DOI: 10.7860/JCDR/2022/57026.16806

Original Article

Pathology Section

# Association between Preoperative RBC Parameters with Serum VEGF in Women Diagnosed with Breast Carcinoma: A Case-control Study

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## ABSTRACT

**Introduction:** The complete blood count is the first investigation to be done in every patient with breast carcinoma before surgery. Vascular Endothelial Growth Factor (VEGF) plays a major role in angiogenesis, metastasis and progression of tumours.

**Aim:** To assess Red Blood Cell (RBC) parameters in breast carcinoma patients and controls, and to evaluate its relation with serum VEGF.

**Materials and Methods:** The present study was a case-control study, conducted in Department of Pathology of JSS Medical College and Hospital, Mysuru, Karnataka, India from November 2019 to April 2021. Preoperative venous blood samples were collected and run in an automated analyser Mindray CAL-6000 for all haematological parameters. Preoperative serum samples were collected and serum VEGF was estimated using the Enzyme Linked Immunosorbent Assay (ELISA) method. Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) version 22.0 to evaluate the association between RBC parameters and serum VEGF using Mann-Whitney U test.

**Results:** A total of 80 samples were evaluated, which included 40 preoperatively diagnosed breast cancer cases and 40 age and sex matched controls. RBC parameters such as RBC, Haemoglobin (Hb), Haematocrit (Hct), red cell indices Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), reticulocytes, Red cell Distribution Width (RDW) and nucleated RBCs (nRBCs) were measured. The nRBCs and serum VEGF of cases were significantly higher than controls. A statistically significant association between patients with low Hb and high serum VEGF was found.

**Conclusion:** A higher percentage of breast carcinoma cases with anaemia in the present study was significantly associated with high serum VEGF, reflecting anaemia induced hypoxia may trigger the tumour cells to secrete VEGF.


**Keywords:** Anaemia, Haemoglobin, Hypoxia, Red blood cell, Vascular endothelial growth factor

## INTRODUCTION

Amongst the simple routine tests performed prior to breast cancer surgery, haematology profile is one of the important tests. RBC parameters which include RBCs, Hb, Hct, red cell indices, reticulocytes, RDW and nRBCs are measured. The analysis of RBC parameters is used to detect anaemia, type of anaemia, response of the body to anaemia and breast cancer. Angiogenesis is the process of forming new blood vessels from an existing vascular network in response to hypoxia, which is required for tumour growth, invasion, and spread [1]. The most effective endothelial cell mitogen and a regulator of vascular permeability ie., VEGF, induces angiogenesis and has emerged as a potent tool in the prognosis [2]. Patients with initial-stage of breast tumours with high levels of VEGF have a higher risk of relapse or mortality than patients with tumours that are less angiogenic [3].

It is known that in anaemia, there is decrease in Hb and RBC leading to hypoxia especially in the growing tumour cells. It has been observed that, in cancer patient Hb and Hct are linked to a greater risk of heart failure [4]. But it is not clearly known whether there is a relationship between the RBC parameters and serum VEGF levels in breast carcinoma. The novelty of present study was to know whether alteration in RBC parameters that results in hypoxia can affect the serum VEGF levels which may alter the prognosis of breast cancer patients.

The objective of the present study was, the analysis of RBC parameters in breast cancer patients and healthy controls, comparing with serum VEGF in breast cancer to elucidate any association between them.

## MATERIALS AND METHODS

A case-control study was conducted from November 2019 till April 2021 in the Department of Pathology of JSS Medical College and Hospital, Mysuru, Karnataka, India. All procedures performed were in accordance with the ethical standards of the Institutional Research Committee (JSS/MC/PG/5189/2019-20) and the 1964 Helsinki declaration. Ethical approval was obtained by the Institutional Ethical Committee (IEC) of JSS Medial College and Hospital, Mysuru, Karnataka, India.

**Inclusion criteria:** Forty preoperatively diagnosed breast carcinoma patients either by fine needle aspiration or core needle biopsy were collected and taken as cases. Forty healthy and age matched female volunteers attending general health checkup (without history of breast carcinoma) were included as controls.

**Exclusion criteria:** Women with postoperative diagnosis of breast carcinoma (preoperative diagnostic test done in other Institute), serum sample collected but underwent operation in other hospital, benign breast disease, postchemotherapy cases were excluded from the study.

