Evaluation of soluble CD279 (sCD279) and CD274 (sCD274) in Iraqi patients with Acute Myeloid Leukemia (AML) with Toxoplasmosis

1Ali A. Issa, 1Ali N. Yaseen* and 2Muhammed A. H. Aldabagh

1Department of Sciences, College of Basic Education, Mustansiriyah University, Iraq.
2Medical Research Unit, College of Medicine, University of Al-Nahrain. Baghdad-Iraq
ms.bio2012@yahoo.com

ABSTRACT

Toxoplasma gondii has been suggested as an important opportunistic pathogen in immunocompromised patients. Possible associations of parasite infection with cancer risk have recently attracted much attention. Published studies concerning the association between Toxoplasma gondii infection and leukemia risk have generated inconsistent results. In the present study, we aimed was to investigate the sero-prevalence of the anti-Toxoplasma gondii IgG antibodies in Iraqi patients with Acute Myeloid Leukemia (AML) with Toxoplasmosis and to clarify the role of sCD249 and sCD274 in Iraqi AML patients with toxoplasmosis. Enzyme Linked Immunosorbent Assay (ELISA) was used to detect anti-T. gondii IgG antibodies in the sera of 120 patients with AML and 50 apparently healthy controls. The results showed that 49(41%) samples of sera patients have been founded AML with toxoplasmosis, 71(59%) samples have AML, 20(40%) cases have control toxoplasmosis (those patients were had toxoplasmosis but showing no symptoms) and 30 (60%) cases samples were considered as a control group without any infections. Sera (sCD279 and sCD274) levels were determined by ELISA using a quantitative sandwich enzyme immunoassay technique. The results showed that levels of sCD279 and sCD274 levels were significantly higher in patients group than healthy subjects (P<0.01).

Keywords: Toxoplasma gondii, Toxoplasmosis, Leukemia, AML, CD279, CD274

Introduction

Toxoplasma gondii is a protozoan parasite that causes a disease called toxoplasmosis. It is a very common parasitic infection in humans and other warm-blooded animals. It is a wide-spread parasite reported to infect about one-third of the world population.

Human tumors, including hematological malignancies, have developed multiple strategies for escape from the host immune system. Mechanisms used by tumors for escape have been extensively investigated in the last decade, and a better understanding of these mechanisms has facilitated the development of novel therapies.
aimed at arresting tumor immune evasion. One of the more recently discovered mechanisms of immune suppression operating in cancer involves immune cell intrinsic checkpoints that are induced on the surface of activated T cells. \(^4\) Several such checkpoint molecules serving as negative regulators of activated T cells are known, including cytotoxic T-cell antigen-4 (CTLA-4), programmed death-1 (PD-1) or CD279, T cell immunoglobulin mucin-3 (TIM-3), lymphocyte activation gene-3 (LAG-3), B and T cell lymphocyte attenuator (BTLA) and others \(^4\).

Leukemia is a malignant tumor of the hematopoietic system, which often has a poor prognosis. The mechanisms are not clear. Several factors, such as radiation \(^5\) and chemical carcinogens exposure \(^6\), have been reported to be risk factors for leukemia, and internal factors, such as gene variation \(^7\).

Acute myeloid leukemia (AML) is heterogeneous diseases and aggressive type of leukemia and the most common type of acute leukemia in adults \(^8\) characterized by cytogenic mutations that modulate proliferation, differentiation and apoptosis as well in myeloid lineages in bone marrow resulting in an increase in the number of abnormal myeloblasts \(^9\).

Immune checkpoints are regulatory pathways that are induced in activated T cells and regulate the amplitude as well as the quality of T-cell antigen responses. These pathways are balanced by co-stimulatory and inhibitory signals and are critical in preventing autoimmunity and uncontrolled T-cell expansion that could facilitate oncogenic mutations \(^3\).

