

Leucopenia with HHV6 in Cancer Patient

Receiving Chemotherapy

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Abstract:

The aim to study the relationship between leucopenia in cancer patients undergoing chemotherapy that infected with HHV6. The study was conducted according to a cross sectional study and included the study population, the total number of 200 cases, the age of individuals between 1-95 years in Middle Euphrates Cancer Center in Najaf Governorate for the period between November 2020 to May 2021. Whole blood sample to investigate the leucopenia through a CBC test and serum sample to detect HHV6 Ag as well as anti-viral IgM, IgG antibody using ELISA The comparison included statistical analysis between positive and negative HHV6 IgM, HHV6 IgG, and HHV6 antigen in the two groups using the chi-sequer method. Our study included patients suffering from leucopenia, where the highest age group was (61-80), which gave (31,7%) cases for male. As for females, the highest age group (41-60) was given (37.2%). The study included three degrees of leucopenia (mild, moderate, and severe). The number of mild patients was (23%), the number of moderate patients was (24%), and the number of severe patients was (53%). The ELISA test aims to diagnose IgM, IgG, HHV6. The antibodies to HHV6-IgM were positive in cancer patients with leucopenia (53%), while antibodies to HHV6-IgM positive in cancer patients without leucopenia were (12%).

The IgG antibody screening in cancer patients with leucopenia revealed that (61%) had positive results for HHV6-IgG antibodies. The same test was done for the second group. The test HHV6-IgG antibodies positive (15%) out of 100 samples. The HHV6-Ag was positive in cancer patients with leucopenia in (51%), While the HHV6-Ag was positive in cancer patients without leucopenia (11%). A statistical comparison was carried out between the research groups: the first group consisting of cancer patients with leucopenia and the second group of cancer patients without leucopenia. There are significant differences in the results between the first and second groups, as

well as significant differences in the positive HHV6 IgM, HHV6 IgG, HHV6 antigen results between the first and second groups, as well as significant differences in the negative results between these two groups (P value < 0.5).

Keyword: HHV6, leucopenia, ELISA, IgM, IgG.

INTRODUCTION:

Leucopenia is a condition that results from a decrease in the number of WBCs in the peripheral blood. It manifests itself clinically as weariness, dizziness, appetite loss, and other non-specific, as well as recurrent infection [1]. This is a common occurrence at specific stages of some infectious diseases, and it is indicating a breakdown of one of the body's defense mechanisms [2]. This can result in fatigue and symptom combinations, as well as an increased risk of infection, which can be deadly. Leucopenia can cause cancer treatment to be delayed or decreased, perhaps resulting in subtherapeutic levels and treatment failure [3].

Chemoradiotherapy most often cause myelosuppression and leucopenia [4]. Antimicrobials, analgesics, anti-inflammatories, antihistamines, and anticonvulsants, among others, cause leukopenia by destroying normal bone marrow or lowering white blood cell counts [6]. Autoimmune diseases such as SLE and RA contribute to leucopenia by destroying healthy white blood cells (WBCs) [6]. Additionally, it was demonstrated that WBCs are insufficient in bone marrow diseases such as leukemia, vitamin B12 or folate deficiency, myelofibrosis, myelodysplastic syndrome, and aplastic anemia. On the other hand, virus infection can temporarily impair BM function, resulting in leucopenia [7].

Chemotherapy-induced myelosuppression, particularly leucopenia or neutropenia, is the most common adverse effect of cancer treatment. Chemotherapy-induced leucopenia can result in serious infections and anemia, which can disrupt treatment schedules by delaying chemotherapy. Leucopenia and its associated complications are clinically significant problems that have a significant impact on the efficacy of chemotherapy and the patient's quality of life [8]. Leucopenia can be caused by a variety of conditions, including Colorado tick fever, meningococcemia, pneumococcal pneumonia, and salmonellosis. Leucopenia also be caused by malnutrition [9].