Venous sample in EDTA vacutainers and serum samples were collected after informed consent under aseptic precautions. EDTA samples were run in automated analyser Mindray CAL-6000 and the data was collected for RBC parameters such as RBC, Hb, Hct, MCV, MCH, MCHC, reticulocytes, RDW and nRBCs. Serum was separated by centrifuging the blood for 10 minutes at 1500 rpm, and stored at -80° C in a deep freezer. RayBio® Human VEGF-A

Enzyme Linked Immunosorbent Assay (ELISA) Kit was used to estimate serum VEGF by sandwich ELISA method.

## STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS 22.0 statistical package for Windows. Continuous data were expressed as mean, median, standard deviation and range, while categorical data were presented as numbers and percentages. The distribution of variables was checked with the Kolmogorov Smirnov test. The categorical parameters between cases and controls were compared with Mann-Whitney U test. Receiver Operating Characteristic (ROC) curve analysis was performed to determine the cut-off of serum VEGF to divide the cases with low and high VEGF levels. The p-values <0.05 was considered statistically significant.

## RESULTS

Age distribution in cases ranged from 34 to 85 years with a mean±age of 52.33±12 years. Age distribution in controls ranged from 30 to 74 years with a mean age of 52±12 years with no significant differences between cases and controls (p=0.92).

On comparing the RBC counts, both cases and controls had low and high RBC counts [Table/Fig-1]. However, percentage of cases with low RBC counts (17.5%; 7/40) were higher than controls (10%; 4/40). There were equal number of cases and controls with high RBC counts. Mean RBC count was slightly lower among cases (4.2±0.5×$10^6$/uL) than controls (4.3±0.5×$10^6$/uL), which was not a statistically significant (p=0.689). There was no statistically significant difference in the mean Hb level between cases (11.6±1.6 g/dL) and controls (11.9±1.5 g/dL) (p=0.391). But the percentage of cases (50%;20/40) with low Hb (<12g/dL) was slightly higher than controls (47.5%; 19/40) [Table/Fig-1]. In cases, 70% (28/40) of them had Hct in normal range and 30% (12/40) had low Hct; whereas among controls 52.5% (21/40) had normal Hct and 47.5% (19/40) had low Hct. Mean Hct had no statistically significant difference (p=0.452) [Table/Fig-1].

The majority of cases and controls had MCV, MCH and MCHC within normal ranges and their mean values had no significant difference [Table/Fig-1]. The percentage of cases with low MCV and MCH were 7.5% (3/40) and 15% (6/40) respectively. Similarly, the percentage of controls with low MCV and MCH were 30% (12/40) and 40% (10/40) respectively. Equal percentage of cases and controls had high MCH. All cases and controls had MCHC within normal range (18-48 g/L). The mean MCHC of cases were slightly higher (33±1.3 g/L) than controls (31.8±4.7 g/L), however there was no statistically significant difference (p=0.105).

In 77.5% (31/40) of cases and 57.5% (23/40) of controls had RDW in normal range [Table/Fig-1]. Mean RDW was low in cases (13.8±2.0) than controls (14.4±1.9) but the difference was statistically not significant (p=0.211). Mean reticulocyte counts were within normal ranges in both cases and control with no significant differences [Table/Fig-1]. The mean nRBCs were significantly high in cases (0.19±0.1) than controls (0.02±0.01) with a p-value of 0.008.

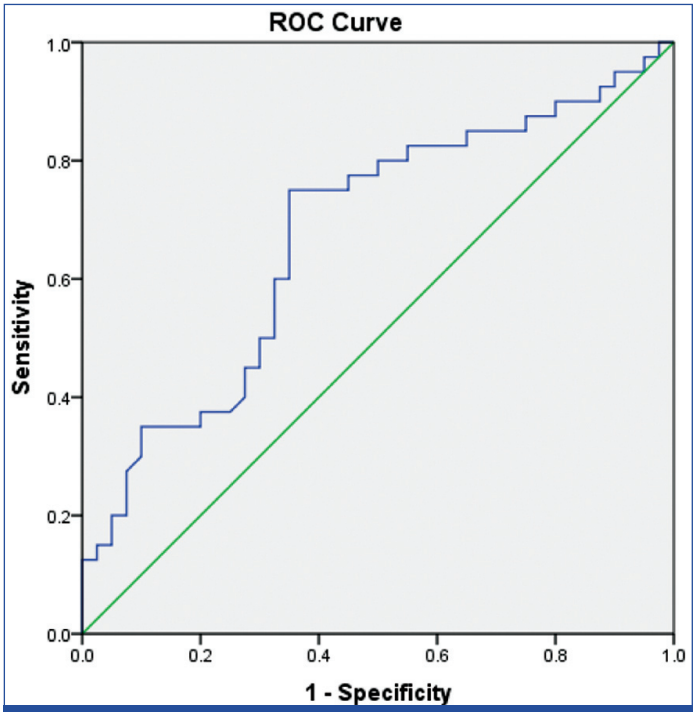
Mean serum VEGF of cases (100.7±33.91 pg/mL) were significantly higher than control (56.4±9.77 pg/mL) with a p-value of 0.013. Median serum VEGF of cases were significantly higher-70.4 (145.5-103.8) pg/mL than controls-44.3 (37.4-83.8) pg/mL with a p-value of 0.007. On statistical analysis, it was found that the VEGF values were not normally distributed hence median value was taken into consideration.