The CD279/CD274 (PD-1/PD-L1) pathway participates in creating and maintaining tumor-associated immunosuppression \(^10\). Tumors effectively convert this normally protective pathway, which is responsible for guarding against inflammation-induced tissue injury, to one that now protects the tumor from immune intervention. Tumors corrupt the PD-1/PD-L1 pathway by bombarding activated PD-1+ T cells with the ligand, thus inducing functional T-cell paralysis \(^11, 12\).

For quite a long time, the roles of parasitic infection in the genesis of tumors have attracted much attention. For example, the parasites *Clonorchis sinensis*, *Opisthorchis viverrini*, and *Schistosoma haematobium* are associated with a number of cancers, such as nasopharyngeal carcinoma, leukemia, and hepatocellular carcinoma \(^13\).

Toxoplasmosis in immunocompetent individuals is generally asymptomatic, but it often leads to serious pathological effects in immunocompromised patients (e.g., people with HIV/AIDS or transplant patients) \(^14\). Usually, infection with *T. gondii* is regarded as an established risk factor for poor obstetric history and is one of the major causes of congenitally acquired infections \(^15\). Moreover, *T. gondii* infection has also been implicated in the development of several disorders, such as liver cirrhosis \(^16\), epilepsy \(^17\), and even schizophrenia \(^18\).
Recently, reports have indicated a possible association of *T. gondii* infection with cancer risk \(^{19, 20}\). The main aim of the current study was to detect the sero-prevalence of the anti-*Toxoplasma gondii* IgG antibodies in Iraqi patients with Acute Myeloid Leukemia (AML) with Toxoplasmosis and to clarify the role of sCD279 and sCD274 in Iraqi patients with AML with Toxoplasmosis.

**Materials and Methods**

**Subjects and Samples**

This study was included 120 samples of patients with Acute Myeloid Leukemia (AML) attending to oncology center of Sadr educational hospital in Basra governorate, Iraq. During the period from December 2019 to June 2020. Out of this sample, a group of 50 healthy subjects were considered as control group. The age of all patients and healthy subjects were ranged from 20 – 79 year. Five ml of venous blood were collected from each subject (patients and control) and placed in gel tube, the serum was separated and divided in ependorff tubes then stored at -20°C until it is used.

**Serological tests**

1- **ELISA *T. gondi* – IgG**: The sera of all samples (Patients and control) were tested with the presence of specific IgG antibodies of *Toxoplasma gondii*, via ELISA kits which had supported by (Bioactiva Company, Germany) and applied the test according to the manufacturer’s instructions.

2- **Serum Level of CD279**: Serum levels of CD279 was measured by using specific enzyme-linked immunosorbent assay (ELISA) kit (R&D Company, USA), according to the manufactures protocol.

3- **Serum Level CD274**: Serum levels of CD274 was measured by using specific enzyme-linked immunosorbent assay (ELISA) kit (R&D Company, USA), according to the manufactures protocol.

**Statistical Analysis:**

The Statistical analyses were done by Statistical Package for the Social Sciences for Science (SPSS) version 2010. The statistical tests were included Descriptive statistical tables, Mean, Standard Deviation, under P>0.05 and P<0.01 to considered statistically significant.

**Results**

In the sum up examine results, the study samples showed that 49(41%) samples of sera patients have been founded AML with toxoplasmosis, 71(59%) samples have AML, 20(40%) cases have control toxoplasmosis (those patients were had
toxoplasmosis but showing no symptoms) and 30 (60%) cases samples were considered as a control group without any infections (Table 1).

The cut-off value of positive IgG (10 IU/ml) in all studied groups. The results recorded in the table 1 were shown higher results of levels of IgG in AML with toxoplasmosis group as 28.6±6.4 IU/ml, followed by positive control group 19.5±4.2 IU/ml, and negative control group with value 3.9±2.8 IU/ml, while AML group presented low results of this antibody 3.8±2.6 IU/ml.

