-treatment Serum Homocysteine levels in cases (mean 11.23 ± 2.164 ng/ml) were significantly higher (p value 0.0046) than 3 months post-treatment serum Homocysteine levels (Table-7; mean 10.06 ± 2.219 ng/ml) and also higher (p value <0.0001) than 6 months post-treatment serum Homocysteine levels (Table-8; mean 8.682 ± 1.760 ng/ml). But after treatment, the serum levels of Homocysteine were found to be decreased significantly after 3 months and 6 months (Fig-2 & Fig-3).

DISCUSSION

Ovarian cancer is a heterogeneous, rapidly progressive and highly lethal disease despite of its relatively low incidence rate. Thus, early diagnosis and good screening tests are essential for the treatment.

Cancer antigen 125 (CA-125) is considered as well-established tumour marker for epithelial ovarian cancer as because it plays an important role in initial diagnosis, as a surrogate marker of clinical response during therapy and during follow-up, but the sensitivity and specificity of CA-125 is low. Bruijn et al. reported rapid decrease in CA125 levels after chemotherapy given to the patients with advanced stage of ovarian cancer [17].

Folate may consider as a potential protective agent for cancer because of its important roles in nucleotide synthesis, as well as in methylation of molecules like DNA, RNA, proteins, and phospholipids. Rapidly dividing cancer cells require more amount of folate for maintenance of DNA synthesis. A case-control study in the United States conducted on 124 ovarian cancer patients has found no associations between dietary folate intake and risk of development of ovarian cancer [18].

Vitamin B12 may enhance the risk of developing carcinogenesis as it has role in one carbon metabolism pathway which is essential for DNA synthesis, repair and methylation. Holly R. Harris et. al reported no association between vitamin B12 and risk of developing ovarian cancer in his study [19].

Normally, cells meet their methionine requirement by synthesizing it from homocysteine. But malignant cells are characterized by high growth rate, and thus the methionine requirement increases in these cells because of increased protein synthesis and transmethylation reactions. Methionine cycle disruptions may relate to intracellular SAM levels and can alter cytosine methylation in DNA which leads to activation of proto-oncogenes, repression of tumour suppressor genes and induction of malignant transformation. Thus, Homocysteine is suggested as a potential marker for several types of cancer. In several studies, it was shown that patients with acute lymphoblastic leukaemia, colorectal, ovarian, pancreatic, and head and neck squamous cell carcinomas had elevated total plasma Homocysteine level [20]. However, in a study by Yesim ozkan et. al did not support this hypothesis for lung cancer [21].

Our study was designed and aimed at finding a new set of biological markers which will help in early diagnosis, treatment and follow up in clinically and radiologically diagnosed cases of epithelial ovarian cancer. Each parameter levels at diagnosis were compared with the serum levels of same parameters in 3 months and 6 months follow up respectively after receiving the treatment. Serum levels of CA125 & Homocysteine were found to be high at the time of diagnosis but significantly decreased after 3 months & 6 months of treatment. Serum levels of Folate & Vitamin B12 were found to be low at the time of diagnosis but significantly increased after 3 months & 6 months of treatment. Thus, in this study we found association between above mentioned parameters and ovarian cancer.

Limitations

A larger sample size with more cases would have been a better representation of study population. The effects of dietary practices were not taken into account which may helped us to understand the correlation of serum parameters with disease severity better.

CONCLUSION

The selected parameters play an important role in the development of carcinoma. It was noticed that the serum levels of CA125 & Homocysteine were significantly decreased & serum levels of Folate & Vitamin B12 were significantly increased after 3 months & 6 months of treatment. Hence, concluded that these parameters should be included in the routine diagnostic workup for epithelial ovarian cancer. An interplay of above markers with disease severity may be studied in a larger patient population so that their role in prognostication of the disease may be explored & can emerge as potential diagnostic tools in epithelial ovarian cancer.

