Potential role of toxoplasmosis to change interleukin 12 level in leukemia patients

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Abstract
Toxoplasma gondii is an opportunist pathogen in which the reactivation of a dormant infection can cause death in immunocompromised patients. The goal of this study is to investigate the possible risk-factor of Toxoplasma infection and its possible correlation with Interleukin-12 (IL-12) cytokine in leukemia patients and according the leukemia types. In this study, 185 patients were enrolled. All serum samples were tested by using enzyme-linked immunosorbent assay (ELISA) technique for the measurement of anti-Toxoplasma (IgG, IgM) antibodies and IL-12 levels. The serological examination revealed that 63 (51.2%) leukemia patients were positive to anti-Toxoplasma IgG compared with the sera of the control group which was 48 (77.4%). Their positivity rates for anti-Toxoplasma IgM in leukemia patients was 3 (2.4%) while it was 2 (3.23%) in the control group. The mean level of IL-12 in leukemia patients infected with toxoplasmosis was the significant 188.865 ±2.845 pg/ml, being higher than the mean value in the positive control which was 85.330±2.834 pg/ml. Furthermore, the seroprevalence of anti-Toxoplasma IgG was the highest in the age group (41-50) years in patients with leukemia. There were no significant differences between age and leukemia patients infected with toxoplasmosis. Since most immunosuppressive patients are exposed to various possible risk factors including Toxoplasma, primary infection or reactivation, so it is important to diagnose and treat toxoplasmosis in leukemia patients to reduce the consequences of this infection.

Keywords: Toxoplasmosis; Leukemia; Interleukin-12

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Introduction
Toxoplasma gondii is an obligate intracellular protozoan parasite with a global distribution in humans and other warm-blooded animals. Transmission to humans occurs through ingestion of T. gondii oocysts shed into the environment by cats, or by eating meat of infected animals. Under normal immune conditions, T. gondii infection is
frequently asymptomatic, but in individuals who are immunocompromised, such as in patients with AIDS, the parasites can become widely disseminated, causing severe toxoplasmosis and encephalitis (Ajioka, 2007).

Leukemia is a group of blood cancers that usually begin in the bone marrow and result in high numbers of abnormal blood cells. These blood cells are not fully developed and are called blasts or leukemia cells. Diagnosis is typically made by blood tests or bone marrow biopsy (National cancer institute, 2014). Leukemia has many types; it has four major types including acute lymphatic (ALL), chronic lymphatic (CLL), acute myeloid (AML), and chronic myeloid leukemia (CML) (Nevo and Tatarsky, 1986). In Iraq, leukemia represents the fourth common cancer in both male and female. Iraqi Cancer Board recorded 13951 cases of leukemia in Iraq in the years from 1991 to 2009 representing 6.59% of new cases (Mjali et al., 2019). The epidemiological relationship among cancer and inflammation has been well established. Many cancers arise at the site of chronic inflammation and inflammatory mediators are always produced in tumors (Coussens and Werb, 2002; Balkwill et al., 2005).

Serum concentrations of anticancer cytokines such as Interleukin-12, a pro-inflammatory cytokine, have been investigated (Floros and Tarhini, 2015). IL-12 is required for antimicrobial responses (Langrish et al., 2004) and plays a major role in resisting Toxoplasma (Lieberman et al., 2004). In patients with cancer, immune function is reduced and this is the major reason for the rise of Toxoplasma antibodies and duration of chemotherapy (Wang et al., 2015). Thus, it is essential to diagnose toxoplasmosis infection in cancer patients to reduce the burden on the immune system when both diseases are present.

Material and Methods

Subjects and Blood Collection

In this study, 185 samples were enrolled from October, 2018 till April, 2019 (14 samples from outpatient clinics as negative control group, 48 samples were taken from outpatient clinics as control groups and 123 samples of patients with leukemia who attended to Baghdad Teaching Hospital in the Medical City Hospital in Baghdad from different governorates in Iraq). Their ages range between 11-50 years. Samples of blood of (5ml) were taken from vein of all patients. The sample was collected in sterilized Gel Clot activator vacuum tubes and left for 30 minutes at room temperature, centrifuged at 3000 rounds per minute (rpm) for 10 minutes and dispensed into Eppendorf- tubes and stored at -20 ºC.

