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# Assessing the Impact of Vitamin D Supplementation on the Absolute Cell Counts in Cancer Patients Receiving Chemotherapy in a Tertiary Care Hospital: A Prospective Case-Control Study

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**KEYWORDS**

Vitamin D; cancer; chemotherapy; absolute cell counts of WBC;

**ABSTRACT**

**Background:** Several clinico-epidemiological studies have shown a correlation between lower serum 25-hydroxy Vitamin D [25 (OH) D] levels and an increased risk of colorectal, breast, ovarian, and prostate cancers. The aim of this study was to assess the Vitamin D levels of cancer patients with confirmatory clinical diagnosis of breast, colorectal, gastric and ovarian cancers even before the initiation of cancer therapy and to observe & analyse the absolute cell counts of white blood cells which include Absolute Neutrophil Count-ANC, Absolute Lymphocyte count-ALC, Absolute Eosinophil count-AEC and Absolute Monocyte count-AMC in both the study and control groups.

**Methods**: A prospective case- control study was conducted between September 2022 to August 2023 in the Department of Oncology, King George Hospital, a tertiary care hospital situated in Visakhapatnam. Serum 25-hydroxy Vitamin D [25 (OH) D] levels were tested for a total of 194 cancer patients belonging to breast, ovary, gastric and colorectal cancers. The Maglumi 4000 Plus analyser instrument was used for the 25-OH Vitamin D assay. The 25-OH Vitamin D assay is a competitive chemiluminescence immunoassay. Vitamin D insufficiency is defined as having a serum 25 (OH) D level between 20 to 30 ng/mL, while patients with a level below 20 ng/mL were classified as having Vitamin D deficiency. Patients who had a Vitamin D level lower than 10 ng/mL were categorized as having severe Vitamin D deficiency. The total sample size was calculated to be 194 using the Raosoft calculator. Of the 194 patients, 5 were not willing to participate in the study, 9 patients missed the Vitamin D dose and 7 patients did not complete chemotherapy cycles due to various reasons. Hence a total of 173 patients were included in the study and among them, 160 patients were finally included in the study as 13 were having sufficient levels of Vitamin D, out of which 80 patients were included in the study/treatment group who were supplemented with Vitamin D3 60000IU capsules and the rest were included in the control group who were not supplemented with Vitamin D3. The Absolute cell counts of WBCs like the Absolute neutrophil Count, Absolute lymphocyte count, Absolute eosinophil count and Absolute monocyte count were observed and studied in both the study and control groups.

**Results:** In a total of 173 patients belonging to breast cancer, colorectal cancer, gastric and

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ovarian cancers, 13(7.5%) patients had sufficient levels of Vitamin D, 19(11%) patients had insufficient levels, 71(41.04%) had deficient and 70 (40.06%) patients had severely deficient levels of Vitamin D. The mean (±standard deviation) Vitamin D level was 12.30(±5.44) among the 160 cancer patients included in the study. Vitamin D deficiency was more prevalent among breast (91.3%) and ovary cancers (90%) which is evident that a high prevalence of vitamin D insufficiency is observed in patients with newly diagnosed breast cancer and ovarian cancers. Females were more deficient (90%) when compared to males (84%). Forty-six 46% in Stage III were and 44% in Stage II had vitamin D deficiency. 49% of Stage II and 48% of Stage IV were found to be severely deficient. Throughout the patient's visits to their respective cycles at different time points for receiving chemotherapy, it was observed that the study group compared to the control groups had an impact on the ANC,ALC,AEC and AMC among the breast, colorectal and gastric carcinomas.

**Conclusion**: Vitamin D deficiency was well established in cancer patients in our study. Vitamin D deficiency in cancer patients has an impact on the prognosis of cancer patients. Overall, a total of 141/160 patients (87%) had deficiency in Vitamin D levels. Vitamin D supplementation in the study group definitely had an impact on the ANC, ALC, AEC and AMC parameters majorly among the breast, colorectal and gastric cancers.

**INTRODUCTION:**

Vitamin D is a fat soluble vitamin and is synthesized from 7-dehydrocholesterol under the skin exposed to sunlight, and is taken through diet, or a supplement, and it is metabolized in the liver to 25- hydroxyvitamin D (25[OH]D), a biomarker of vitamin D status. The 25(OH)D is further activated in the kidneys by 1-alpa-hydroxylase to 1,25-dihydroxyvitamin D (1,25[OH]2D), which facilitates calcium absorption and is associated with bone health.Most tissues, as well as cancers, possess 1-αhydroxylase, which converts blood 25(OH)D to 1,25(OH)2D. They also have vitamin D receptors (VDR), a steroid hormone nuclear receptor that regulates various genes within a cell. This regulation is hypothesized to prevent cancer relapse and progression through the inhibition of cell proliferation, angiogenesis, and metastasis. Vitamin D is also believed to induce apoptosis and differentiation.[1]Thus, the aim of this study is to study the true role of vitamin D levels in cancer patients with solid tumours belonging to breast, ovary, gastric and colorectal cancers.Cancer is the second-leading cause of death in the USA having a significant deleterious impact on individual patients and society at large. Approximately one in two men and one in three women will develop cancer in their lifetime.[2]A low Vitamin D status is becoming increasingly common worldwide. Studies have shown a strong association between Vitamin D deficiency and chronic as well as acute conditions, from basic science to clinical applications.[3] Vitamin D's primary role is to regulate the metabolism of calcium and phosphate, which is crucial for bone remodeling. However, recent research has shown that low sunlight exposure and Vitamin D deficiency can also increase the risk of various non-skeletal diseases, including cancer.[4]

The aim of this study was to study the prevalence of Vitamin D deficiency among newly diagnosed cancer patients and whether the concurrent supplementation with Vitamin D along with chemotherapy would have an impact on the absolute cell counts of WBCs.

**Methodology:**

**Study design:**

A prospective cross-sectional study was carried out in a tertiary care teaching hospital between September 2022 and August 2023 who were registered in the Department of Medical Oncology.The sample size for the study was calculated using the Raosoft sample size online calculator. With a population size of 390 patients (derived from the prevalence data of the hospital in the previous year prior to the study) and a confidence interval of 95%, the sample size was calculated to be 194.A baseline serum Vitamin D was assessed for all the patients including the study and control groups.Vitamin D insufficiency is defined as having a serum 25 (OH) D level between 20 to 30 ng/mL, while patients with a level below 20 ng/mL were classified as

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having Vitamin D deficiency. Patients who had a Vitamin D level lower than 10 ng/mL were categorized as having severe Vitamin D deficiency. The Absolute cell counts of WBCs like the Absolute Neutrophil count, Absolute Lymphocyte count, Absolute Eosinophil count and Absolute Monocyte count were observed and studied in both the study and control groups. The study was approved by the TheInstitutional Ethics Committee, King George Hospital, Visakhapatnam Reg No., ECT/197/Inst/KGH/2013/RR-20. All the study participants have provided a written informed consent for their willingness to participate in this study.

**Study participants:**

Patients who are 18 years or older with a confirmatory clinical diagnosis belonging to breast, ovary, gastric and colorectal cancers with Vitamin D levels assessed at the initiation of chemotherapy after obtaining the informed consent in addition to the regular haematological, serological and biochemical tests done to assess the baseline organ function before initiation of chemotherapy.Patients who have undergone previous cancer treatments such as radiotherapy, chemotherapy, hormonal therapy, or cancer surgeries (excluding diagnostic biopsies and cytology) were excluded from the study.Patients with active pregnancy, renal osteodystrophy, or documented osteoporosiswith a history of rickets were deemed ineligible to participate in the study. Patients with a history of taking Vitamin D supplementation of any formulation in the past 1 year were excluded.