Sarcoidosis can affect the bone marrow and result in fever, anemia, and leucopenia [1] (Kalajian *et al.*, 2009).

Human herpesvirus 6 is a double-stranded DNA virus that was initially identified in immunocompromised patients with lymphoproliferative disorders. [10]. The salivary glands in humans, they have been identified as a reservoir for HHV6 infection. According to studies [11], HHV-6 is highly infectious to T cells. Human herpesvirus 6 is mostly found in humans. While they can infect a wide number of cell types. Herpesviruses have evolved a variety of strategies for ensuring their survival in latently infected cells and evading host immunity during successful reproduction [12]. Saliva is the principal reservoir for virus transmission. HHV-6 has been identified following organ donation, despite there being no examples of virus transmission through blood transfusions or nursing. [13]. Primary HHV6 infection is most common in infants and children under the age of six months, with fever-induced seizures being a major cause in children aged six to 24 months. Acute HHV-6 infection is uncommon in immunocompetent people, but it can cause a mononucleosis-like illness with fever, leucopenia, and hepatitis. [14].

HHV-6, like other herpesviruses, infects a wide variety of cells and then goes dormant [15]. HHV-6, unlike other herpesviruses, can be chromosomally integrated, which is the preferred form of vertical transmission, despite the fact that it only affects about 1% of newly infected people [16]. The HHV-6 (ciHHV-6) virus, which they received from their parents, is expected to harm 1% to 2% of the general population [17]. When diagnosing HHV-6 encephalitis, chromosomally integrated HHV-6 has the potential to significantly complicate a clinician's ability to interpret laboratory results. Because ciHHV-6 DNA can be found in all leukocytes, whole-blood polymerase chain reaction (PCR) detection is practically certain in these patients [18].

The most well-known clinical condition in adults is HHV6 encephalitis, which exclusively affects those who are highly immunocompromised, such as HSCT or SOT recipients. Despite being the most dreaded HHV6 infection consequence, it is still very uncommon. In a retrospective study of HSCT users in 2019, the Mayo Clinic observed a 1.7 % (9/531) incidence of HHV-6 encephalitis. While the incidence was low, these patients had a 50% mortality rate and a high proportion of long-term neurologic deficits among those who survived [19].

In addition, the literature is divided on the relevance of HHV-6 in critically sick patients who do not have baseline immunosuppression. HHV6 DNA has been identified in the blood of people who are very sick but don't show any symptoms of the virus. HHV-6 DNA has been found in more than a quarter of septic shock patients, according to certain investigations. The relationship has not been proven to reactivate and is thought to contribute to a poor prognosis in these people [20]. Idiopathic pneumonitis and hepatitis have also been linked to HHV-6. There is inadequate evidence to suggest pathophysiologic causality in the majority of cases when a link between HHV-6 and other clinical entities (e.g., multiple sclerosis, pityriasis rosea) has been discovered [21].

Diagnosis of leukocyte and its differential count, which is usually performed as part of a complete blood count, or CBC, is necessary for the accurate diagnosis of quantitative leukocyte disorders. In the event of leukopenia, the total leukocyte count will decrease [22].

Methods

Patients: The study population included patients (cross-sectional study). Blood samples (200 cases) at the Middle Euphrates Center for Cancer. A serum sample was collected from a cancer patient who was undergoing chemotherapy. were collected (period between November, 2020 to May, 2021). Of 200 cases, there were 111 females and 89 males, and the patients' age ranged from 1-95 years. where the individuals were divided into two groups: first, included 100 cancer patients with leucopenia the second group consisted of 100 cancer patients without leucopenia including 59 % females and 41% males, while the second group was 52% females and 48% males from cancer patients without leucopenia >

Ethical Approval: Samples of blood were taken from the individuals enrolled in this study after obtaining the oral consent of them. The acceptance of the study protocol has been made by the ethical committee.