ROC analysis was done and area under the curve was 0.674, serum VEGF of 72.3 pg/mL was taken as cut-off value with a sensitivity of 47.5% and specificity of 70% with p-value of 0.007 [Table/Fig-2]. Considering 72.3 as cut-off value, cases were categorised into low VEGF and high VEGF categories respectively and were compared with RBC parameters.

The mean serum VEGF in patients with low VEGF category was 29.4, 48.7 and 49.1 pg/mL when RBC counts were low, normal and high respectively. Similarly, the mean serum VEGF among high VEGF category was 83.6, 119.8 and 99.6 pg/mL, respectively. Absence of association between low, high and normal RBC counts and serum VEGF categories were observed [Table/Fig-3a,4].

| Parameters | Range | Cases n (%) | Controls n (%) | p-value* |
|---|---|---|---|---|
| RBCs (million/µL) | <3.8 (Low) | 7 (17.5) | 4 (10) | 0.689 |
| | 3.8-4.8 (Normal) | 26 (65) | 29 (72.5) | |
| | >4.8 (High) | 7 (17.5) | 7 (17.5) | |
| | Mean RBCs | 4.2±0.5 | 4.3±0.5 | |
| Hb (g/dL) | <12 (Low) | 20 (50) | 19 (47.5) | 0.391 |
| | 12-16 (Normal) | 20 (50) | 21 (52.5) | |
| | Mean Hb | 11.6±1.6 | 11.9±1.5 | |
| Hct (percentage) | <35 (Low) | 12 (30) | 19 (47.5) | 0.452 |
| | 36-45 (Normal) | 28 (70) | 21 (52.5) | |
| | Mean Hct | 37.5±8.8 | 36.3±4.0 | |
| MCV (fL) | <80 (Low) | 3 (7.5) | 12 (30) | 0.415 |
| | 80-100 (Normal) | 37 (92.5) | 28 (70) | |
| | Mean MCV | 82.8±10 | 80.8±11 | |
| MCH (pg) | <27 (Low) | 6 (15) | 10 (40) | 0.135 |
| | 27-32 (Normal) | 33 (82.5) | 29 (57.5) | |
| | >32 | 1 (2.5) | 1 (2.5) | |
| | Mean MCH | 28.0±2.7 | 26.9±3.7 | |
| MCHC (g/L) | 18-48 (Normal) | 40 (100) | 40 (100) | 0.105 |
| | Mean MCHC | 33.0±1.3 | 31.8±4.7 | |
| RDW (percentage) | 11.6-14 (Normal) | 31 (77.5) | 23 (57.5) | 0.211 |
| | >14 (High) | 9 (22.5) | 17 (42.5) | |
| | Mean RDW | 13.8±2.0 | 14.4±1.9 | |
| Reticulocytes (percentage) | 0.5-2.5% (Normal) | 40 (100) | 40 (100) | 0.135 |
| | Mean reticulocytes | 0.6±0.1 | 0.8±0.1 | |
| Mean nRBCs (percentage) | | 0.19±0.1 | 0.02±0.01 | 0.008 |
| Mean VEGF (pg/mL) | | 100.7±33.91 | 56.4±9.77 | 0.013 |
| Median VEGF (pg/mL) | | 70.4 (145.5-103.8) | 44.3 (37.4-83.8) | 0.007 |

**[Table/Fig-1]:** Distribution of RBC parameters between cases and controls.
*Mann-Whitney U test, p-value <0.05 was considered as significant




**[Table/Fig-2]:** Receiver operating characteristic (ROC) analysis for VEGF to Predict Breast Cancer (Area Under Curve 0.674).