The patients who had insufficient (20-30ng/ml), deficient (10-20ng/ml) and severely deficient (<10ng/ml) were included in the study. They were supplemented with a dose of 60000IU of Vitamin D3 capsulesweekly for the first 2 months as a loading dose and then monthly for the next 4 months as the maintenance dose. Of the 194 patients, 5 were not willing to participate in the study, 9 patients missed the Vitamin D dose and 7 patients did not complete chemotherapy cycles due to various reasons. Hence a total of 173 patients were included in the study and among them, 160 patients were finally included in the study as 13 were having sufficient levels of Vitamin D. Among the 160 patients, 80 subjects were taken in the study group and were given Vitamin D3 supplementation and other 80 subjects were taken as controls, not supplemented with Vitamin D3 through randomizationin order to mitigate the selection bias. Out of the 160 study participants, 58 patients belonged to breast cancer, 42 belonged to gastric cancers, 40 belonged to colorectal and 20 patients were diagnosed to have ovary cancer.

**Sample collection:**

A 3mL blood sample was drawn from a peripheral vein, regardless of fasting or dietary requirements, using a red-top serum separator vacutainer. The plasma was separated and stored at 2–8 C refrigeration. Frozen samples were thawed only once to avoid analyte deterioration. The samples containing precipitates should be centrifuged before performing the assay. Care was taken that no heat-inactivated samples are not used and also that they are free from fibrin or any particulate matter. The sample volume required for a single determination of 25-OH Vitamin D is 100 µL.The Maglumi 4000 Plus analyser instrument was used for the 25-OH Vitamin D assay. The 25-OH Vitamin D assay is a competitive chemiluminescence immunoassay.The 25-OH Vitamin D assay is a two-incubation chemiluminescence immunoassay for the quantitative determination of total 25-OH Vitamin D in human serum.The average turnaround time for obtaining a report was 48-72 hours.

All the blood parameters(CBC) were analysed using the Horiba Automatic Haematology Analyser in the KGH Central lab. ANC, ALC, AMC and AEC were calculated by multiplying the WBC count with the percentage of neutrophils, lymphocytes, monocytes andeosinophils respectively.

**Categorization of the patients:**

The patients' sociodemographic details including the age, gender, height, weight, body mass index and body surface area,residence (rural/urban), educational status, occupation, family history of cancer, whether alcoholic, smoker, tobacco use, marital status, number of children were recorded.Age at menarche, age at first and second pregnancies, menopausal age& history of hysterectomydetails were taken among breast and ovary cancer patients. The diagnosis, type of cancer, staging, grading, type of chemotherapy (adjuvant/neoadjuvant),

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JCHR (2023) 13(6), 2655-2669 | ISSN:2251-6727

tumor size and drugs given during the chemotherapy for the prescribed duration were also recorded. Patients who have given the sample for the Vitamin D test were informed about their status and also about the importance of having normal range of Vitamin D levels. The patients were advised by the medical oncologist that their decision to take Vitamin D supplements would not affect the course of their tumor-directed therapy. Patients were educated that Vitamin D supplementation to those with Vitamin D deficiency would improve the outcome and also would reduce the chemotherapy related chemotoxicities.The chemotherapy drugs used in the treatment of breast cancer were Adriamycin + Cyclophosphamide and Docetaxel + Cyclophosphamide.In colorectal cancer Oxaliplatin+ Capecitabine capsules, in gastric cancers the treatment cycles included Oxaliplatin + Capecitabine, Gemcitabine+ Carboplatin, Cisplatin + Gemcitabine depending upon the staging and severity of the cancers. In ovarian cancers, Paclitaxel+ Carboplatin drugs were administered.

**Results:**

From the total sample size of 194 patients who were tested for Vitamin D levels, 160 patients were included in the study. Among the 160 patients, 80 subjects were taken in the study group and were given Vitamin D3 supplementation and other 80 subjects were taken as controls, not supplemented with Vitamin D3 through randomization in order to mitigate the selection bias. Among the 160 study participants, equal no. of study and control group patients were taken. 58 patients were diagnosed with breast cancer, of which 29 belonged to study and 29 belonged to the control group. 42 patients were diagnosed with colorectal cancers, of which 21 were in the study and rest were in control group. 40 patients were diagnosed to havegastric cancers, of which 20 belonged to the study group and the other 20 patients were included in the control group. 20 patients were diagnosed to have ovary cancer, out of which 10 were in the study group and the other 10 in control group. Among the 160 study participants, 103 (64%) were female, and 57(35%) were male patients. The median age at diagnosis was found out to be 51 years with a range from (18-83) years. A total of 86(53.7%) patients belonged to the rural background and 74(46.2%) belonged to urban areas. 49 patients (30.6%) were smokers and 62(38.7%) were alcoholics. Among the patients diagnosed with solid tumours54(33.7%) belonged to stage 1V (T4), followed by stage III (T3) 52(32.5%). A total of 108(67.5%) patients have received adjuvant chemotherapy and 42(26.25%) patients received neoadjuvant chemotherapy. 10(6.25%) patients received palliative care.

**Table 1: Demographic details of the patient:**

| Category | Variables | n=160, n(%) |
|---|---|---|
| **Age(median)=51yrs** | <51 | 85(53.1) |
| | >=51 | 75(46.8) |
| **Gender** | Male | 57(35) |
| | Female | 103(64) |
| **Residence** | Rural | 86(53.7) |
| | Urban | 74(46.2) |
| **Educational qualification** | Elementary school | 40(25) |
| | Secondary school | 50(31.25) |
| | Intermediate | 4(2.5) |
| | Graduate | 5(3.1) |
| | Post graduate | 1(0.62) |
| | No qualification | 60(37.5) |
| **Smoker** | No.of smokers | 49(30.6) |
| **Alcoholic** | No.of alcoholics | 62(38.7) |

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JCHR (2023) 13(6), 2655-2669 | ISSN:2251-6727

| | | |
|---|---|---|
| **Type of cancer** | Breast | 58(36.25) |
| | Colorectal | 42(26.25) |
| | Gastric | 40(25) |
| | Ovary | 20(12.5) |
| **Cancer clinical staging** | Tx | 2(1.25) |
| | T0 | 1(0.62) |
| | T1 | 4(2.5) |
| | T2 | 47(29.3) |
| | T3 | 52(32.5) |
| | T4 | 54(33.7) |
| **Type of chemotherapy** | NACT | 42(26.25) |
| | ACT | 108(67.5) |
| | NACT PALLIATIVE | 7(4.3) |
| | ACT PALLIATIVE | 3(1.8) |