Inclusion Criteria: Cancer patient who undergo chemotherapy with leucopenia and cancer patient without leucopenia

Study Parameters: All suspected Cancer patients undergo chemotherapy subjected to the following:

Samples Collection: The samples that obtained from patients were divided into two types of tubes: gel serum and EDTA tubes. The whole blood samples (1ml) that transferred to EDTA tubes were mixed well for several times for CBC test. Serum samples: prepared by allowing blood samples (4 ml) to be clotted at room temperature for about one hr. After that, centrifugation for 15 min at 3000 rpm, the serum was separated in a new plain tube for immunological test (HHV6 Ags, HHV6_IgM and IgG)

Hematological methodology: By detecting and measuring variations in electrical impedance in conductive liquid, the impedance method (coulter-method) sizes and counts cells (Elite3 hematology analyzerv/ Germany).

Serological technique (ELISA): The serum samples of patients were obtained for screening of the presence of HHV6 Ag, HHV6_IgM and IgG antibodies by ELISA using commercial kits (SUNLOMG_CHINA).

Statistical Analysis: The application of analysis to determine the statistical significance of the data included the Chi-square test, P value of <0.05. It depends on SPSS 24.

Results:

Gender distribution of leucopenia disease in overall patients according to age groups

Our current study included 100 patients suffering from leucopenia. 41 males (41%) and 59 females (59%), where the highest age group was (61-80), which gave 13 (31,7%) cases, followed by the age group (41 – 60), which gave 12 cases. (29.2%) of the total number of males. As for females, the age group was given (41_60). 22 cases (37.2 %), followed by the age group (21 – 40), including 16 cases (27.1%).

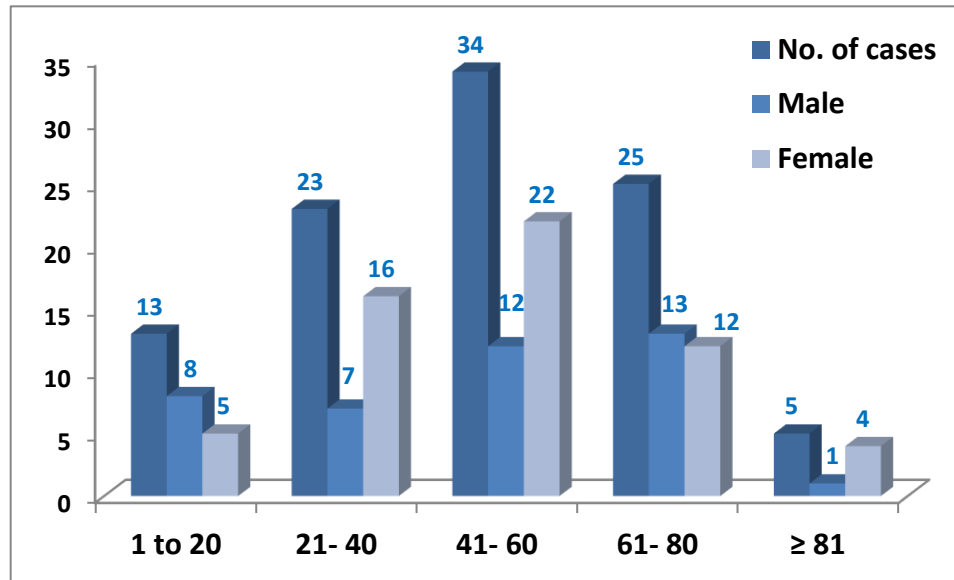


Figure (1): gender distribution of leucopenia disease in overall patients according to age groups chi_square is 5.9479. p value is .653064. The difference is *not* significant (p -value >0.05).

Degree of Leucopenia in cancer patients

The current study included three degrees of leucopenia (mild, moderate, and severe). The number of mild patients was 23 (23%), the number of moderate patients was 24 (24%), and the number of severe patients was 53 (53%).