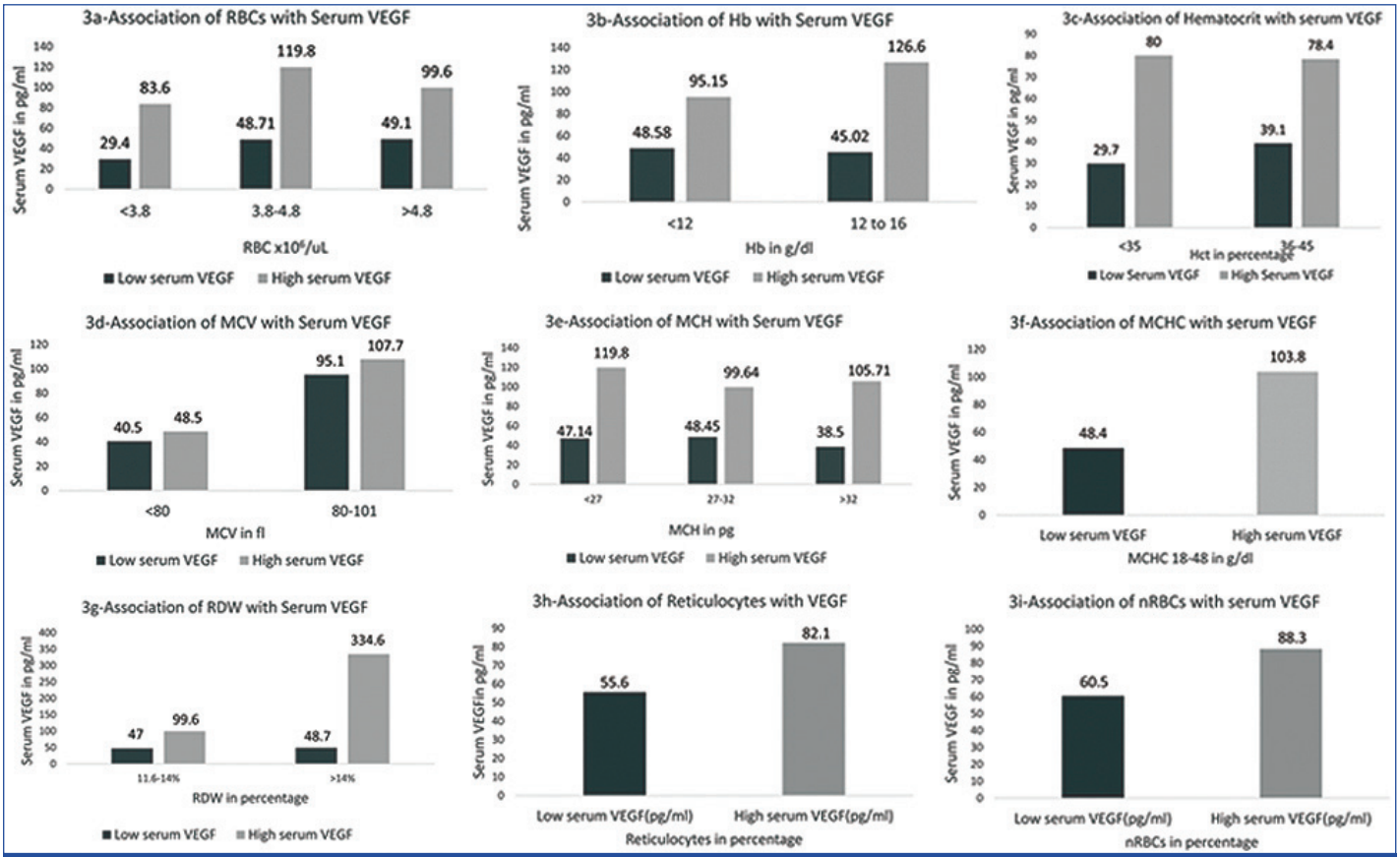
Among patients with Hb <12, the mean serum VEGF was 48.5 and 95.1 pg/mL in low and high VEGF category respectively. A statistically significant association between patients with low Hb and high serum VEGF with a p-value of 0.015 was found. Among patients with normal Hb, the mean serum VEGF was 45 and 126.6 pg/mL in low and high VEGF category respectively and observed no association between these categories [Table/Fig-3b,4].

Among patients with Hct<35, the mean serum VEGF was 29.7 and 80.0 pg/mL low and high VEGF category respectively. Similarly, when Hct was in normal range, the mean serum VEGF was 39.1 and 78.4 pg/mL, respectively. There was no association between low and normal Hct with serum VEGF categories [Table/Fig-3c,4].

The mean serum VEGF, among patients in low and high VEGF categories was compared with different categories of MCV, MCH and MCHC without statistically significant association [Table/Fig-3d-f,4].