**Figure 1:**

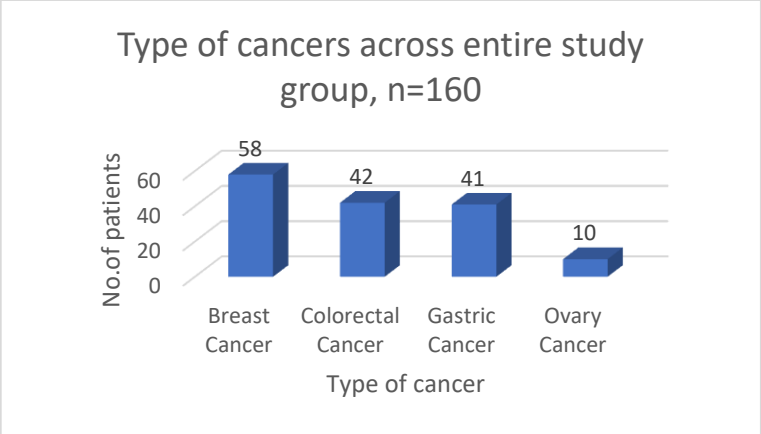


**Figure 2:**

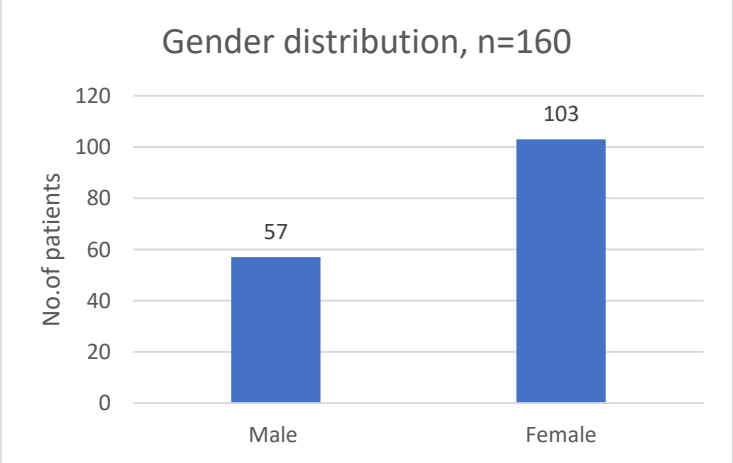

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**Figure 3:**

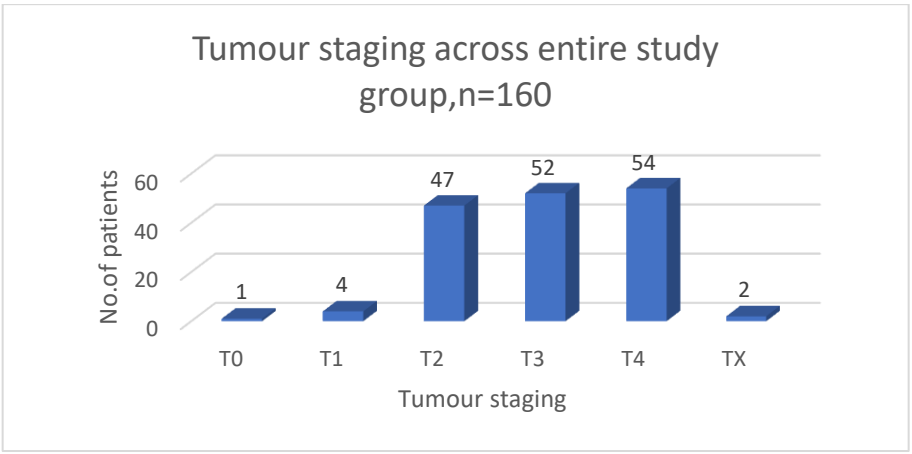



**Figure 4:**

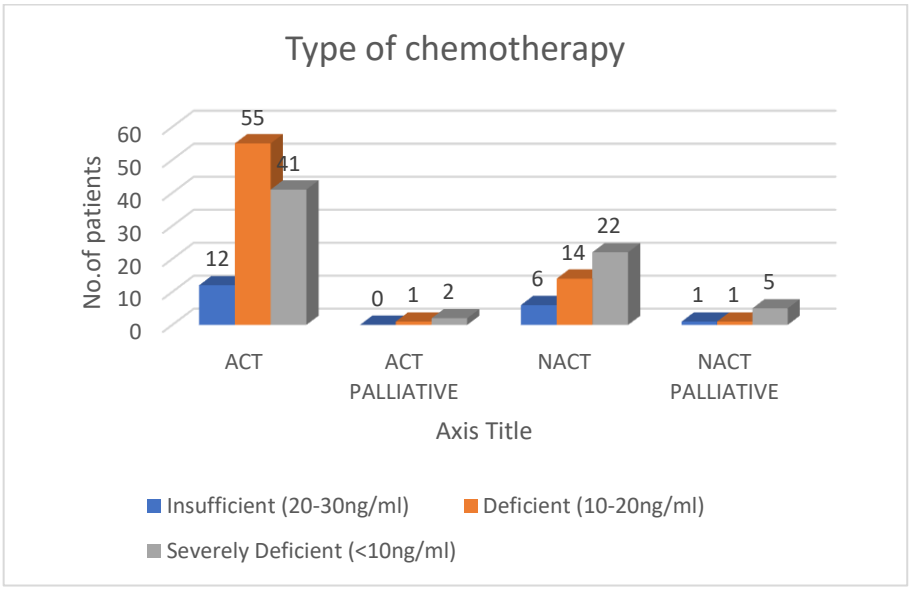
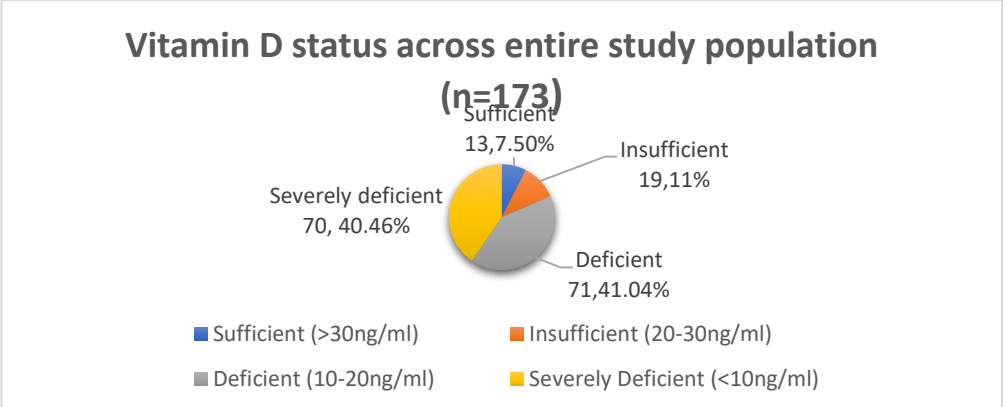



**Figure 5:**



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www.jchr.org

JCHR (2023) 13(6), 2655-2669 | ISSN:2251-6727

**Table 2:Vitamin D status among the major and demographic variables:**

| Variables | Category | Insufficient(20-30ng/ml),n(%) | Deficient(10-20ng/ml),n(%) | Severelydeficient (<10ng/ml),n(%) | Total |
|---|---|---|---|---|---|
| **Age** | <51 | 15(17.6) | 31(36.4) | 39(45.8) | 85 |
| | >=51 | 4(5.3) | 40(53.3) | 31(41.3) | 75 |
| **Gender** | Male | 9(15.7) | 27(47.3) | 21(36.8) | 57 |
| | Female | 10(9.7) | 44(42.7) | 49(47.5) | 103 |
| **Type of cancer** | Breast | 5(8.6) | 24(41.3) | 29(50) | 58 |
| | Colorectal | 7(16.6) | 21(50) | 14(33.3) | 42 |
| | Gastric | 5(12.5) | 16(40) | 19(47.5) | 40 |
| | Ovary | 2(10) | 10(50) | 8(40) | 20 |
| **Cancer clinical staging** | Tx | 0 | 1(50) | 1(50) | 2 |
| | T0 | 0 | 0 | 1(100) | 1 |
| | T1 | 0 | 2(50) | 2(50) | 4 |
| | T2 | 3(6.38) | 21(44.68) | 23(48.93) | 47 |
| | T3 | 11(21.15) | 24(46.15) | 17(32.69) | 52 |
| | T4 | 5(9.25) | 23(42.59) | 26(48.14) | 54 |
| **Type of chemotherapy** | NACT | 6(14.2) | 14(33.3) | 22(52.3) | 42 |
| | ACT | 12(11.11) | 55(51) | 41(38) | 108 |
| | NACT PALLIATIVE | 1(14.2) | 1(14.2) | 5(71.4) | 7 |
| | ACT PALLIATIVE | 0(0) | 1(33.3) | 2(66.6) | 3 |