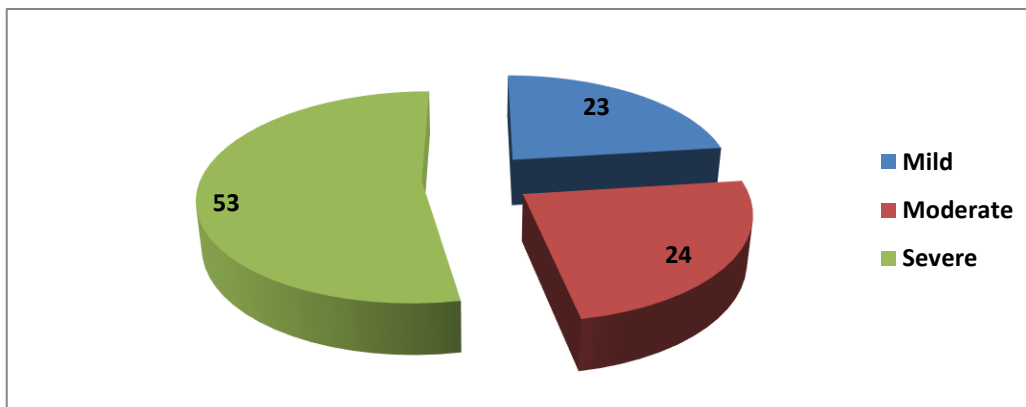


Figure (2): Leucopenia degrees in cancer patients

The degree of Leucopenia in cancer patients according to age groups

The three degrees of leucopenia were divided depending on the age groups adopted in the study, where the age group (41_60) was the highest in mild leucopenia, as it gave out of 22 patients 10 patients (45.4%), followed by the age group (61_80), which gave 9 patients (40.9%). The moderate leucopenia showed that the highest age group was (21_40), as it gave 10 patients out of 26 patients (38.4%), followed by the age group (41_60), which gave 7 patients (26.9%). For the severe leucopenia, the age group (41-60) was the highest, giving 17 out of a total of 52 patients (32.6%).

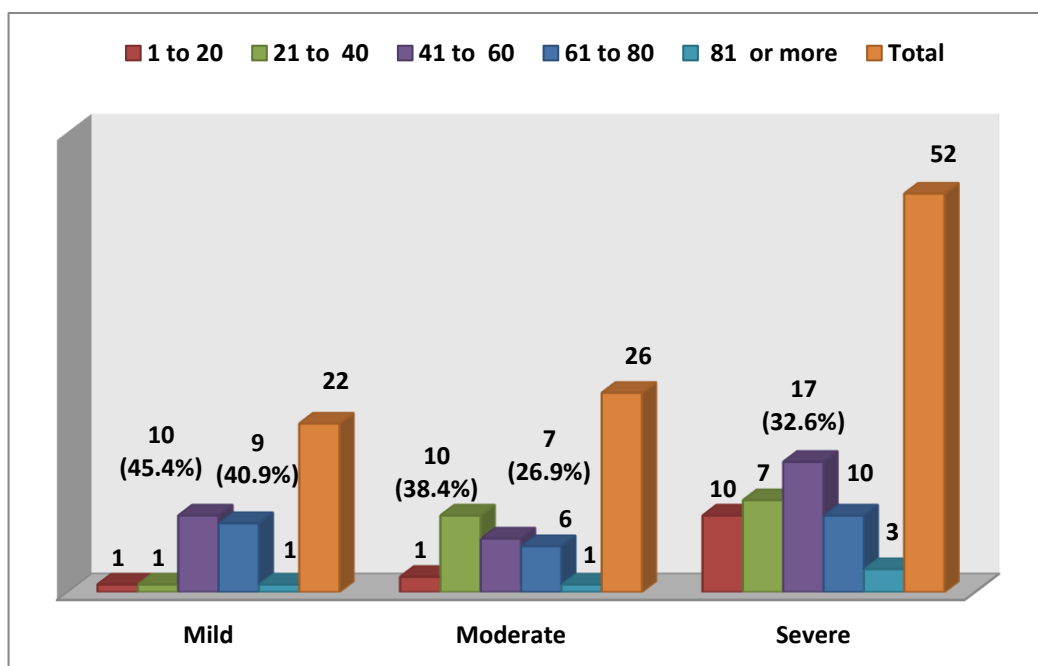


Figure (3): The degrees of leucopenia in cancer patients according to age groups. Chi-square is 13.6422. *P*-value is .324139. There is *no* significant result at $p > 0.05$.