In breast carcinoma cases with normal RDW, the mean serum VEGF in low and high VEGF categories were 47 and 99.6 pg/mL respectively. Likewise, when the RDW was high the mean serum VEGF in low and high VEGF categories were 48.7 and 334.6 pg/mL respectively. On statistical analysis, there was no association between any of these categories [Table/Fig-3g,4].

All the cases in this study had normal reticulocyte counts: the means serum VEGF levels were 55.6 and 82.1 pg/mL in low and high VEGF category, respectively [Table/Fig-3h,4]. Amongst patients with nRBCs,




**[Table/Fig-3]:** Association of RBC parameters with serum VEGF; 3a)-Association of RBCs with Serum VEGF; 3b) Association of Hb with Serum VEGF; 3c) Association of Haematocrit with serum VEGF; 3d) Association of MCV with Serum VEGF; 3e) Association of MCH with Serum VEGF; 3f) Association of MCHC with serum VEGF; 3g) Association of RDW with Serum VEGF; 3h) Association of Reticulocytes with VEGF; 3i) Association of nRBCs with serum VEGF.
*Statistical analysis by Mann-Whitney U test, p-value <0.05 was considered as significant

| Variables | Low VEGF (pg/mL) | p-value | High VEGF (pg/mL) | p-value |
|---|---|---|---|---|
| **RBCs (million/µL)** | | | | |
| <3.8 (Low) | 29.4 | 0.69 | 83.6 | 0.299 |
| 3.8-4.8 (Normal) | 48.71 | 0.566 | 119.8 | 0.354 |
| >4.8 (High) | 49.1 | 0.167 | 99.6 | 0.675 |
| **Hb (g/dL)** | | | | |
| <12 (Low) | 48.58 | 0.79 | 95.15 | 0.015 |
| 12-16 (Normal) | 45.02 | 0.561 | 126.6 | 0.355 |
| **Hct (percentage)** | | | | |
| <35 (Low) | 29.7 | 0.595 | 80.0 | 0.487 |
| 36-45 (Normal) | 39.1 | 0.262 | 78.4 | 0.669 |
| **MCV (fL)** | | | | |
| <80 (Low) | 40.5 | 0.752 | 95.1 | 0.644 |
| 80-100 (Normal) | 48.5 | 0.771 | 107.7 | 0.845 |
| **MCH (pg)** | | | | |
| <27 (Low) | 47.14 | 0.464 | 119.8 | 0.844 |
| 27-32 (Normal) | 48.45 | 0.372 | 99.64 | 0.718 |
| >32 (High) | 38.5 | 0.891 | 105.71 | 0.589 |
| **MCHC (g/L)** | | | | |
| 18-48 (Normal) | 48.4 | 0.555 | 103.8 | 0.663 |
| **RDW (percentage)** | | | | |
| 11.6-14 (Normal) | 47.0 | 0.774 | 99.6 | 0.477 |
| >14 (High) | 48.7 | 0.227 | 334.6 | 0.248 |
| **Reticulocytes (percentage)** | | | | |
| 0.5-2.5 (Normal) | 55.6 | 0.465 | 82.1 | 0.219 |
| **nRBCs (percentage)** | | | | |
| 0.19±0.1 | 60.5 | 0.068 | 88.3 | 0.253 |

**[Table/Fig-4]:** Association of RBC parameters with low and high serum levels in patients with breast carcinoma.
*Mann-Whitney U test, p-value <0.05 was considered as significant

the mean serum VEGF were 60.5 and 88.3 pg/mL in low and high VEGF category respectively [Table/Fig-3i,4]. Association between either reticulocyte count or nRBCs with serum VEGF levels, were not noticed.

## DISCUSSION

Breast cancer is the most common invasive malignancy and the second leading cause of tumour-related deaths among women

globally [5]. RBC parameters are one of the initial tests requested for all patients with breast cancer to detect anaemia and its associated changes. In this study, RBC parameters in both breast carcinoma cases and healthy controls were assessed. These were then compared with a potential breast cancer progression risk indicator-serum VEGF [6]. Growth of tumour requires supply of many new blood vessels in order to provide and support the metabolic activity; these growing tumours secrete many growth factors, including VEGF to recruit new blood vessels [7]. This stimulates proliferation of endothelial cells and formation of new vessels called angiogenesis [8,9]. VEGF receptor system is a primary tumour angiogenesis regulator and it has been resulted in progression and metastasis of the tumour [10,11]. Inhibition of VEGF results in suppression of development and metastasis of tumour [12].