The mean (±standard deviation) Vitamin D level was 12.30(±5.44) among the 160 cancer patients included in the study. 19/160 (11%) patients had insufficient levels of Vitamin D, 71/160 (44%) had deficient levels and 70/160 (43%) patients had severely deficient Vitamin D levels.Vitamin D deficiency was more prevalent among breast (91.3%) and ovary cancers (90%) which is evident that a high prevalence of vitamin D insufficiency is observed in patients with newly diagnosed breast cancer and ovarian cancers and it may be linked pathophysiologically with the development or progression of the two types of cancer.Females were more deficient (90%) when compared to males (84%). Forty-six46% in Stage III were and 44% in Stage II had vitamin D deficiency.49% of Stage II and 48% of Stage IV were found to be severely deficient.

**Vitamin D impact on all the absolute cell counts of WBC:**

**Absolute Neutrophil Count (ANC)**

The Absolute Neutrophil Count (ANC) is a calculated value that assesses the number of white blood cells, percentage of neutrophils, and percentage of band cells in a patient's blood sample at a given time. This parameter is commonly used to evaluate immune function and determine the likelihood of acute bacterial infections. It is particularly useful in assessing the risk of opportunistic bacterial infections in immunosuppressed patients with malignancy who are receiving chemotherapy. Teramukai et al. (2009) found a correlation between higher neutrophil counts and reduced overall and progression-free survival in advanced non-small-cell lung cancer patients.[5]This highlights the prognostic value of ANC in cancer management. Patients with acute febrile illness have significantly higher ANC levels, indicating potential acute bacterial infections[6]. In a study on infectious diseases, Nascimento et al. (2010) found that obese pediatric patients had higher absolute neutrophil counts, indicating a potential association between ANC and inflammatory conditions[7]. Intrahepatic cholangio-carcinoma (IHCC) patients with high ANC have poor recurrence-free survival and a high risk of recurrence[8]. Levels of ANC have been investigated as a diagnostic

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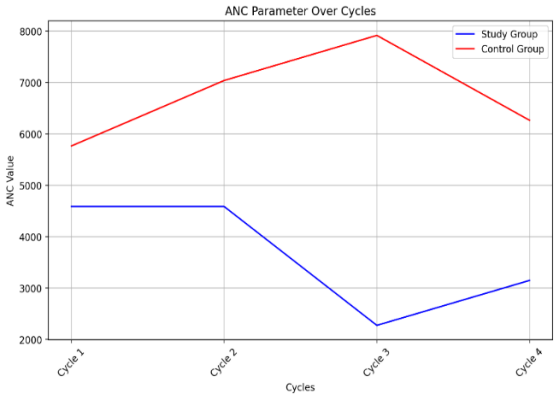
JCHR (2023) 13(6), 2655-2669 | ISSN:2251-6727

marker for lung cancer. They are found to be increased in lung cancer patients compared to healthy individuals[9].These findings suggest that ANC may have prognostic and diagnostic significance in cancer, as well as potential associations with tumor characteristics.

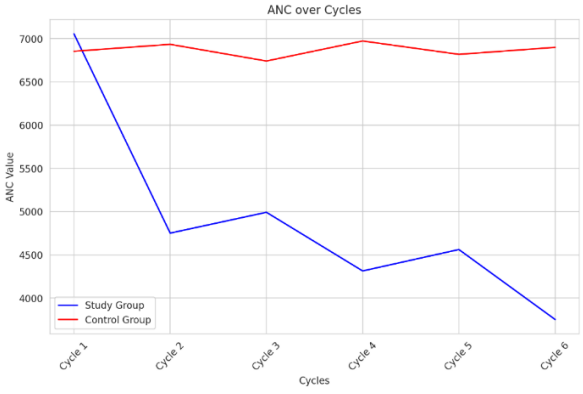
In the present study, the ANC parameter was analysed both in the study and treatment groups among the breast, colorectal, gastric and ovarian cancers. The normal range of ANC is 2500-6000 cells/microlitre.

**Figure 6(a-d):**

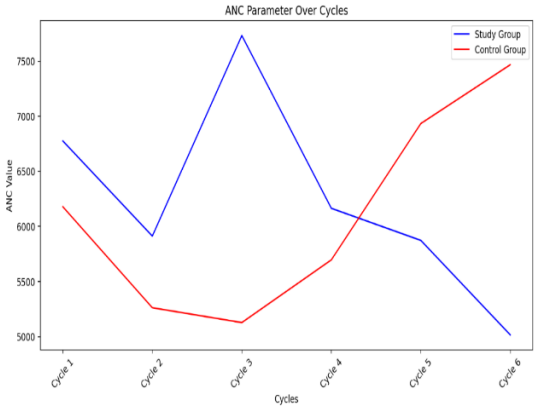
Breast cancer (a):



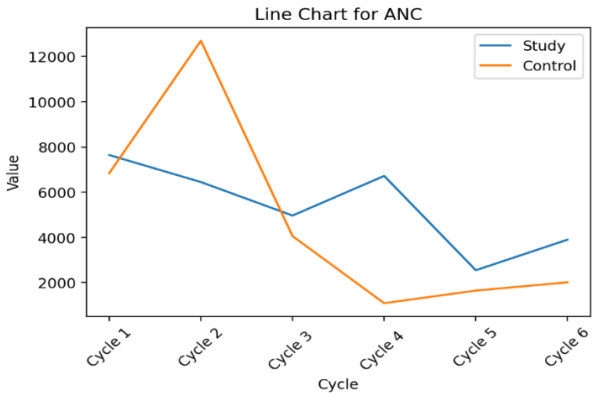

Colorectal cancer (b):




Gastric cancer (c):




Ovary cancer (d):




In breast cancer, the ANC parameter shows a decrease in the study group and an increase in the control group over the treatment cycles. In colorectal cancer, study group has a significant drop in the cycles 1-6, whereas the control group maintains a steadier line. The study group's significant mid-cycle drop could reflect a transient response to treatment, (chemotherapy + Vitamin D supplementation) whereas the control group'ssteadiness suggests no such effect.(only chemotherapy). In gastric cancerthe study group's line peaks up in cycle 2 and then drops from cycle 3 to a normal range, indicating significant changes between cycles, whereas the control group's line shows an increase outside the normal range.In ovary cancerthe ANC parameter in the study group shows a decreasing trend across the cycles, with the control group again showing fluctuations.

**Absolute Lymphocyte Count:**

Absolute lymphocyte count (ALC) has been studied in various cancers to determine its prognostic value. It is a measure of the number of lymphocytes present in a certain volume of blood. Lymphocytes are a type of white blood cell that plays an essential role in the immune system by identifying and attacking foreign invaders such as bacteria, viruses, and other pathogens.