**Table 1: Levels of IgG antibodies (IU/ml) for all study groups.**

| Groups                        | No. of Samples | %  | Mean ± SD  | Lower value | Upper value |
|-------------------------------|----------------|----|------------|-------------|-------------|
| AML with Toxoplasmosis        | 49 /120        | 41 | 28.6±6.4   | 15.9        | 44          |
| AML                           | 71 /120        | 59 | 3.8±2.6    | 2           | 9           |
| Positive control               | 20 /50         | 40 | 19.5±4.2   | 13.3        | 29          |
| Negative control               | 30 /50         | 60 | 3.9±2.8    | 1           | 9           |

Table (2) shows the comparisons in the means of the IgG among all studied groups, highly significant differences (P < 0.01) were registered when comparing the values of IgG for the patient’s AML with toxoplasmosis and AML only, positive control and negative control.

**Table 2: Multiple comparisons of the IgG concentrations (IU/ml) for potential couples among studied groups.**

| Parameter        | Group(1)                | Group(j)       | P-value | Sig. |
|------------------|-------------------------|----------------|---------|------|
| IgG              | (AML) with Toxoplasmosis | AML            | 0.000   | HS   |
|                  |                         | Positive control | 0.000  | HS  |
|                  |                         | Negative control | 0.000  | HS |
|                  | AML                     | Positive control | 0.000  | HS  |
|                  |                         | Negative control | 0.000  | HS |
|                  | Positive control        | Negative control | 0.000  | HS |

HS: Highly Significant at P< 0.01.

Table (3) showed the mean values of sCD279 in all the groups, AML patients has registered the highest value 207.24±46.05 pg/ml then AML with toxoplasmosis patients 203.81±62.93 pg/ml, finally, negative and positive control groups has 164.19±47.13 pg/ml, 158.64±62.35 pg/ml respectively, also the table was referred to the highest and lowest response of sCD279 levels.
Table 3: Levels of sCD279 (pg/ml) for all study groups.

| Groups                          | No. of Samples | Mean± SD         | Lower value | Upper value |
|--------------------------------|----------------|-----------------|-------------|-------------|
| AML with Toxoplasmosis         | 49             | 203.81±62.93     | 63.31       | 186.24      |
| AML                            | 71             | 207.24±46.05     | 87.37       | 148.67      |
| Positive control                | 20             | 158.64±62.35     | 83.12       | 186.89      |
| Negative control                | 30             | 164.19±47.13     | 36.19       | 162.61      |

Table 4 referred to the differences of the means for sCD279 among all studied groups, the results did record high significant difference at probability of $P< 0.01$ when comparing the level of sCD279 in AML patients with toxoplasmosis and the groups of positive and negative control respectively, while no significant differences at probability of $P<0.05$ were recorded when comparing the AML with Toxoplasmosis group with AML and negative control.

Table 4: Multiple comparisons of the sCD279 concentrations (pg/ml) for potential couples among studied groups.

| Parameter        | Group(1)                          | Group(j)       | P-value | Sig.   |
|------------------|-----------------------------------|----------------|---------|--------|
| sCD279           | AML with Toxoplasmosis            | AML            | 0.74    | NS     |
|                  | Positive control                  | AML            | 0.000   | HS     |
|                  | Negative control                  | Positive control| 0.000  | HS     |
|                  | Positive control                  | Negative control| 0.000  | HS     |
| Positive control | Negative control                  |                | 0.72    | NS     |

HS: Highly Significant at $P< 0.01$; NS: No Significant at $P> 0.05$

Table (5) showed high level of sCD274 in the AML group 156.08±58.20 pg/ml compared AML with Toxoplasmosis group 150.88±66.15 pg/ml, positive control group 139.21±53.50 pg/ml negative control group 138.80±48.43 pg/ml respectively.

Table 5: Levels of sCD274 (pg/ml) for all study groups.

| Groups                  | No. of Samples | Mean± SD        | Lower value | Upper value |
|-------------------------|----------------|-----------------|-------------|-------------|
| AML with Toxoplasmosis  | 49             | 150.88±66.15    | 63.31       | 186.24      |
Table (6) illustrates the differences between studied groups that found no significant differences (\(P>0.05\)) appear between a group of AML with Toxoplasmosis and AML, positive, negative control.