In the present study, serum VEGF by sandwich ELISA method was estimated and found to be significantly elevated in cases than controls. Present study results were consistent with Lawicki S et al., and Ali EM et al., findings [13,14]. The preoperative detection of serum VEGF may support in identifying the severity and prognosis of breast cancer cases [12]. Statistically, 72.3 pg/mL as a cut-off for serum VEGFwas derived and thereby all breast carcinoma cases were divided into low VEGF and high VEGF categories. In the current study, similar mean age in cases and controls was found. Mean age of present study was comparable with Okuturlar Y et al., Raghunathachar Sahana K et al., and Harano K et al., [15-17].

Low RBC count and Hb, the indicators of anaemia were observed in both cases and controls. However, the high percentage of cases had low RBC count and low Hb than those of controls. This indicates that the anaemia which is prevalent in women in developing country like India [18] probably due to lower socio-economic status and poor nutrition was aggravated in breast carcinoma patients [19]. Metastasis to the bone marrow from breast cancer and nutritional deficiency induced by anorexia can be associated with suppression of erythropoiesis resulting in anaemia [4,20]. While in the present study Hb<12 was in 50% (20/40) of cases, Rana APS et al., has reported 60% and Zhang Y et al., in 25.2% [4,21]. High RBC count among controls could be due to dehydration or on anaemia treatment. It has been reported that some of the breast carcinoma tumour cells secrete erythropoietin that can result in high RBC and Hb values [22].

In the present study, association between RBC counts and serum VEGF categories was not noticed. Surprisingly the serum VEGF was low in patients with low RBC count than patients with normal RBC count. In contrast Bhatta SS et al., have reported inhibition of serum VEGF with antiangiogenic therapy increased erythropoiesis and increased RBCs [23]. Nevertheless, in patients with low Hb, a significant association with high serum VEGF was found, upholding the previous reports that low Hb results in hypoxia which in turn stimulates secretion of VEGF [22]. Majority (97%) of the oxygen supply to tissues are derived from oxygen bound to haemoglobin. Therefore, in anaemia cases, low haemoglobin levels might increase hypoxia in tumours [22]. Zhang Y et al., have reported that preoperative anaemia in breast carcinoma patients was associated with poor prognosis [21].

Another method of detecting anaemia was by estimation of Hct, which is the percentage of RBCs in total volume of blood. In contrast to Hb and RBC count, the percentage of patients with low Hct was less compared to controls in the present study. It has been reported that Hb was a more accurate method than Hct in assessing anaemia, which could be the cause of contrasting results [24]. The mean Hct in cases were higher than in controls and similar values were published by Rana APS et al., [4]. Statistical analysis revealed lack of association between Hct and serum VEGF.

Erythrocyte indices such as MCV, MCH and MCHC provide a clue to the different types of anaemia [25]. Mean erythrocyte indices were within normal range in this study and a similar observation was noted in previous study [4]. Erythrocyte indices showed that majority of the cases had normocytic normochromic anaemia while in controls microcytic hypochromic anaemia was more prevalent in this study. Previous study has reported that one of the causes for normocytic normochromic anaemia was breast cancer [26] which substantiates the reason for majority of cases with normocytic normochromic anaemia. The findings in the present study that mean erythrocyte indices of cases were higher than the controls contrasted with the findings of Akinbami A et al., [27]. Low serum VEGF levels was detected in patients with decreased MCV than those with normal MCV range. Those patients with decreased MCH had high VEGF than those with normal and high MCH. However, association between erythrocyte indices and serum VEGF was not found.

The RDW is a parameter to measure the variability of the size of the circulating RBCs [25]. Both the percentage and mean RDW were lower in cases compared to controls indicating that the RBCs in cases were more homogenous than controls in the current study. However, Seretis et al., reported higher RDW in cases than controls [28]. The presence of varied types of RBCs such as microcytes, normocytes and reticulocytes found in controls resulted in high RDW. The reticulocytes and nRBCs representing the immature erythrocytes are either low or not normally found in peripheral blood respectively. Even though there was no increase in reticulocytes in the present study, nRBCs were significantly increased in cases than controls. Increased reticulocytes and presence of nRBCs indicates increased erythropoiesis in bone marrow found in patients as a response to anaemia [29]. In peripheral blood, presence of nRBCs has shown to be associated with various medical conditions like solid cancers, haematological malignancies, cardiovascular conditions, infections, haemorrhage etc., [30,31]. An association of serum VEGF with RDW, reticulocytes or nRBCs was not observed in this study. On reviewing the literature, studies analysing the association between all the RBC parameters (except for Hb) and serum VEGF were not found to the best of the author's knowledge.