Journal of Chemical Health Risks

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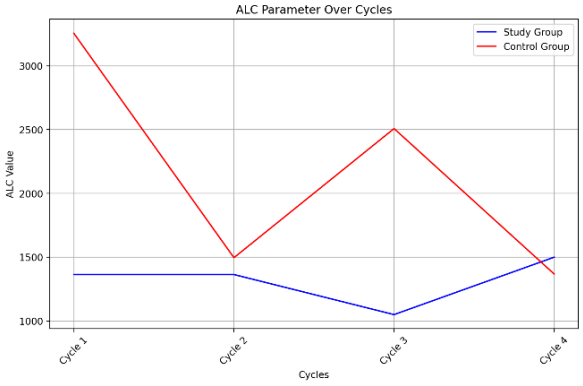
JCHR (2023) 13(6), 2655-2669 | ISSN:2251-6727

Normal ranges for absolute lymphocyte count can vary slightly depending on the laboratory and the specific population being tested. However, in general, a normal ALC for adults is typically in the range of 1,000 to 4,000 lymphocytes per microliter. An abnormally high or low ALC (absolute lymphocyte count) may suggest the presence of an underlying health condition. For instance, an elevated ALC might be a response to infections, autoimmune diseases, or some types of leukaemia. Conversely, a reduced ALC may be linked to bone marrow-related issues like chemotherapy, radiation therapy, or certain viral infections such as HIV. The absolute lymphocyte count (ALC) has been identified as a significant prognostic indicator in various types of lymphomas and leukemias.[10]Extranodal natural killer/T-cell lymphoma has been associated with overall survival and progression-free survival in relation to ALC.[11]In metastatic or recurrent breast cancer (MBC), a high ALC at diagnosis is an independent prognostic factor for better overall survival.[12]Additionally, the ALC has been researched in relation to other types of cancers, including breast cancer. In this context, it has been utilized alongside absolute monocyte count as a measure of systemic inflammation, which could potentially have prognostic significance, as per the findings of (Wen et al. in 2015).[13]The absolute lymphocyte count (ALC) has proven to be a significant prognostic indicator in several blood cancers. It plays a vital role in determining the risk level and predicting the outcome for patients. Its connection to immune status and systemic inflammation highlights its potential usefulness in various clinical settings.

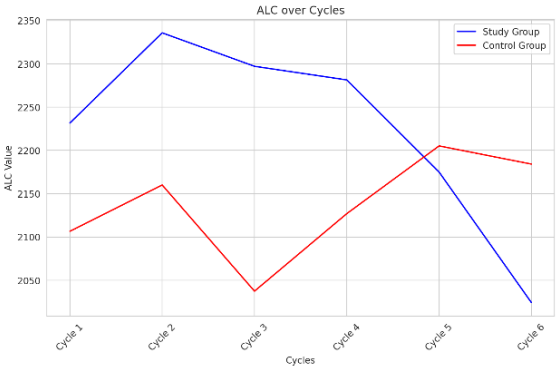
In our present study, ALC was monitored in both the study and control groups.a normal ALC for adults is typically in the range of 1,000 to 4,000 lymphocytes per microliter

**Figure 7(a-d):**

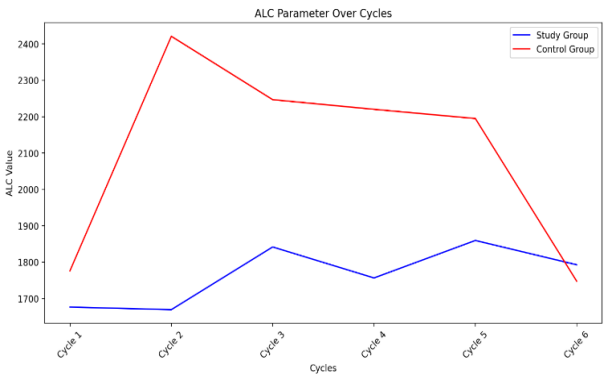
Breast cancer (a):



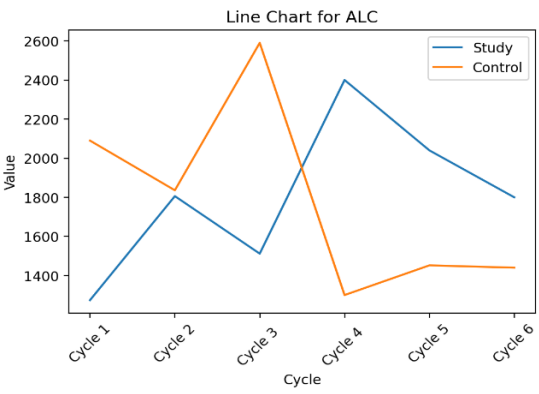

Colorectal cancer (b):




Gastric cancer (c):




Ovary cancer (d):



Journal of Chemical Health Risks

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In breast cancer, the ALC parameter shows a decrease in the study group and an increase in the control group over the treatment. The study group's decrease might reflect a treatment-related decline, whereas the control group's increase could be attributed to normal fluctuations or absence of treatment influence.

In colorectal cancer, a downward trend in both groups with the study group's more pronounced decline indicates a stronger reaction or progression of the condition being studied and they remain in normal ranges.In gastric cancers, both study and control groups display fluctuations across the cycles and remain within the normal range at the end of cycle 6. In ovary cancer, the ALC parameter in both the study and control groups shows fluctuations across the cycles, and show no significant changes.

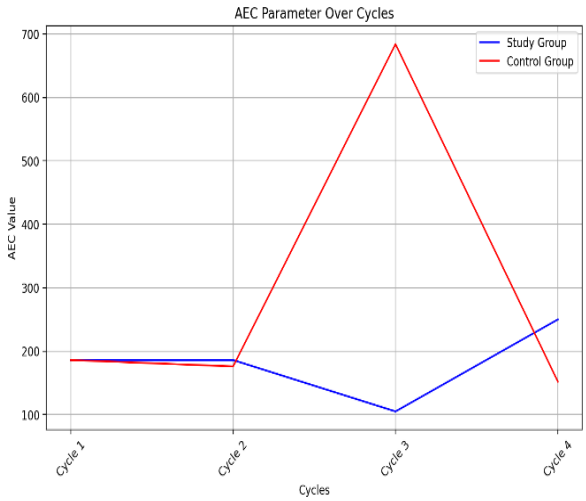
**Absolute Eosinophil count:**

The absolute eosinophil count (AEC) is a measure of the number of eosinophils present in a specific volume of blood. Eosinophils are a type of white blood cell that plays a significant role in the immune system, especially in responses to allergic reactions and parasitic infections.Normal ranges for the absolute eosinophil count can vary, but a typical range for adults is usually between 50 and 300 eosinophils per microliter.An elevated AEC, known as eosinophilia, can be associated with allergic conditions, parasitic infections, autoimmune diseases, and certain types of cancers. Childhood urticaria can be assessed for disease severity and management by measuring elevated absolute eosinophil count and serum IgE levels.[14]In COVID-19 patients, lower levels of eosinophil count, also known as eosinopenia, were linked with higher mortality rates.[15] Additionally, in patients with chronic obstructive pulmonary disease (COPD), a higher eosinophil count can be indicative of a positive response to bronchodilators.[16] Patients with non-small cell lung cancer (NSCLC) treated with immune checkpoint inhibitors (ICIs) have better clinical outcomes and longer progression-free survival (PFS) and overall survival (OS) when their baseline absolute eosinophil count (AEC) is high (≥130/μL).[17]

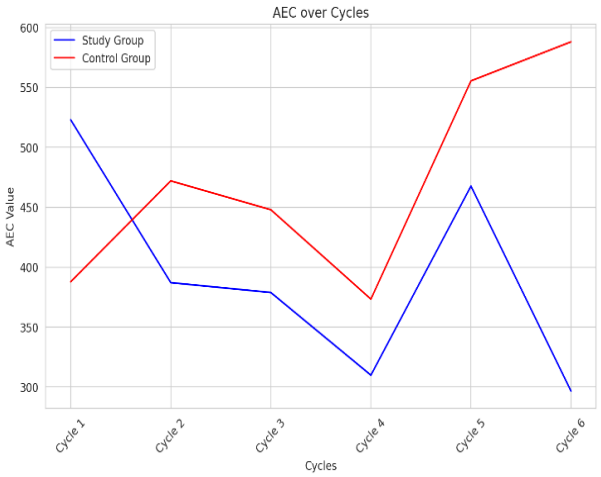
In the present study AEC was monitored both in study and control groups.

