

Intestinal parasitic infections among leukemia patients after chemotherapy and estimation of Interleukin-2 (IL-2) level

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ABSTRACT

Background: Intestinal parasites are common cause of infectious disease in leukemia patient receiving anticancer therapy, additionally, immunosuppression therapy in such patients may exacerbate the symptoms related to these parasitic infection.

Objectives: The current study aimed to detect the prevalence of intestinal parasite infection in leukemia patients after chemotherapy and assess the levels of IL-2 in those patients.

Methods: 100 stool and serum samples were collected from leukemia patients after receiving chemotherapy from (1-79 years) ages and both sexes. And 25 samples from healthy individuals as a control group. In this cross-sectional study, samples were collected from September 2023 to March 2024 at Kirkuk Oncology and Hematology center. The method used was the direct smear and concentration method (sedimentation by formal ether and flotation by using a saturated salt solution) for the investigation of intestinal parasites and the Sandwich Enzyme-Linked Immunosorbent Assay (Sandwich-ELISA) technique for the determination of level in serum.

Results: The results showed that the total infection percentage of intestinal parasites was 28% in leukemia patients. Five species of intestinal parasites were recorded: Entamoeba histolytica (50%), Blastocystis hominis (21.42%), Entrobium vermicularis (14.28%), and (7.14%) patients infected with Giardia lamblia and Ascaris lumbricoides. The percentage of infection in females was higher than that of males, and higher prevalence of IPI was in (1-9) age group. The statistical analysis showed significant differences ($P < 0.05$) between the percentage of intestinal parasitic infection with age and type of leukemia in leukemia patients, while no significant differences ($p > 0.05$) between the percentage of intestinal parasite and sex.

The results showed a significant increase ($P < 0.05$) in IL-2 in leukemia patients after chemotherapy treatment infected with intestinal parasites compared to non-infected patients.

Conclusion: The findings of this study indicated that intestinal parasitic infections are still a public health problem specially in immunocompromised leukemia patients in Kirkuk City, Iraq

Keywords: Oncology, Kirkuk, parasite, Hematology.

INTRODUCTION

Intestinal parasites are organisms that live in the digestive system of humans and other hosts. The diseases they cause are known as intestinal parasitic infections. The prevalence of intestinal parasitic infections (IPI) is high all around the world, and it remains a significant cause of morbidity and mortality in underdeveloped countries, particularly in public health [1]. Protozoa and Helminths are two common categories of intestinal parasites living in humans' small and large intestines [2]. Intestinal parasite infections represent significant public and socioeconomic problems that adversely impact the health and survival of the impoverished in developing countries [3]. Over fifty percent of the world's population is estimated to experience pain, discomfort, and substantial financial hardship as a result of parasitosis [2]. Most parasitic infections are concentrated in developing countries, particularly sub-Saharan Africa, due to inadequate water supply, poor environmental sanitation, rapid population growth, and other economic and social challenges [4]. It is important to note that many common intestinal parasitic infections in humans are acquired through fecal-oral transmission; this can happen directly through hand-to-oral contact or indirectly through contaminated food and water [5]. Annually, more than 10.5 million new cases of intestinal parasites are reported. The most common parasites include Ascaris lumbricoides, hookworms, Trichuris trichiura, Entrobium vermicularis, Cryptosporidium parvum, Blastocystis hominis, Giardia lamblia, Entamoeba histolytica, and Schistosoma species [6].

Leukemia is a group of hematological malignancies caused by the rapid and high production of abnormal white blood cells; because these abnormal white blood cells take up space and are ineffective immune cells, they eventually inhibit the bone marrow's capacity to create enough platelets, red blood cells, and functional white blood cells[7]. leukemia constitutes 2.5% of all new cancer cases and 3.1% of global cancer mortality[8]. Leukemia is categorized into four primary subgroups: acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), and chronic lymphoblastic leukemia (CLL). Leukemia, especially acute leukemia (AL), is a highly prevalent malignant tumor characterized by a life-threatening condition[9]. Within the population of cancer patients, specifically those with leukemia, the immunosuppressive effects caused by the illness and/ or the anticancer therapy used increase susceptibility to intestinal parasite infections, especially opportunistic parasites[10]. However, immunocompromised leukemic individuals fail to eliminate parasites upon exposure, resulting in a more severe or widespread infection[11]. Parasitic infections present problems to patient health due to the life-threatening chronic diarrhea and severe clinical signs they produce in immunocompromised patients. However, the routine detection of these infections is frequently ignored during chemotherapy or illness management [12].