Table 6: Multiple comparisons of the sCD274 concentrations (pg/ml) for potential couples among studied groups.

| Parameter          | Group(1)                        | Group(j)         | P-value | Sig. |
|--------------------|---------------------------------|------------------|---------|------|
| sCD274             | AML with Toxoplasmosis          | AML              | 0.17    | NS   |
|                    |                                 | Positive control | 0.25    | NS   |
|                    |                                 | Negative control | 0.3     | NS   |
|                    | AML                             | Positive control | 0.73    | NS   |
|                    |                                 | Negative control | 0.83    | NS   |
|                    | Positive control                 | Negative control | 0.65    | NS   |

NS: No Significant at \(P>0.05\)

Discussion

One of the most critical problems in leukemia is infectious diseases which may lead the patient succumbs to sudden death. The active infection may alter the normal immune response of the host. Granulocytes and macrophages play a main role in immune surveillance in innate immune system \(^{(21)}\).

In this study, \(T. \) gondii was investigated in serum samples of patients with acute myeloid leukemia (AML) and control using ELISA. Only 49 out 120 (41%) with AML patients were positive for \(T. \) gondii and 20 out 50 (40%) in control group; this result is agree with study reported by Yazar et al. \(^{(22)}\) in which patient's neoplasia demonstrate superior value of \(T. \) gondii IgG antibodies seropositive (52.9%).

In a study, Gharavi et al. \(^{(23)}\) which have their results are consistent with the results of the current study investigated the anti-\(T. \) gondii IgG antibodies in chronic myeloid leukemia and acute myeloid leukemia patients and healthy individuals in Iran, using ELISA method and their results showed that 56.4% of the leukemia individuals were seropositive for anti- \(T. \) gondii IgG while 42.4% of the healthy individuals were seropositive for the same antibody.

Furthermore, other research showed \(T. \) gondii IgG antibodies in 114 (45.2%) cancer patients were positive, in control group, 92 (36.5%) cases revealed seropositive for IgG antibodies \(^{(24)}\). Furthermore, Gharavi et al. \(^{(25)}\) assessed IgG anti-\(T. \) gondii in renal transplant recipients, before and after transplantation. ELFA method detected 65 (63.7%) pre-transplantation specimens as positive IgG. Moreover, additional studies indicated that the difference frequency of positive IgG antibodies between the patients with cancer and the control group was significant \(^{(26)}\), and this was confirmed by the results of the current study.
The mechanisms by which *T. gondii* starts tumorigenesis are vague. Reports appeared that *T. gondii* can trade miRNAs into its host cell, which might regulate the hosts’ gene expression, and thus cause cancer onset (27). By modifying host miRNA expression, *T. gondii* infection has been reported to initiate and develop brain carcinoma (28). Since the genes involved in apoptosis or anti-apoptosis were both targeted by the differentially expressed miRNAs, the change in balance of power between the miRNAs targeting host apoptosis genes and those modulating host anti-apoptosis genes leads to the fate of the host apoptosis process (29). The above evidence might be helpful in elucidating the roles of *T. gondii* in the genesis of leukemia.

Our results suggest that *T. gondii* infection might be a risk factor for leukemia, providing new insight into the etiology of leukemia. Future studies with large sample sizes in different geographic areas are needed to confirm this conclusion.

Immune checkpoints are regulatory pathways induced in activated T lymphocytes that regulate antigen responsiveness. These immune checkpoints are hijacked by tumors to promote dysfunction of anti-tumor effector cells and consequently of tumor escape from the host immune system (30).

In AML, the CD279/CD274 pathway is hijacked by malignant cells to facilitate immune escape. Many studies have demonstrated up-regulation of the CD279/CD274 pathway in AML and the negative impact of this amplification on disease control. In mice injected with an AML cell line (C1498), the percentage of CD8+ T-cells expressing CD279 dramatically increased in the liver, a major site of C1498 dissemination (31). Similarly, when C1498 cells were injected into mice and allowed to grow in vivo, CD274 expression on T cells increased compared to baseline (32).