**Figure 8 (a-d):**

Breast cancer (a):




Colorectal cancer (b):



Journal of Chemical Health Risks

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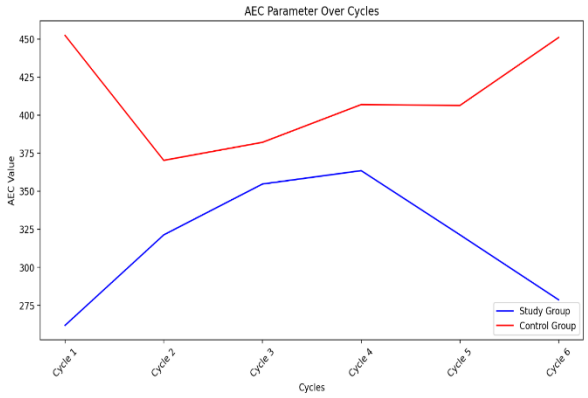
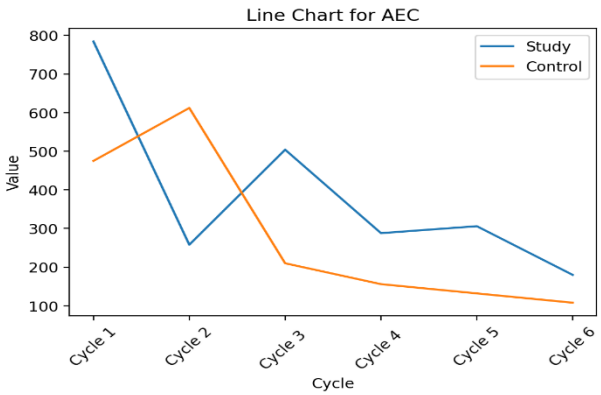
JCHR (2023) 13(6), 2655-2669 | ISSN:2251-6727

Gastric cancer (c):




Ovary cancer (d):




In breast cancer, the AEC parameter remains within the normal range, but in the control group shows an increase, and again drops at the cycle 3 possibly due to the treatment effect. But in the study group was moresteady and within normal range throughout the of chemotherapy which shows that there was a significant change showing a positive effect of Vitamin D along with chemotherapy. In colorectal cancer, the study group shows a fluctuating trend with a notable dip and recovery,suggesting a specific point in time where there is a decrease or dip in the observed values. The study group exhibits variability with a notable mid-cycle dip, suggesting a response to an intervention, while the control group's stability implies a baseline condition and increases above the normal range of AEC.

In gastric cancer, the study group shows an increase in AEC upto cycle 4 and then drops to a normal range showing a positive effect of Vitamin D supplementation., suggesting a response to the intervention, while the control group shows fluctuations, a decrease in the first few cycles and again a rise. In ovary cancer, the AEC parameter in the study group shows a decreasing trend across the cycles. This suggests that, over time or across measurement points, the absolute eosinophil count is consistently decreasing in individuals with ovarian cancer. The decreasing trend might be indicative of a response to chemotherapy along with Vitamin D supplementation,while the control group shows fluctuations.

**Absolute monocyte count:**

The absolute monocyte count (AMC) is a measure of the number of monocytes in a specific volume of blood. Monocytes are a type of white blood cell that plays a role in the immune system, particularly in responding to infections and inflammation. Normal ranges for the absolute monocyte count can vary, but a typical range for adults is usually between 200 and 800 monocytes per microliter. An elevated absolute monocyte count (AMC) may indicate an infection, chronic inflammatory condition, autoimmune disease, or certain cancers. Conversely, a decreased AMC may be seen in certain conditions such as bone marrow disorders or immune system deficiencies. The absolute monocyte count (AMC) has prognostic significance in various types of lymphoma, including extranodal marginal zone B-cell lymphoma of the MALT lymphoma[18]angioimmunoblastic T cell lymphoma (AITL)[19] and diffuse large B-cell lymphoma (DLBCL).[20] Various cancer types, including extranodal natural killer/T-cell lymphoma, breast cancer, hepatocellular carcinoma, lung adenocarcinoma, and esophageal cancer have been linked to poor prognosis with absolute monocyte count.[21] The absolute monocyte count has been associated with adverse pathology in radical prostatectomy specimens. It has been identified as an independent diagnostic and prognostic biomarker for prostate cancer, indicating its relevance in the context of urological malignancies.[22] Overall, the absolute monocyte count has emerged as a valuable prognostic indicator in various medical conditions, particularly in the context of cancer, infectious diseases, and urological malignancies, highlighting its clinical significance in risk stratification and patient outcome prediction.

Journal of Chemical Health Risks

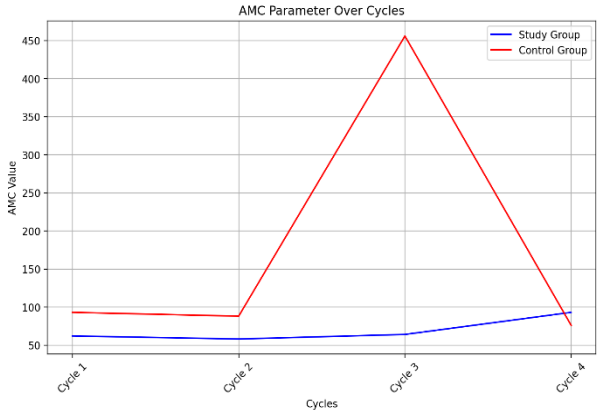
www.jchr.org

JCHR (2023) 13(6), 2655-2669 | ISSN:2251-6727

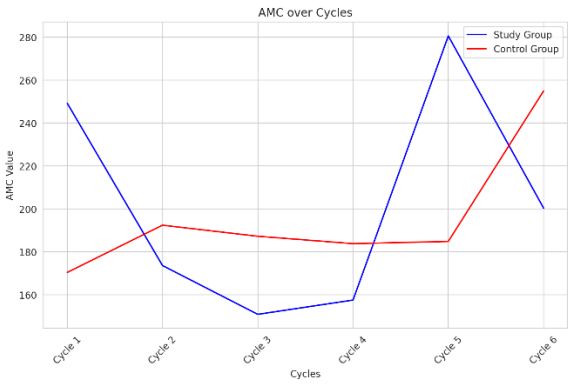
Absolute monocyte count was monitored at different time points when the patients come for receiving the chemotherapy. The normal range of AMC is between 200 and 800 monocytes per microliter.