A statistical comparison of IgM results between the first group (with lymphocytopenia and leukopenia) and the second group (without lymphocytopenia and leukopenia) by using the chi_ square method

A statistical comparison was carried out between the research groups: the first group consisting of cancer patients with leucopenia and the second group of cancer patients without leucopenia. The comparison included statistical analysis between positive and negative IgM results in the two groups using the chi-square method.

There are currently significant differences in the results between the first and second groups, as well as significant differences in the positive IgM results between the first and second groups, as well as large differences in the negative results between the first and second groups.

Table (1): A statistical comparison of IgM results between the first group (with leucopenia and the second group (without leucopenia) by using the chi_ square method.

Cancer patient	No. of cases	IgM+	IgM -	Row Totals
Leucopenia	100	53(53%)	47 (47 %0	200
without leucopenia	100	12 (12 %)	88 (88 %)	200
Column Totals	200	65	135	400 (the Grand Total)

A statistical comparison of IgG results between the first group (with Leucopenia) and the second group (without Leucopenia) by using the chi_ square method.

A statistical comparison was carried out between the research groups: the first group consisting of cancer patients with leucopenia and the second group of cancer patients without leucopenia.

The comparison included statistical analysis between positive and negative IgG results in the two groups using the chi- square method. There are currently significant differences in the results between the first and second groups, as well as significant differences in the positive IgG results between the first and second groups, as well as large differences in the negative results between the first and second groups.

Table (2): A statistical comparison of IgG results between first group (with leucopenia) and the second group (without Leucopenia) by using the chi_ square method.

Cancer patient	No. of cases	IgG+	IgG -	Row Totals
Leucopenia	100	61 (61%)	39 (39%)	200
without Leucopenia	100	15 (15%)	85 (85%)	200
Column Totals	200	76	124	400 (Grand Total)

Chi square is 44.9066. The p value is < 0.00001 . The difference is *significant* (p -value < 0.05 .).

A statistical comparison of HHV6 Ag results between the first group (with Leucopenia) and the second group (without Leucopenia) by using the chi_ square method.

A statistical comparison was carried out between the research groups: the first group consisting of cancer patients with leucopenia and the second group of cancer patients without leucopenia. The comparison included statistical analysis between positive and negative HHV6Ag results in the two groups using the chi- square method. There are currently significant differences in the results between the first and second groups, as well as significant differences in the positive HHV-6 results between the first and second groups, as well as large differences in the negative results between the first and second groups.

Table 3. A statistical comparison of HHV6 Ag results between the first group (with Leucopenia) and the second group (without Leucopenia) by using the chi_ square method.

Cancer patient	No. of cases	HHV6-Ags +	HHV6-Ags -	Row Totals
Leucopenia	100	51 (51%)	49 (49%)	200
Without Leucopenia	100	11 (11%)	89 (89%)	200
Column Totals	200	62	138	400 (Grand Total)

Chi square is 37.4007. p value is < 0.00001 . The difference is *significant* (p -value < 0.05 .).

Discussion:

The axes of the study were: the distribution of disease and patients in terms of gender and age groups to the degree of leucopenia. The second axis is statistical comparisons between the two groups using chi. Square. Cancer patients in the current study were distributed, depending on gender, into the first group suffering from leucopenia, and they were divided according to the age groups approved in the study.