### Limitation(s)

The limitation of the present study includes small sample size and lack of demonstration of VEGF induced angiogenesis in breast carcinoma patients with anaemia. Therefore, it is recommended to conduct similar studies with large sample size at various geographic locations and further investigate the extent of angiogenesis in breast carcinoma patients with and without anaemia.

## CONCLUSION(S)

Anaemia is prevalent in elderly women due to various causes in developing country like India, which can be aggravated by breast carcinoma. Either as a compensatory mechanism to anaemia leading to increased bone marrow erythropoiesis or irritation due to metastasis into bone marrow had significant increase in nRBCs in the peripheral blood of breast carcinoma patients. A higher percentage of breast carcinoma cases with anaemia in the present study was significantly associated with high serum VEGF, reflects anaemia induced hypoxia may trigger the tumour cells to secrete VEGF.

### Acknowledgement

Authors would like to acknowledge (a) the infrastructure support provided by Department of Science and Technology to CEMR Laboratory (CR-FST-LS-1/2018/178) and to Department of Biochemistry (SR/FST/LS-1-539/2012); (b) the laboratory facilities provided by CEMR laboratory (DST-FIST supported centre), Department of Biochemistry (DST-FIST supported department), and Special Interest Group on Cancer Biology and Cancer Stem Cells (SIG-CBCSC), JSS Academy of Higher Education and Research (Mysore, Karnataka, India).