**Figure 9 (a-d):**

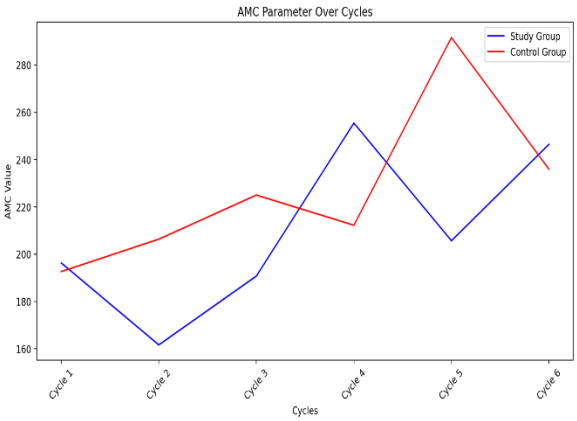
Breast cancer (a):



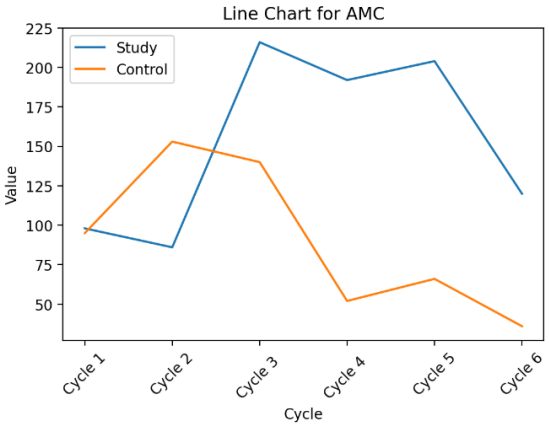

Colorectal cancer (b):




Gastric cancer (c):




Ovary cancer (d):




In breast cancer, the AMC parameter shows a decrease in the study group and an increase in the control group over the treatment cycles. This decrease might be interpreted as apotential response to the treatment that the study group is undergoing. A reduction in the absolute monocyte count could be indicative of treatment-related effects on the immune system. In the control group, the AMC parameter shows an increase. This increase may be interpreted as the absence of any impact from chemotherapy alone. In colorectal cancer, the study group's AMC values fluctuate more across treatment cycles. This fluctuation may suggest variable responses to treatment or different stages of disease progression within the study group. The AMC graph indicates more variation in absolute monocyte counts across treatment cycles for the study group. This variability could be indicative of dynamic responses to the treatment. The control group's monocyte counts remained more constant, suggesting less variability without treatment influence. In gastric cancer,the AMC parameter for the study group appears to have a slight downward trend, while the control group's values fluctuate with no clear pattern. This could suggest that the study group is experiencing a decrease in the parameter over time. In ovary cancer, similar to ALC, the AMC parameter also exhibits fluctuations in both study and control groups across the cycles.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 2655-2669 | ISSN:2251-6727

**Discussion:**

Within the last several years, frequency of Vitamin D testing has multiplied substantially all over the world, since it has been shown to have an important role in many diseases and conditions.Recent epidemiological and clinical studies strongly support that vitamin D supplementation is associated with reduced cancer risk and favourable prognosis. Vitamin D is known to have analytical problems, such as hydrophobicity, low circulating concentrations, and the ability to bind to lipids, albumins, and Vitamin D binding protein. Additionally, there are multiple vitamin D metabolites present in the blood, which can result in variable ratios of 25(OH)D2 and 25(OH)D3. Furthermore, Vitamin D exhibits significant preanalytical variability, as its concentration is strongly influenced by seasonal changes, exposure to sun, type of clothing, and use of sun block creams.The Boston Medical School has completed a great many research studies on vitamin D. The following statementfrom their Professor Hollick, "If women obtained adequate levels of vitamin D there would be 25 per cent less deaths from breast cancer.Hollick is not alone in stressing the importance for women and breast cancer. St Georges Hospital in London calculated from their studies that women withlow levels of Vitamin D in their breast tissue have a 354 per cent greater risk ofbreast cancer, this means they have 4 and a half times the breast cancer risk.Cancer patients taking chemotherapy should therefore consider monitoring their vitamin Dstatus throughout the treatment period as well as aggressive supplementation to maintain adequate levels which areassociated with a better prognosis.

Inflammation plays an important role in tumorigenesis. Studieshave indicated that Vitamin D exhibits anti-inflammatory effectswithin TME to inhibit cancer initiation and progression.Moreover, inflammatory factors including TNF-α,IL-6 and IL-8 were significantly inhibited by 1,25(OH)2D3 inprostate primary epithelial cells, indicating the beneficial role ofanti-inflammatory action of vitamin D in prostate cancer. Aboveall, vitamin D exerts anti-inflammatory effects through suppressionof the production and action of inflammatory mediators such ascytokines, chemokines and PGs, and inhibition of MAPK and NFκB signalings in cancer cells, macrophages as well as the epithelial cells, all of which might contribute to the prevention of cancerprogression and inflammatory progress.Vitamin D is a multifunctional precursor of the potent steroidal hormone calcitriol (1α,25-dihydroxyvitamin D3, 1,25(OH)2D3). As most foods contain little vitamin D, there are certain diseases which need vitamin D as a dietary supplement to replenish the deficiency, especially for the elderly and children to maintain adequate Vitamin D store for bone health and autoimmunity.

Regarding the important role of TME(tumour micro environment) in cancer initiation, progression, metastasis andrecurrence, Vitamin D might be used as a therapeutic agent targeting the TME to assist clinical treatment and prognosis for many kinds ofcancer.Inflammation plays an important role in tumorigenesis. Studies have indicated that vitamin D exhibits anti-inflammatory effects within TME to inhibit cancer initiation and progression. Before receiving any cancer-directed therapy, over two-thirds of adult cancer patients have a deficiency in Vitamin D. Women and patients with cancer of the upper gastrointestinal tract are at a higher risk.[23]

Thus, in our study we attempted to monitor whether concurrent administration of Vitamin D along with the chemotherapy had influence on the absolute cell counts and found that Vitamin D definitely had an impact on the ANC, ALC, AECand AMCparameters majorly among the breast, colorectal and gastric cancers.

**Conclusion:**

Overall, a total of 141/160 patients (87%) had deficiency in Vitamin D levels. Vitamin D deficiency was more prevalent among breast (91.3%) and ovary cancers (90%) which is evident that a high prevalence of vitamin D insufficiency is observed in patients with newly diagnosed breast cancer and ovarian cancers. Females were more deficient (90%) when compared to males (84%). Forty-six 46% in Stage III were and 44% in Stage II had vitamin D deficiency. 49% of Stage II and 48% of Stage IV were found to be severely deficient. It is essential to focus on populations that are deficient in Vitamin D and have a high rate of related health issues. While Vitamin D is not a panacea, it can be a valuable and safe supplementary treatment for

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 2655-2669 | ISSN:2251-6727

various diseases and stages of life, such as pregnancy, childhood, and old age. Therefore, public health initiatives should be promoted to prevent severe vitamin D deficiency.

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**Conflicts of interest:**
There are no conflicts of interest.