REFERENCES

1. Shabir S, Gill PK. Global scenario on ovarian cancer – Its dynamics, relative survival, treatment, and epidemiology. Adesh University J Med Sci Res. 2020;2(1):17–25.
2. Arjmand M, Zahedi Avval F. Clinical biomarkers for detection of ovarian cancer. J Mol

Cancer, 2019;2(1):3-7.
3. Montagnana M, Benati M, Danese E. Circulating biomarkers in epithelial ovarian cancer diagnosis: from present to future perspective. Ann Transl Med 2017 Jul;5(13):276
4. Prasad AE, Nandennavar M I, Ganesh M S et al. Demographic and clinicopathologic profile of malignant epithelial ovarian tumours. Int J Reproduction Contracept Obstet Gynecol. 2017 Mar;6(3):856-860
5. Hennessy BT, Coleman RL, Markman M. Ovarian cancer. Lancet. 2009;374 (9698):1371–1382.
6. Cannistra SA. Cancer of the ovary. N Engl J Med. 2004;351(24):2519–29.
7. Dong X, Men X, Zhang W, Lei P. Advances in tumour markers of ovarian cancer for early diagnosis. Indian J Cancer. 2014;51(7): 72-76
8. Moyer VA. Screening for ovarian cancer: U.S. Preventive Services Task Force reaffirmation recommendation statement. Ann Intern Med. 2012;157(12):900-904
9. Sarojini S, Tamir A, Lim H et al. Early Detection Biomarkers for Ovarian Cancer. J Oncol. 2012;1-15.
10. Guo Y, Lu Y, Jin H. Appraising the role of circulating concentrations of micro-nutrients in epithelial ovarian cancer risk: A Mendelian randomization analysis. Sci Rep. 2020;10(1):7356
11. Choi S-W, Mason JB. Folate and carcinogenesis: An integrated scheme. J Nutr. 2000;130(2):129–132
12. Pieroth R, Paver S, Day S, Lammersfeld C. Folate and its impact on cancer risk. Curr Nutr Rep. 2018;7(3):70–84
13. Zhang D, Wen X, Wu W, Guo Y, Cui W. Elevated homocysteine level and folate deficiency associated with increased overall risk of carcinogenesis. PLoS One. 2015;10(5): 0123423
14. Salazar-Martinez E, Lazcano-Ponce EC, Gonzalez Lira-Lira G, Escudero-De los Rios P, Hernandez-Avila M. Nutritional determinants of epithelial ovarian cancer risk: a case-control study in Mexico. Oncology 2002; 63:151–157
15. Scholler N, Urban N. CA125 in ovarian cancer. Biomark Med. 2007;1(4):513–23
16. Rifai N, Horvath AR, Wittwer CT. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 6th ed. Boston: Elseiver; 2018
17. Sen U, Sankaranarayanan R, Mandal S, Ramanakumar AV, Parkin DM, Siddiqi M. Cancer patterns in eastern India: the first report of the Kolkata cancer registry. Int J Cancer. 2002;100(1):86-91
18. Kushi LH, Mink PJ, Folsom AR, Anderson KE, Zheng W, Lazovich D, et al. Prospective study of diet and ovarian cancer. Am J Epidemiol 1999;149(1):21–31
19. Harris H R, Cramer D W, Vitonis A F, DePari M, Terry K L. Folate, vitamin B6, vitamin B12, methionine and alcohol intake in relation to ovarian cancer risk. Int J Cancer. 2012;131(4): 518–529
20. Bostwick DG, Tazelaar HD, Ballon SC, Hendrickson MR, Kempson RL. Ovarian epithelial tumours of borderline malignancy: a clinical and pathologic study of 109 cases. Cancer. 1986;58(9):2052–2065.
21. Berek JS, Hacker NF. Practical gynecologic oncology. 3rd ed. Baltimore, MD: Lippincott Williams & Wilkins; 2000.