The study showed in general that the number of females constituted 59%, while with regard to males, there were 41%, in which (61-80) and then followed by (41-60) were the highest in leukopenia cases that revealed 31.7% and 29.2% respectively in males.

The reason here may be due to age plays a role in the presence of some diseases, especially cancerous diseases and also perhaps these age groups represent the largest number in the study group, so they gave a high percentage in the event that the last age group >80 gave a lower percentage due to the lack of cases.

As for females, the age group (60-41) was the largest in the number of cases, 37.2%, followed by the age group (21-40), which gave 27.1%. It is noticeable in the current study that there are no significant differences between males and females in terms of the number of cases or age groups that suffer from the disease, which indicates that both sexes are exposed to these diseases, as well as most age groups, are also exposed to the occurrence of the disease.

The occurrence of these diseases, therefore, may be due to other reasons, such as genetic, environmental, immune, psychological, and nutritional factors for patients The current study also included the division of cases of leucopenia into three degrees: mild, moderate, and severe, depending on the results obtained.

[23] Found that amplified neutrophils, reduction in the both lymphocytes and monocytes, and a raise in N/L in circulating blood were observed in stomach cancer patients after total or subtotal gastrectomy. The changes in these principles were mainly noticeable in the direct postoperative stage and tend to be restore as time passed in both gender during the postoperative period. In this study's large sample size, we establish considerable gender effects on changes in the proportion of blood WBC subsets subsequent surgery. For a few days female patients exhibited after surgery, circulating neutrophils more, fewer lymphocytes, resulting in greater N/L, and fewer monocytes, indicating the condition of an immune-compromised become further, than male

patients. Except for the entire amount of WBC and the proportion of monocytes, there were no important variations in preoperative values between sexes.

Where this division was relied upon according to the approved medical and scientific criteria, and it is inferred that patients suffer from the development of leucopenia, either for pathological or immune reasons, On the other hand, because of the toxic effects of chemotherapy for cancer patients due to the damage that occurs in the bone marrow, which leads to a reduction in the production of leukocyte. Alternatively, a defect in the post-production stages, such as the stages of synthesis, differentiation, and others.

The current study included three groups of leucopenia (mild, moderate, and severe). The number of mild patients was (23%), the number of moderate patients was (24%), and the number of severe patients was (53%). We find that more than half of the cancer patients who undergo chemotherapy in this study group may suffer from leukopenia as a result of the effects of chemotherapy directly or indirectly, such as the toxic effect on the bone marrow and its other effects on the activity of white cells. In addition to other immune or inflammatory factors, or the percentage of viral and microbial infections. In addition, the degrees of leukopenia were distributed depending on the age groups studied the relationship between the degrees and levels of leucopenia and the number of patients, where the age group (41_60) was the highest in mild leucopenia (45.4%)], followed by the age group (61_80), which gave (40.9%). The moderate leucopenia showed that the highest age group was (21_40), as it gave (38.4%), followed by the age group (41_60), which gave (26.9%). For the severe leucopenia, the age group (41-60) was the highest, giving (32.6%). We found that although age may play an important role in leukopenia degrees, the current distribution might not indicate a direct relationship between age and cases of leukopenia, and there may be a relationship between the number of chemotherapy doses and the severity of cancer in patients that play an important role in severity. Leukopenia is exacerbated regardless of the patient's age.

[24] indicated Cyclophosphamide is a widespread cancer chemo-therapy treatment, but the immunosuppression associated with it results in leukopenia.[25,26] (Huang *et al.*, 2017.; Sun *et al.*, 2017) indicated cyclophosphamide can simply inhibit normal hematopoietic cell division and proliferation, resulting in impaired restitution or harm to hematopoietic organization, decreased

nucleated cells, and decreased hematopoietic reconstitution activity. Leukopenia is by far the most frequently observed adverse effect. [27]. It was indicated that cyclophosphamide was used to induce leukopenia in cancer rodents in order to mimic clinical and pathological physiology.