**REFERENCES:**

1. Feldman, D., Krishnan, A. V., Swami, S., Giovannucci, E., & Feldman, B. J. (2014, April 4). The role of vitamin D in reducing cancer risk and progression. *Nature Reviews Cancer*, *14*(5), 342–357. https://doi.org/10.1038/nrc3691
2. Centers for Disease Control and Prevention. An update on cancer deaths in the United States. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Preven-tion, Division of Cancer Prevention and Control, 2020
3. Amrein, K., Scherkl, M., Hoffmann, M., Neuwe-rsch-Sommeregger, S., Köstenberger, M., Tmava Berisha, A., Martucci, G., Pilz, S., & Malle, O. (2020, January 20). Vitamin D deficiency 2.0: an update on the current status worldwide. *European Journal of Clinical Nutrition*, *74*(11), 1498–1513. https://doi.org/10.1038/s41430-020-0558-y
4. Jeon, S. M., & Shin, E. A. (2018, April). Exploring vitamin D metabolism and function in cancer. *Experimental & Molecular Medicine*, *50*(4), 1–14. https://doi.org/10.1038/s12276-018-0038-9
5. Teramukai S. , Kitano T. , Kishida Y. , Kawahara M. , Kubota K. , Komuta K. et al.. Pretreatment neutrophil count as an independent prognostic factor in advanced non-small-cell lung cancer: an analysis of japan multinational trial organisation lc00-03. European Journal of Cancer 2009;45(11):1950-1958. https://doi.org/10.1016/j.ejca.2009.01.023
6. S, A, Latif., Hamid, Iqbal., Jawad, Zafar., T., Chughtai., Saadat, Parveen., Aayza, Mujahid. Evaluation of Absolute Neutrophil Count, Band Count and Morphological Changes in Neutrophils in Bacterial Infections. Pakistan Journal of Medical and Health Sciences, (2023). doi: 10.53350/pjmhs202317436
7. Nascimento H. , Rocha S. , Rêgo C. , Mansilha H. , Quintanilha A. , Santos-Silva A. et al.. Leukocyte count versus c-reactive protein levels in obese portuguese patients aged 6-12 years old. The Open Biochemistry Journal 2010;4:72-76. https://doi.org/10.2174/1874091x01004010072
8. Akira, Watanabe., N., Harimoto., Kenichiro, Araki., Norio, Kubo., Takamichi, Igarashi., Mariko, Tsukagoshi., Norihiro, Ishii., Takahiro, Yamanaka., Tomoharu, Yoshizumi., Ken, Shirabe. Absolute Neutrophil Count Predicts Postoperative Prognosis in Mass-forming Intrahepatic Cholangiocarcinoma.. Anticancer Research, (2019). doi: 10.21873/ANTICANRES.13197
9. Chang, Ik, Yoon., Chang, Ik, Yoon., Soeun, Park., Yoon, Jin, Cha., Soong, June, Bae., Chihwan, Cha., Da, Young, Lee., Sung, Gwe, Ahn., Joon, Jeong. Associations between absolute neutrophil count and lymphocyte-predominant breast cancer. bioRxiv, (2019). doi: 10.1101/551465
10. Porrata L., Ristow K., Colgan J., Habermann T., Witzig T., Inwards D.et al.. Peripheral blood lymphocyte/monocyte ratio at diagnosis and survival in classical hodgkin's lymphoma. Haematologica 2011;97(2):262-269. https://doi.org/10.3324/haematol.2011.050138
11. Huang J., Li Y., Xia Y., Wang Y., Wei W., Zhu Y.et al.. Prognostic significance of peripheral monocyte count in patients with extranodal natural killer/t-cell lymphoma. BMC Cancer 2013;13(1). https://doi.org/10.1186/1471-2407-13-222
12. Aya, Sawa., Hiroko, Bando., Reina, Kamohara., N, Takeuchi., Azusa, Terasaki., Mai, Okazaki., Akiko, Iguchi-Manaka., Hisato, Hara. Absolute Lymphocyte Count as an Independent Prognostic Factor in Metastatic Breast Cancer: A Retrospective Study. Oncology, (2022). doi: 10.1159/000526963
13. Wen J. , Ye F. , Huang X. , Li S. , Yang L. , Xiao X. et al.. Prognostic significance of preoperative circulating monocyte count in patients with breast cancer. Medicine 2015;94(49):e2266.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2023) 13(6), 2655-2669 | ISSN:2251-6727

https://doi.org/10.1097/md.0000000000002266

14. Poorana, B., S., Chidambaranathan., P, K, Kaviarasan. Serum IgE and absolute eosinophil count-A valuable serological markers in childhood urticaria. IP Indian journal of clinical and experimental dermatology, (2023). doi: 10.18231/j.ijced.2023.006
15. Kunal, Gaur., S., Natarajan., Mohinī, Guptā. Importance of absolute eosinophil count in covid-19 patients. Journal of diagnostic pathology and oncology, (2022). doi: 10.18231/j.jdpo.2022.041
16. Muhammad, Naveed., Malik, Nadeem, Azam, Khan., Altaf, Hussain., Muhammad, Shahid, Akhtar, Khan., Muhammad, Zahid, Hussain. Association of Absolute Eosinophil Count and Post-Bronchodilator Reversibility in the Chronic Obstructive Pulmonary Disease Patients. Pakistan Armed Forces Medical Journal, (2023). doi: 10.51253/pafmj.v73i2.4546
17. Enrico, Caliman., Sara, Fancelli., Carlotta, Ottanelli., Francesca, Mazzoni., Luca, Paglialunga., Daniele, Lavacchi., Marta, Rita, Gatta, Michelet., Elisa, Giommoni., Brunella, Napolitano., F., Scolari., Luca, Voltolini., Camilla, E., Comin., Serena, Pillozzi., Lorenzo, Antonuzzo. Absolute eosinophil count predicts clinical outcomes and toxicity in non-small cell lung cancer patients treated with immunotherapy.. Cancer treatment and research communications, (2022). doi: 10.1016/j.ctarc.2022.100603
18. Yun, Li., C, Shang., Y., Ren., Li, Wang., Jie, Li., W, Xu. P1118: prognostic significance of absolute monocyte count and lymphocyte to monocyte ratio in mucosa-associated lymphoid tissue (malt) lymphoma. HemaSphere, (2022). doi: 10.1097/01.HS9.0000847340.31656.2a
19. Yu-Qiong, Yang., Yu-Qiong, Yang., Jin-Hua, Liang., Jia-Zhu, Wu., Li, Wang., Xiaoyan, Qu., Lei, Cao., Xiaoli, Zhao., Dong-Ping, Huang., Lei, Fan., Jianyong, Li., Wei, Xu. Elevated absolute monocyte count predicts unfavorable outcomes in patients with angioimmunoblastic T-cell lymphoma..Leukemia Research, (2016). doi: 10.1016/J.LEUKRES.2015.12.010
20. Lihui, Han., Yibin, Jia., Qingxu, Song., Nana, Wang., Jin-Xing, Wang., B, Bai., Xiu-Lan, Chen., Chao, Wang., Y., Cheng. Prognostic significance of preoperative absolute peripheral monocyte count in esophageal squamous cell carcinoma. Diseases of The Esophagus, (2016). doi: 10.1111/DOTE.12401
21. Li N. , Zhang L. , Song H. , Zhang J. , Weng H. , & Zou L.. Prognostic impact of absolute lymphocyte count/absolute monocyte count ratio and prognostic score in patients with nasal-type, extranodal natural killer/t-cell lymphoma. Tumor Biology 2017;39(5):101042831770550. https://doi.org/10.1177/1010428317705503
22. Hayashi T. , Fujita K. , Nojima S. , Hayashi Y. , Nakano K. , Ishizuya Y. et al.. Peripheral blood monocyte count reflecting tumor-infiltrating macrophages is a predictive factor of adverse pathology in radical prostatectomy specimens. The Prostate 2017;77(14):1383-1388. https://doi.org/10.1002/pros.23398
23. Pandey, A., Singh, A., & Singh, S. (2020). Prevalence of Vitamin D deficiency in treatment-naive individual consecutive cancer patients. *Cancer Research, Statistics, and Treatment*, *3*(1), 25. https://doi.org/10.4103/crst.crst_113_19