ELISA kits (Acon Toxoplasma IgG ELISA (I231-1091), IgM ELISA (I231-1101) was used to determine the anti- T. gondii antibodies (IgG and IgM). All serum samples then were divided into 4 groups: apparently healthy people, patients with toxoplasmosis, leukemia patients with toxoplasmosis, and leukemia patients without toxoplasmosis. As well as, samples were tested for serum mean titer of IL-12 by using the RayBio® Human IL-12 (P70) ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IL-12 (P70) in serum,
plasma. Chi-square test was used to significant compare between percentage and least significant difference –LSD test was used to significant compare between means in this study.

Results

Serological examination of anti \textit{T. gondii} antibodies IgG and IgM in Leukemia patients and control groups

The result shows that the percentage of seropositive anti-IgG and IgM in leukemia patient was 51.2% and IgM 2.4% respectively, and about half of samples of leukemia were infected with toxoplasmosis.

Table (1) Serological examination of anti \textit{T. gondii} antibodies IgG and IgM in Leukemia patients and control groups

<table>
<thead>
<tr>
<th>Anti-IgG</th>
<th>Control</th>
<th>Leukemia patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO</td>
<td>%</td>
</tr>
<tr>
<td>IgG (+)</td>
<td>48</td>
<td>77.4%</td>
</tr>
<tr>
<td>IgG (-)</td>
<td>14</td>
<td>22.6%</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>100%</td>
</tr>
<tr>
<td>IgM (+)</td>
<td>2</td>
<td>3.23%</td>
</tr>
<tr>
<td>IgM (-)</td>
<td>60</td>
<td>96.77%</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>100%</td>
</tr>
</tbody>
</table>

The mean levels of IgG in different types of Leukemia according to toxoplasmosis

The different type of leukemia that might affect by the level of \textit{T. gondii} IgG in leukemia patients was investigated. The result showed that the higher mean titer of IgG was in CML leukemia type which was (14.60 ±1.348 IU/ml) followed by type AML that is seropositive to anti-\textit{T. gondii} IgG with mean titer (11.32±1.172 IU/ml) and then followed by type ALL that is seropositive to anti-\textit{T. gondii} IgG which was (11.13±2.171 IU/ml) and finally the type NHL that is seropositive to anti-\textit{T. gondii} IgG and the mean titer was (8.05±1.577) with statically significant differences (P ≤ 0.05).
Table (2): The mean levels of IgG in different types of Leukemia according to toxoplasmosis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Toxo (-ve)</th>
<th>Toxo (+ve)</th>
<th>P-Value</th>
<th>Significant P ≤ 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.87 b ± 0.228</td>
<td>8.70 b ± 0.476</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>ALL</td>
<td>0.61 b ± 0.222</td>
<td>11.13 ab ± 2.171</td>
<td>0.001</td>
<td>S</td>
</tr>
<tr>
<td>CLL</td>
<td>1.20 b ± 0.591</td>
<td>9.27 ab ± 1.800</td>
<td>0.001</td>
<td>S</td>
</tr>
<tr>
<td>AML</td>
<td>5.56 a ± 1.725</td>
<td>11.32 ab ± 1.172</td>
<td>0.007</td>
<td>S</td>
</tr>
<tr>
<td>CML</td>
<td>2.30 b ± 0.889</td>
<td>14.60 a ± 1.348</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>NHL</td>
<td>0.90 b ± 0.251</td>
<td>8.05 b ± 1.577</td>
<td>0.001</td>
<td>S</td>
</tr>
<tr>
<td>LSD P≤0.05</td>
<td>2.385</td>
<td>4.291</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean levels of IL-12 in control groups and Leukemia patients according to toxoplasmosis

The mean level of IL-12 in leukemia patients infected with *T. gondii* was (188.865±2.845 pg/ml) which is higher than mean level in control groups infected with toxoplasmosis (147.980±2.714 pg/ml). The differences in the levels of IL-12 among the leukemia patients and control subjects showed significant differences (P ≤ 0.05).