Expression of CD279 and its ligands is also increased in hematopoietic cells of patients with AML. One study of 124 patients with myeloid malignancies, including 69 with myelodysplastic syndrome (MDS) and 9 with AML, sampled at various stages of treatment found that the CD274 mRNA expression level was upregulated by ≥2 fold in 36% and 25% of CD34+ cells in MDS and AML, respectively, compared to CD34+ normal control cells. PD-L2 was also upregulated in a smaller proportion of CD34+ cells, i.e., 12% in MDS and 33% in AML (33). In a smaller subset of patients, mRNA expression correlated perfectly with CD274 expression on CD34+ cells by immunohistochemistry. Expression levels of CD274, CD273, and CD279 were also increased in peripheral blood mononuclear cells (PBMCs). In fact, expression levels of CD273 and CD279 were higher in PBMCs than in CD34+ cells (33). Another cohort of 154 patients with AML demonstrated no significant increase in surface CD274 expression on leukemia cells at initial diagnosis compared to healthy controls.

References

1 - Dubey JP, Jones JL. Toxoplasma gondii infection in humans and animals in the United States. Int J Parasitol 2008; 38:1257-78.

2 - Tenter AM, Heckeroth AR, Weiss LM. Toxoplasma gondii: From animals to humans. Int J Parasitol 2000; 30:1217-58.

3 - Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. Science. Mar 25; 2011 331(6024):1565–70.
4- Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate antitumor immunity. Curr Opin Immunol. Apr; 2012 24(2):207–12.

5- Brown N, Finnon R, Manning G et al: Influence of radiation quality on mouse chromosome 2 deletions in radiation-induced acute myeloid leukaemia . Mutat Res Genet Toxicol Environ Mutagen, 2015; 793: 48–54.

6 - Talbott EO, Xu X, Youk AO et al: Risk of leukemia as a result of community exposure to gasoline vapors: A follow-up study. Environ Res, 2011; 111(4): 597-602.

7-Zhang XX, Du YF, Zhai YJ et al: A common genetic variation in CEBPE and acute lymphoblastic leukemia: A meta-analysis of the available evidence . Onco Targets Ther, 2015; 8: 2443–51.

8-Teague RM and Kline J. Immune evasion in acute myeloid leukemia: current concepts and future directions. Journal for ImmunoTherapy of Cancer, 2013; 1:13.

9- Dohner H, Weisdorf DJ. and Bloomfield C.D. Acute myeloid leukemia. N. Engl. J. Med. 2015; 373: 1136-1152.

10- Chen DS, Irving BA, Hodi FS. Molecular pathways: next-generation immunotherapy--inhibiting programmed death-ligand 1 and programmed death-1. Clin Cancer Res. Dec 15; 2012 18(24):6580-6587.

11- Thompson RH, Gillett MD, Cheville JC, Lohse CM, Webster WS, et al. Costimulatory B7-H1 in renal cell carcinoma patients: Indicator of tumor aggressiveness and potential therapeutic target. Proc Natl Acad Sci U S A. Dec 7; 2004 101(49):17174–9.

12- Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. Jun 28; 2012 366(26):2443–54.

13- Oh JK, Weiderpass E: Infection and cancer: Global distribution and burden of diseases. Ann Glob Health, 2014; 80(5): 384–92.

14-Ahmadpour E, Daryani A, Sharif M et al: Toxoplasmosis in immunocompromised patients in Iran: A systematic review and meta-analysis. J Infect Dev Ctries, 2014; 8(12): 1503–10.

15- Kamal AM, Ahmed AK, Abdellatif MZ et al: Seropositivity of toxoplasmosis in pregnant women by ELISA at Minia University Hospital, Egypt. Korean J Parasitol, 2015; 53(5): 605–10.
16- Zajkowska A, Garkowski A, Czupryna P et al: Seroprevalence of parvovirus B19 antibodies among young pregnant women or planning pregnancy, tested for toxoplasmosis. Przegl Epidemiol, 2015; 69(3): 479–82, 597–600.