Interleukin 2 (IL-2) is a 15.5 kDa cytokine composed of four α -helix bundles[13]. It is primarily produced by CD4+ T cells following antigen stimulation and a smaller amount by CD8+ cells, natural killer T cells, mast cells, monocytes, and myeloid dendritic (mDCs) cells. Pleiotropic reactions in the immune system occur when IL-2 acts as a protein, which regulates the pro- and anti-inflammatory processes (modulates both innate and adaptive immune responses) that has immunostimulatory or immunoinhibitory action depending on the specific target cell[14]. Alteration in normal IL-2 signaling or expression can lead to leukemia development; however, IL-2 itself is not the primary etiology of the illness[14]. IL-2/IL-2R can promote the survival and proliferation of leukemia cells by providing growth signals, but the specific effects may vary depending on the type of leukemia[15].

2. METHODOLOGY

Study population

A total of 100 serum and stool samples were collected from leukemia patients after receiving chemotherapy, from different ages and both sexes (male&female), and 25 samples from healthy people as a control. Samples were collected from the 1st of September 2023 to the 1st of March 2024 at Kirkuk Oncology and Hematology center.

Sample collection

One stool sample was taken from each study participant (leukemia patients and control group) using a sterile, dry, and clean stool cup (container). Macroscopically examined for colour, odor and the presence of blood, mucus, adult worm or proglottid[16]. Serum samples were collected from each leukemia patient and control. Using sterile syringes, 5 ml of venous blood was obtained and placed into sterile gel tubes after coagulation at room temperature; the blood tubes were centrifuged for 5–10 minutes at 3000 rpm to separate the blood serum. Then, the serum was placed into Eppendorf tubes and kept in a deep freezer (-20 C) in the laboratory until the time of work.

Serological Tests

The stool sample was examined microscopically after proceeding fresh stool sample by three methods: direct wet mount (saline and iodine preparation), sedimentation by Formal-Ethyl Acetate techniques[17] and flotation method[18]. The Sandwich enzyme-linked immunosorbent assay (ELISA) kit was used for the accurate quantitative detection of Human Interleukin 2 (also known as IL2) in the serum sample, according to the manufacturer's direction (human ELISA, BT LAB).

Statistical analysis

Data were gathered, calculated, tabulated, and statistically analyzed using SPSS, a statistical computer program with ANOVA test and T-test. Duncan's multiple range test was used to compare the differences between means under the probability level 0.05 ($P < 0.05$).

Ethical approval

The research was carried out according to the ethical principles outlined in the Helsinki Declaration. Approval was obtained from the Directorate of Health in Kirkuk before taking the sample. A local ethics committee reviewed and approved the study protocol, subject information, and consent form using document number 604 (including the number dated September 20, 2023) to obtain this approval.

3. RESULTS AND DISCUSSION

Table (1)show the proportion of intestinal parasite infections among leukemia patient after chemotherapy treatment in the Kirkuk City and their correlation with epidemiological parameters.125 clinical samples were included in the study,100 samples from leukemia patients after receiving chemotherapy from ages (1-79) and 25 samples from (apparently) healthy controlscollected at Kirkuk Oncology and Hematology Center from the 1st of September 2023 to the 1st of March 2024. the result of the microscopical examination revealed that 28% leukemia patients , the current total percentage of infection was higher than recorded in the other researchers, such as in Turkey[19] in which 91 leukemic patients examined,intestinal parasites were diagnosed in nine (9.9%) patient . In Szczecin, Poland [20] from 50 hematological patients intestinal protozoan was detected in only eight (16%) patients.This may be attributed to the different settings and locations from where the samples were obtained, the sample size, and the duration of the research, potentially influenced by differing living situations, nutrition, health, and climatic and economic factors. In addition to the various age groups, the number of models examined and the procedures used to identify intestinal parasites, such as only using the direct approach without including additional concentration methods examination of faeces by sedimentation or flotation methods[21].