Table (3): The mean level of IL-12 in control groups and Leukemia patients according to toxoplasmosis

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Toxo (-ve)</th>
<th>Toxo (+ve)</th>
<th>P-Value</th>
<th>Significant P ≤ 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.330 ± 2.834</td>
<td>147.980 ± 2.741</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>Leukemia</td>
<td>173.307 ± 1.670</td>
<td>188.865 ± 2.845</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant P≤0.05</td>
<td>S</td>
<td>S</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean levels of IL-12 in different types of Leukemia according to toxoplasmosis

According to the types of leukemia, the highest mean levels of IL-12 in patients infected with toxoplasmosis was in type ALL (203.25±6.704 pg/ml), and low mean level was in type CLL (178.59±6.310 pg/ml). This result is statically significant with P≤ 0.05 in contrast with positive control groups (147.98±2.741 pg/ml).
Table (4): The mean levels of IL-12 in different types of Leukemia according to toxoplasmosis.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Toxo (-ve)</th>
<th>Toxo (+ve)</th>
<th>P-Value</th>
<th>Significant P≤0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.33 d ± 2.834</td>
<td>147.98 d ± 2.741</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>ALL</td>
<td>180.78 a ± 4.442</td>
<td>203.25 a ± 6.704</td>
<td>0.011</td>
<td>S</td>
</tr>
<tr>
<td>CLL</td>
<td>163.44 c ± 2.954</td>
<td>178.59 c ± 6.310</td>
<td>0.041</td>
<td>S</td>
</tr>
<tr>
<td>AML</td>
<td>168.16 bc ± 3.090</td>
<td>186.82 bc ± 4.959</td>
<td>0.004</td>
<td>S</td>
</tr>
<tr>
<td>CML</td>
<td>176.22 ab ± 2.857</td>
<td>195.53 ab ± 7.134</td>
<td>0.020</td>
<td>S</td>
</tr>
<tr>
<td>NHL</td>
<td>172.82 ab ± 2.679</td>
<td>184.38 bc ± 3.713</td>
<td>0.019</td>
<td>S</td>
</tr>
<tr>
<td>LSD P≤0.05</td>
<td>9.204</td>
<td>15.670</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Toxoplasmosis generally is asymptomatic in immunocompetent individuals, while it leads to many pathological effects in immunocompromised patients e.g., people with HIV/AIDS or transplant patients (Tenter et al., 2000; Ahmadpour et al., 2014; Dubey, 2008). Seropositive of anti-*T. gondii* IgG has more prevalence in cancer patients. The *T. gondii* IgG positivity rate was detected in leukemia cancer group (36.0%), in breast cancer group (56.6%), in thyroid cancer group (44.6%) and in rectal cancer group (54.0%) (Molan and Rasheed, 2016). In compare with other study in leukemia patients, the result demonstrated that IgM and IgG anti-*T. gondii* antibodies were 10 (5.9%) and 96 (56.4%), respectively. In line with, control group, result showed IgM and IgG anti-*T. gondii* antibodies were 3(1.8%) and 72 (42.4%). It is in triggering to note the level of IgG and IgM antibodies were consistently moderate higher in leukemia patients than control groups (p<0.05).

This is indicative of possible reactivation of *T. gondii* infection in leukemia patients (Gharavi et al., 2017). Other study revealed that the seropositive anti-*T. gondii* IgG in 114 (45.2%) cancer patients, and seropositive anti-*T. gondii* IgM were 26 (10.3%); in control group was 92 (36.5%) cases and 15 (6%) cases revealed seropositive for IgG and IgM antibodies (Ghasemian et al., 2007). The result of present study show that the higher positive for anti-*T. gondii* IgG titer was in leukemia patients compare with control groups.