17 - Ngoungou EB, Bhalla D, Nzoghe A et al: Toxoplasmosis and epilepsy – systematic review and meta-analysis. PLoS Negl Trop Dis, 2015; 9(2): e0003525.

18- Torrey EF, Bartko JJ, Yolken RH: Toxoplasma gondii and other risk factors for schizophrenia: an update. Schizophr Bull, 2012; 38(3): 642–47.

19- Cong W, Liu GH, Meng QF et al: Toxoplasma gondii infection in cancer patients: prevalence, risk factors, genotypes and association with clinical diagnosis. Cancer Lett, 2015; 359(2): 307–13.

20 - Thomas F, Lafferty KD, Brodeur J et al: Incidence of adult brain cancers is higher in countries where the protozoan parasite Toxoplasma gondii is common. Biol Lett, 2012; 8(1): 101–3.

21- Gharavi MJ, Ashraf F, Vosough PA, Rokni MB. Survey of intestinal parasitic infection in leukemic children and evaluation of their serum immunoglobulins. Iran J Public Health 2003;32:19-21.

22- Yazar, S., Yaman, O., Eser, B., Altuntas, F., Kurnaz, F. and Sahin, I. Investigation of anti-Toxoplasma gondii antibodies in patients with neoplasia. J. Med. Microbiol(2004), 53: 1183-1186.

23- Gharavi MJ, Roozbehani M, Mandeh Z. Detection of anti-Toxoplasma gondii antibodies in chronic myeloid leukemia and acute myeloid leukemia patients, Veterinary World, (2017) ,10(9): 1063-1065.

24- Ghasemian, M., Maraghi, S.H., Saki, J. and Pedram, M. Determination of antibodies (IgG, IgM) against Toxoplasma gondii in patients with cancer. Iran. J. Parasitol.,(2007), 2(4):1-6.

25- Gharavi, M.J., Jalali, S., Khademvatian, S.H. and Heydari, S. Detection of IgM and IgG anti-Toxoplasma antibodies in renal transplant recipient using ELFA, ELISA and ISAGA methods: Comparison of preand post-transplantation status. Ann. Trop. Med. Parasitol. (2011),105(5): 367-371.

26- Yuan, Z., Gao, S., Liu, Q., Xia, X., Liu, X., Liu, B. and Hu, R. Toxoplasma gondii antibodies in cancer patients. Cancer Lett., (2007),254(1): 71-74.

27 - Sacar MD, Bagci C, Allmer J: Computational prediction of microRNAs from Toxoplasma gondii potentially regulating the hosts’ gene expression. Genomics Proteomics Bioinformatics, 2014; 12(5): 228–38.
28- Thirugnanam S, Rout N, Gnanasekar M: Possible role of Toxoplasma gondii in brain cancer through modulation of host microRNAs. Infect Agent Cancer, 2013; 8(1): 8

29- He JJ, Ma J, Wang JL et al: Analysis of miRNA expression profiling in mouse spleen affected by acute Toxoplasma gondii infection. Infect Genet Evol, 2015; 37: 137–42.

30 - Alison Sehgal, Theresa L. Whiteside, Michael Boyiadzis, PD-1 Checkpoint Blockade in Acute Myeloid Leukemia Expert Opin Biol Ther. 2015 ; 15(8): 1191–1203. doi:10.1517/14712598.2015.1051028.

31 - Zhou Q, Munger ME, Highfill SL, Tolar J, Weigel BJ, Riddle M, et al. Program death-1 signaling and regulatory T cells collaborate to resist the function of adoptively transferred cytotoxic T lymphocytes in advanced acute myeloid leukemia. Blood. Oct 7; 2010 116(14):2484–93.

32 - Zhang L, Gajewski TF, Kline J. PD-1/PD-L1 interactions inhibit antitumor immune responses in a murine acute myeloid leukemia model. Blood. Aug 20; 2009 114(8):1545–52.

33 .Yang H, Bueso-Ramos C, DiNardo C, Estecio MR, Davanlou M, Geng QR, et al. Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. Leukemia. Jun; 2014 28(6):1280–8.