The percentage of intestinal parasitic infection in the females was higher17 (28.8%) than in the males 11 (26.82%).Statistical analysis showed a non-significant difference in the percentage of intestinal parasitic infection between females and malesat $p > 0.05$. These differences are usually attributed to either physiological, usually hormonal in origin, or ecological in humans; the result may be in agreement with another study that provides a novel epidemiological assessment of IPIs in Nepal, indicating the females had a slightly higher prevalence of being infected with parasitic infection as (38.2%)in female and (37.8%) in male[22], and results of study conducted in Babylon, Iraqi[23]the rate of parasitic infection with parasites in female was higher than in males (16.0%).

The findings of the present research reveal the distribution of intestinal parasite infection percentages across various age groups of leukemia patients.It was found that the age group(1-9)years is the age group with the highest percentage ofintestinal parasitic infection(77.8%), Followed by the age group (50-59)years (26.32%), While the lowest rate ofinfection in the age group 70-79 year (14.28%). Statistical analysis showed highly significant differences($p=0.01$) between the age groups in leukemia patients with percentages of intestinal parasitic infection ($p \leq 0.05$).The parasitic infection is more prevalent in younger and older age because their weak immune system, in addition, leukemic patients are predisposed to infection due to immune defects related to the primary disease and as a result of therapy[24]and alsodue to children have increased exposure to the external environment while being in play in proximity to waste sites[25].Result may be in agreement with the result of the study that researched in Turkey, about intestinal parasite infection in leukemic patients, intestinal parasites were diagnosed in nine patients (9.57%) of 91 leukemic patients that highly recorded among patient mean age was (50.9 ± 15.5) [19].The result is also in agreement with the other study conducted in South Khorasan Province, eastern Iran about the prevalence of parasitic infection that reported that nearly half of the students aged 6-11 years were infected with intestinal parasiteswhich may be reported similar finding to our study in which children are the most sensitive group that may be affected by parasites[26]. Additionally, they may also serve as asymptomatic carriers, potentially increasing the risk and causing significant public health problems[26]

According to the types of leukemia there are significant differences ($p < 0.05$) between the parasitic infection and types of leukemia. The higher percentage of 12 (27.91%) of parasites belonged to (ALL) , while the lowest rate of 4 (23.52%) of parasites belonged to (AML) .ALL is the predominant malignancy in pediatric patients[27].Children with underlying malignancies and those receiving chemotherapy have a greater frequency of parasite infections, characterised by more serious complications compared to immunocompetent individuals[28].the our results may be in agreement with the finding of resaserch investigated in Egypt[29] which reported that higher intestinal parasitic infection in ALL .

Table 1: Distribution of intestinal parasitic infection percentage according to epidemiological factors.

variables	Infection		Non-infection		Total		significance
	NO	%	NO	%	NO	%	
gander							
male	11	26.82	30	73.17	41	41.0	(P>0.05)=0.2
female	17	28.8	42	71.1	59	59.0	
Age							
1-9	7	77.8	2	22.2	9	9.0	(P<0.05)=0.01
10-19	4	44.4	5	55.6	9	9.0	
20-29	2	11.1	16	88.9	18	18.0	
30-39	2	22.2	7	77.8	9	9.0	

40-47	3	15	17	85	20	20.0	
50-59	5	26.32	14	73.68	19	19.0	
60-69	4	44.4	5	55.6	9	9.0	
70-79	1	14.28	6	85.7	7	7.0	
Types of leukemia							
ALL	12	27.91	31	72.09	43	43.0	(P<0.05)=0.04
AML	4	23.52	13	76.47	17	17.0	
CLL	7	38.9	11	61.11	18	18.0	
CML	5	22.73	17	77.27	22	22.0	