## REFERENCES


[1] Madu CO, Wang S, Madu CO, Lu Y. Angiogenesis in breast cancer progression, diagnosis, and treatment. J Cancer. 2020;11(15):4474-94.
[2] Shibuya M. Vascular Endothelial Growth Factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: A crucial target for anti- and pro-angiogenic therapies. Genes Cancer. 2011;2(12):1097-05.
[3] Aliustaoglu M, Bilici A, Ustaalioglu BBO, Konya V, Gucun M, Seker M, et al. The effect of peripheral blood values on prognosis of patients with locally advanced gastric cancer before treatment. Med Oncol. 2010;27(4):1060-65.
[4] Rana APS, Kaur M, Zonunsanga B, Puri A, Kuka AS. Preoperative peripheral blood count in breast carcinoma: Predictor of prognosis or a routine test. Int J Breast Cancer. 2015;2015:01-05.
[5] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jema A, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-24. Available from: http://doi.wiley.com/10.3322/caac.21492.
[6] Relf M, Le Jeune S, Scott PA, Fox S, Smith K, Leek R, et al. Expression of the angiogenic factors vascular endothelial cell growth factor, acidic and basic fibroblast growth factor, tumor growth factor beta-1, platelet-derived endothelial cell growth factor, placenta growth factor, and pleiotrophin in human primary breast cancer and its relation to angiogenesis. Cancer Res. 1997;57(5):963-69.
[7] Petrovic N. Targeting angiogenesis in cancer treatments: Where do we stand? J Pharm Pharm Sci. 2016;19(2):226-38.
[8] Carpini JD, Karam AK, Montgomery L. Vascular endothelial growth factor and its relationship to the prognosis and treatment of breast, ovarian, and cervical cancer. Angiogenesis. 2010;13(1):43-58.
[9] Ma J, Hu W, Zhang P, Sun Y, Wang Na, Teng X, et al. The association between VEGF +936C/T and –634G/C polymorphisms and breast cancer susceptibility, tumor growth, and metastases: Evidence from 20,728 subjects. Cancer Investig. 2015;33(7):312-17.
[10] Alitalo K, Carmeliet P. Molecular mechanisms of lymphangiogenesis in health and disease. Cancer Cell. 2002;(3):219-27.
[11] Iyer S, Darley PI, Acharya KR. Structural insights into the binding of vascular endothelial growth factor-B by VEGFR-1D2. J Biol Chem. 2010;285(31):23779-89.
[12] Veeravagu A, Hsu A, Cai W, Hou L, Tse V, Chen X, et al. Vascular endothelial growth factor and vascular endothelial growth factor receptor inhibitors as anti-angiogenic agents in cancer therapy. Recent Pat Anticancer Drug Discov. 2007;2(1):59-71.
[13] Ławicki S, Zajkowska M, Głażewska EK, Będkowska GE, Szmitkowski M. Plasma levels and diagnostic utility of VEGF, MMP-2 and TIMP-2 in the diagnostics of breast cancer patients. Biomarkers. 2017;22(2):157-64.
[14] Ali EM, Sheta M, el Mohsen MA. Elevated serum and tissue VEGF associated with poor outcome in breast cancer patients. Alexandria J Med. 2011;47(3):217-24.
[15] Okuturlar Y, Gunaldi M, Tiken EE, Oztosun B, Inan YO, Ercan T, et al. Utility of peripheral blood parameters in predicting breast cancer risk. Asian Pac J Cancer Prev. 2015;16(6):2409-12.
[16] Raghunathachar Sahana K, Akila P, Prashant V. Quantitation of vascular endothelial growth factor and Interleukin-6 in different stages of breast cancer. Rep Biochem Mol Biol. 2017;6(1):33-39.
[17] Harano K, Kogawa T, Wu J, Yuan Y, Cohen EN, Lim B, et al. Thrombocytosis as a prognostic factor in inflammatory breast cancer. Breast Cancer Res Treat. 2017;166(3):819-32.
[18] Siddiqui MZ, Goli S, Reja T, Doshi R, Chakravorty S, Tiwari C, et al. Prevalence of anemia and its determinants among pregnant, lactating, and nonpregnant nonlactating women in India. SAGE Open. 2017;7(3):215824401772555.
[19] Gascon P, Barrett-Lee PJ. Prevalence of anemia in cancer patients not receiving Antineoplastic Treatment (ANT): Data from the European Cancer Anaemia Survey (ECAS). J Clin Oncol. 2006;24(18_suppl):8565-65.
[20] Chang EI, Chang EI, Thangarajah H, Hamou C, Gurtner GC. Hypoxia, hormones, and endothelial progenitor cells in hemangioma. Lymphat Res Biol. 2007;5(4):237-44.
[21] Zhang Y, Chen Y, Chen D, Jiang Y, Huang Y, Ouyang HD. Impact of preoperative anemia on relapse and survival in breast cancer patients. BMC Cancer. 2014;14(1):844.
[22] Dunst J, Pigorsch S, Hänsgen G, Hintner I, Lautenschläger C, Becke A. Low hemoglobin is associated with increased serum levels of vascular endothelial growth factor (VEGF) in cancer patients. Strahlenther Onkol. 1999;175(3):93-96.
[23] Bhatta SS, Wroblewski KE, Agarwal KL, Sit L, Cohen EEW, Seiwert TY, et al. Effects of vascular endothelial growth factor signaling inhibition on human erythropoiesis. Oncologist. 2013;18(8):965-70.
[24] Keen ML. Hemoglobin and hematocrit: An analysis of clinical accuracy. Case study of the anemic patient. ANNA J. 1998;25(1):83-86.
[25] Dixon S, Nelson DA. RBC Values help pinpoint causes of anemia and other diseases [Internet]. New York: Dotdash Meredith; 2022 [updated 2022 May 23; cited 2022 June 4]. Available from: https://www.verywellhealth.com/mean-corpuscular-hemoglobin-concentration-797200.
[26] Yilmaz G, Shaikh H. Normochromic Normocytic Anemia. Treasure Island (FL): Stat Pearls Publishing; 2022 [updated 2022 March 7; cited 2022 June 6]. Available from: https://pubmed.ncbi.nlm.nih.gov/33351438.
[27] Akinbami A, Popoola A, Adediran A, Dosunmu A, Oshinaike O, Adebola P, et al. Full blood count pattern of pre-chemotherapy breast cancer patients in Lagos, Nigeria. Caspian J Intern Med. 2013;4(1):574-79.
[28] Seretis C, Seretis F, Lagoudianakis E, Gemenetzis G, Salemis NS. Is red cell distribution width a novel biomarker of breast cancer activity? Data from a pilot study. J Clin Med Res. 2013;5(2):121-26.
[29] Hermansen MC. Nucleated red blood cells in the fetus and newborn. Arch Dis Child Fetal Neonatal Ed. 2001;84(3):F211-15.
[30] Schwartz SO, Stansbury F. Significance of nucleated red blood cells in peripheral blood; analysis of 1496. J Am Med Assoc. 1954;154(16):1339.
[31] Danise P, Maconi M, Barrella F, Palma AD, Daniela Avino D, Rovetti D, et al. Evaluation of nucleated red blood cells in the peripheral blood of hematological diseases. Clin Che Lab Med. 2012;50(2).



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**PLAGIARISM CHECKING METHODS:** [Jain H et al.]
- Plagiarism X-checker: Apr 16, 2022
- Manual Googling: Jul 07, 2022
- iThenticate Software: Jul 09, 2022 (7%)

**ETYMOLOGY:** Author Origin

**AUTHOR DECLARATION:**
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Apr 11, 2022**
Date of Peer Review: **Apr 23, 2022**
Date of Acceptance: **Jul 14, 2022**
Date of Publishing: **Aug 01, 2022**