From the result, it appears that all types of leukemia patients infected toxoplasmosis; the incidences were high in type CML and fewer incidences were in type NHL. *T. gondii* is opportunistic and has a role in early stage of leukemia complication (Gharavi et al., 2017). Leukemia patients with toxoplasmosis is consider a main risk factor.
and the severe toxoplasmosis in some patients due to possible secondary infection (Gharavi et al., 2017). It is unknown if the toxoplasmosis was of any significance in the development of the lymphoma (Abdalla, 2017). The result of this study shows that the seropositive to anti-\textit{T. gondii} was in all types of leukemia but the highest rate was in the myeloid line of leukemia (CML, AML).

Cytokines play a key role to control the infection and immunity. The modulation of their function has many potential for therapeutic advantage in several disease and autoimmune pathology. IL-12 is a family of the cytokines and has role in infection, inflammation and autoimmune diseases (Gee et al., 2009). IL-12 was also displayed to decrease the metastatic extent of experimental tumors (Brunda et al., 1993; Cavallo et al., 1999; Mu et al., 1995). IL-12 seemed as one of the furthestmost effective cytokines in mediating antitumor and begins a linkage among innate and adaptive immunity depending on the nature of tumor or the affected tissue (Tugues et al., 2015). Other study shows that IL-12 can be stably expressed in hematopoietic cells in addition when transplanted, transduced cells induce IFN-$\gamma$ making and initiation of natural killer cells, together of which may possibly involve in inhibiting the development of leukemia in vivo (Gautam et al., 2000).

Immune disorders were a deep feature of CLL since first stage immune defects contribute to infectious problems and autoimmune cytopenias (Forconi and Moss, 2015). Furthermore, the progressively immunosuppressive environment in CLL effects disease improvement and treatment efficacy (Riches and Gribben, 2013). The mechanisms essential of these clinical phenomena involve together the innate and adaptive immune system. At the crossroad of innate and adaptive immunity and natural killer NK cells exhibition reduced cytotoxicity (Wild et al., 2015; Huergo-Zapico, 2014). NK cells are at the present known to be part of a bigger group of innate lymphocytes (ILCs) (Artis and Spits, 2015). ILCs play a basic role in the initial immune response, tumor observation and tumorigenesis (Eisenring et al., 2010; Chan et al., 2014). ILCs can play role in tumor development, mainly by producing tumor-promoting cytokines and by supporting an immunosuppressive environment. In AML, at diagnosis, ILCs are significantly deregulated in terms of frequency, subtype composition and function (Trabanelli et al., 2015; Munneke et al., 2014).

\textit{T. gondii} is a chief manipulator of immunity. Later encountering \textit{Toxoplasma} and immune cells, proinflammatory signaling cascades may possibly affectedly triggered within infected cells leading to immune activation or immune subversion. Macrophages and dendritic cells or neutrophils infected with \textit{T. gondii} secrete numerous cytokines, including IL-23 and IL-12 (Langrish et al., 2004). In this study, high serum level of IL-12 was appearing in leukemia patients in compare with controls according to the toxoplasmosis infection with significance differences.

**Conclusion**

In this study, the results revealed a developed incidence of \textit{T. gondii} infection in immunocompromised patients (leukemia patients). The level of IL-12 was significantly higher in the leukemia patients with toxoplasmosis. Thus, it
is essential to diagnose toxoplasmosis in leukemia patients to decrease the imports of toxoplasmosis infection in these patients.

References


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Gee, K., Guzzo, C., Mat, C., Nor, F., Ma, W., & Kumar, A. (2009). The IL-12 family of cytokines in infection, inflammation and autoimmune disorders. Inflammation & Allergy-Drug Targets (Formerly Current Drug Targets-Inflammation & Allergy), 8(1), 40-52.