Table (2) shows the current study revealed five species of intestinal parasites were recorded microscopically: *Entamoeba histolytica* 14 (50%), *Blastocystis hominis* 6 (21.42%), *Entrobium vermicularis* 4 (14.28%), and 2 (7.14%) patients infected with *Giardia lamblia* and *Ascaris lumbricoides*, and 72% of leukemic patients without intestinal parasite infections. The result showed the highest rate of parasite infection was for *E. histolytica* and the lowest percentage for both *Giardia lamblia* and *Ascaris lumbricoides*, which also affects on level of IL-2 in patients with leukemia disease. The frequency of *E. histolytica* is widespread globally, particularly in tropical and subtropical areas like Iraq, and lead to many clinical presentations and conditions, including dysentery, diarrhea, and hepatic liver abscess [30]. The result may disagree with study conducted in Egypt which shows that *B. hominis* was most prevalent [29] while in agreement with [21] the difference in result may be attributed to difference in environment and socio-economical condition that study conducted, and difference in number and size of sample examined.

Table 2: Prevalence of intestinal parasitic infection percentages in leukemia patients after chemotherapy according to species of the parasite (number examined = 100).

Parasite species	No	%
<i>E. histolytica</i>	14	50
<i>B. hominis</i>	6	21.42
<i>E. vermicularis</i>	4	14.28
<i>G. lamblia</i>	2	7.14
<i>A. lumbricoides</i>	2	7.14
Total	28	28.0

Although the role of cytokines in parasitic infections has been widely investigated in animal models, there is limited literature and few clinical works on the importance of Th1 (IL-2) in human parasitic infection. Thus, the present work ELISA system was undertaken to study the levels of cytokine (IL-2) in sera of leukemic patients infected with different types of intestinal parasite infection.

Table 3: Parasite's type effect on serum Human interleukin -2 level

Parasite species	No	Mean of IL-2 (ng/l) ± S E
<i>E. histolytica</i>	14	363.41 ab ±42.54
<i>B. hominis</i>	6	312.9 ab ±42.17
<i>E. vermicularis</i>	4	266.73 ab ±42.53
<i>G. lamblia</i>	2	382.78a ±108.9
<i>A. lumbricoides</i>	2	124.75 b ±94.43
No (IPI) leukemia patient	72	335.8 ab ±30.42
control	25	93.51 c ±2.7

* The same letters mean no significant differences between groups at a potential level ($P \leq 0.05$).

** The different letters mean a significant difference between groups at a potential level ($P \leq 0.05$).

The result of the current study was observed that the level of IL-2 was significantly increased in leukemia patients infected with intestinal parasitic infection when compared with the healthy control group, and statistical analysis shows a highly significant difference ($p < 0.05$) of interleukin-2 level in parasite-infected leukemia patients when compared with the control group, as shown in Table (3). The results of nearly all parasites were higher than the control group (*E.histolytica* 363.4 ± 42.5 ng/l: 93.5 ± 2.7 ng/l control), (*B.hominis* 312.9 ± 42.1 ng/l: 93.5 ± 2.7 ng/l control), (*E.vermicularis* 266.7 ± 42.5 ng/l: 93.5 ± 2.7 ng/l control), (*A.lumbricoides* 124.7 ± 94.4 ng/l: 93.5 ± 2.7 ng/l control) and (*G.lambliia* 382.7 ng/l: 93.5 ± 2.7 ng/l control). The highest concentration of interleukin -2 was shown in *G.lambliia* (382.7 ± 108.9 ng/l) infection when compared with control (93.5 ± 2.7 ng/l). Excretory and secretory antigens produced by the parasite trigger the immune responses, leading to increases in interleukin -2 production; IL-2 is an important marker of immune system activation in living organisms. Its release rate indicates the activation of T- and B-cells and macrophages against parasitic infection. Protein p53 is a tumor suppressor that regulates cell cycle and apoptosis; its mutations are common in leukemia and can lead to altered immune regulation, including changes in IL-2 production. Protein p53 can regulate the expression of IL-2. Under typical conditions, p53 is essential in regulating appropriate immune function, including controlling cytokine synthesis. If p53 is mutated, this regulation can be disrupted, leading to abnormal immune responses, including altered IL-2 levels [31, 32]. The result might be in agreement with studies [33-35], which revealed that there is an association between parasitological infection and an increase in the level of IL-2, in addition to the correlation between leukemia disease and elevation of the rate of IL-2.

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