[28] found that total of 445 patients aged 18 to 80 years were studied, with four to ten periods of chemotherapy established histologically and cytologically. They discovered that 62 of the 85 people who died as a result of a serious infection had severe leukopenia. Mild leukopenia is strongly associated with a greater chance to survive. And demonstrated that female patients were more likely to develop leukopenia.

[29] Indicated that vinorelbine pharmacokinetics chemotherapy is linked not only to body surface area, but also to body mass, serum creatinine, platelet count, and sex.[30] revealed that females had considerably more apparent hematologic toxicity than males did.[31] Reported that higher frequency of hematologic toxicity in women who have a higher baseline body mass index (BMI) than in males, which may alter the delivery of cytotoxic drugs and raise possible toxicity.[32] indicated that ADR-induced lymphocytopenia could be the result of the destruction of lymphocyte precursors and mature lymphocyte populations, resulting in immunosuppression.

As for talking about the division of leukopenia into degrees or levels in terms of the severity of the disease. A statistical comparison was made between the research groups: the first group consisted of cancer patients with leukopenia and the second group of cancer patients without leukopenia. The comparison included statistical analysis between the results of the four parameters IgM, IgG, HHV6 Ag, positive and negative in the two groups using chi-square method. There are currently significant differences in the results between the first and second groups, as well as significant differences in the positive IgM results between the first and second groups, as well as large differences in the negative results between the first and second groups.

This indicates that cancer patients who have decreased immunity because of receiving chemotherapy in large quantities are more likely to have new HHV6 Ag virus infection than the second group that does not suffer from immunodeficiency, or by another explanation, that viral infection has caused a decrease in immunity, and thus the number of infections in cancer patients who suffer from a lack white blood cells is much more than those who do not suffer from a lack of white blood cells.

There are significant differences in the results between the first and second groups, as well as significant differences in the positive IgG results between the first and second groups, as well as large differences in the negative results between the first and second groups.

This indicates that cancer patients who have a low number of lymphocytes and white blood cells as a result of receiving chemotherapy in large quantities are more susceptible to virus reactivation than the second group that does not suffer from lymphocytopenia and leukopenia. As we know that HHV6 Ag has the ability to chromosome integration, and that decreased immunity is one of the important factors that lead to the reactivation of this virus. The result is significant between the first and second groups, as well as significant differences in the positive HHV-6 Ag results between the first and second groups, as well as large differences in the negative results between the first and second groups.

This indicates that cancer patients with leukopenia as a result of receiving large amounts of chemotherapy are more likely to be infected with HHV6 Ag than the group without leukopenia. Or in another explanation, that the viral infection caused leukopenia, and therefore the number of infections in cancer patients with leukopenia is much greater than in those without leukopenia.

HHV-6 is only found in certain types of cancer, but its presence in tumor cells is insufficient to attribute a direct role to the virus in tumorigenesis. Cancer viruses are implicated as causative agents in a large proportion of cases, due to their presence in the majority of tumor cells and virus-induced in vitro cell transformation. This virus, while not directly oncogenic, could indirectly promote tumor cell growth, in some cases by collaborating with other viruses and other factors. HHV-6 could also be an opportunistic virus that thrives in an immunodeficient tumor microenvironment, according to some researchers.

In spite of published studies, it is still too early to conclude that HHV-6 is responsible for several human cancers. As a result of some evidence, it appears that HHV-6 may work in concert with other viruses to cause cancer, such as HPV and EBV; HHV-6 has also been implicated in nodular sclerosis, Hodgkin lymphoma, gastro-intestinal tumors and oral cancers. HHV-6's exact role in tumor growth, however, will require further investigation. As outlined by [33,34]. Chemotherapy.

Cocclusions:

Studies have demonstrated an increase in the frequency of HHV-6 after chemotherapy, both during the course of treatment and at the end of it, which confirms that the virus is reactivated after.

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