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Does toxoplasmosis relate with brain cancer?

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Abstract : The present study investigates the association of toxoplasmosis with brain cancers by identifying IgG and IgM *Toxoplasma* antibodies in brain cancer patients and healthy individuals and discusses the role of some immunological aspects related to these diseases. Seventy- eight patients from the outpatient and inpatient clinics of the Al-Amal National Hospitals for Cancer Management and Neurosurgery Teaching Hospital in Baghdad cityduring the period from June 2015 to June 2016 and 59 healthy blood donor volunteers as control subjects were also enrolled in the study. Analysis for anti-*Toxoplasma* IgG antibodies and anti-*T. gondii* immunoglobulin M (IgM) antibodies were done for diagnosis of toxoplasmosis in all subjects. Levels of INF-γ, IL-4, TNF-α, IL-10 and IL-17 were evaluated in the sera of all subjects by means of ELISA method. In addition ,Glial fibrillary acidic protein (GFAP) means of concentration were assessed. The results showed that 82 out of 137 cancer patients were positive for anti-*Toxoplasma* IgG antibodies while 42 cases were positive among healthy volunteers (control).Most cancer patients were having brain cancer (78 out of 137) and the highest rate of toxoplasmosis was among them (66.66%).Concentration of both IgG and GFAP was increased in cancer and control seropositivity with toxoplasmosis. The relative risk factors of *Toxoplasma* infection were larger twice in brain cancer patients than in healthy control and the odd ratio to get an infection among brain cancer was 3.619 times more than in healthy subjects. Concentration of INF-γ, TNF-α and IL-17 was higher in cancer and healthy control, while IL-10 revealed no significant difference in all groups.IL-4 was increased only in cancer patients. The study concluded that there was a relationship between brain cancer and toxoplasmosis but it needs more investigation to prove that and to guess which one causes progress of the other. Moreover, GFAP may be used to determine the brain injury in toxoplasmosis patients but this also needsa designfor further investigation.
Key words : Toxoplasmosis, brain cancer, cytokines.

Introduction:-

Toxoplasmosis is one of the zoonotic diseases in humans and animals. It is caused by a cosmopolitan, obligate intracellular protozoan (*Toxoplasma gondii*) which infectsa broad range of vertebrate hosts, including up to one-third of the world's population and it is widespread especially in immunocompromised individuals[1]. The infection may pass through the blood stream and ultimately localizes in various organs of the host such as brain, spinal cord, eyes, lungs, liver, spleen, lymph nodes and muscles. As an intracellular parasite, *T.gondii* has evolved wide strategies of mechanisms depending on the subversion of cellular defense to invade and manipulate host cells for its intracellular survival[2].During cell invasion by *Toxoplasma*, many events may be carried out in infected cells by parasitophorous ways (forms by the parasite) to manipulate the functions and pathways of these cells. In addition,new findings reveal that various host genes, participated in

numerouscellular activities, were modified afte r*T. gondii* infection[3].*T. gondii* interferes with numerous molecular pathways, such as cellular differentiation, proliferation and apoptosis[4].

Cancer is a continuous increasing disease worldwide and it is of the most common reasons that leading to death in themost developed and developing countries[5]. There are many causes of cancer[6]. Some pathogen infections such as viruses, bacteria and parasites are considered as one of the most important causes of cancer as they are estimated to form 17.8% of all cancer cases. Continual infections may enhance cancer by increasing mutation rates and inducing inflammatory responses[7].In immunocompetent people,the acute disease recovers spontaneouslywithout treatment, but in immunocompromised people, such as those with HIV, those taking chemotherapy or those who have recently received an organ transplant, the risk of death increases[8].There are numerous studies which recorded a positive relationship between the seroprevalence of *T.gondii* and cancer. It has been detected incancerpatients[9-15].

Serum marker profiles have been frequently investigated to discover many diseases and the ability of the immune system to magnify the appearance of disease by producing relatively great amounts of antibody in response to small amounts of disease which makes it as a natural biosensor[16, 17]. Toxoplasmosis is routinely diagnosed by identifying the parasite specific IgG and IgM antibodies in the serum with serological techniques such as Enzyme-linked immunosorbent assay (ELISA)[15].There are some cases (e.g., AIDS patients and fetal infection in patients with cancer) in which diagnosis needs to be rapid and accurate to start treatment. Serum antibodies have emerged as promising biomarkers for the detection of cancer[16]. Thus, the present study aims to investigate the association of toxoplasmosis with brain cancers by identifying IgG and IgM *Toxoplasma* antibodies in brain cancer patients and healthy individuals and discusses the role of some immunological aspects related with these diseases.

Materials and Methods

Patients and Controls

Seventy- eight patients from the outpatient and inpatient clinics of Al-Amal National Hospitals for Cancer Management and Neurosurgery Teaching Hospital in Baghdad city during the period from June 2015 to June 2016 were enrolled in this study. All of them were subjected to full history taking and clinical examination. The diagnosis of their diseases was done by the consultant medical staff of these two hospitals. The patients were 28 males and 50 females with a mean age of 43.1 ± 4.8 years(ranged from 18 to 62 years). Fifty-nine healthy blood donor volunteers as control subjects were also enrolled in the study. They were 25 males and34 females with a mean age of 40.3 ± 1.9 years (ranged from 20to 57 years) and were compatible with both sex and age of patients (P> 0.05).

Determination of the Anti-*T. gondii* Antibody Positivity

From all participants, 3 ml of venous blood samples were collected and placed in plain tube for serum collection. The serum was divided into aliquots (0.25ml) and stored in the freezer for anti-*Toxoplasma* IgG antibodies analysis using a commercial enzyme immunoassay, *Toxo*IgGELISA kit (Human Gesellschaftfür Biochemica und DiagnosticambH, Max-Planck-Ring, Wiesbaden. Germany).Anti-*T. gondii* IgG antibody levels were expressed as IU/ml anda result equalsto or greater than 32 IU/ml was considered as positive. In addition, positive samples for anti-*T. gondii* IgG antibodies were analyzed for anti-*T.gondii* immunoglobulin M (IgM)antibodies by the commercial enzyme immunoassay, *Toxoplasma* IgM kit, (DRG International Inc., USA). The tests were done according to the manufacturer's instructions.

Measurement of Cytokines and GFAP Level

Levels of INF-γ, IL-4, TNF-α, IL-10 and IL-17 were evaluated in the sera of all subjects by means of ELISA method according to instruction of manufacturers of ready to use kits (Biosource, and Belgium and Cell Company, France). GFAP means level was assessed by following instructions of human GFAP ELISA kit (CUSABIO, BiotechCo., Ltd., China) in all individuals enrolled in this study.

Statistical Analysis

Statistical analysis was carried out by SPSS software (statistical package for social science,version 16, SPSS, Inc., USA). Qualitative data were expressed in numbers and percentages and quantitative data were expressed as mean and standard deviation. The collected data were analyzed using Chi-square test and student's t-test for significance differences. $P < 0.001$ was considered as statistically significant.

Ethical Considerations

The study was approved by the Medical Ethics Committee of Iraqi Ministry of Health.

Results

The results of the present study showed that 82 out of 137 (59.85%) cancer patients were positive foranti-*Toxoplasma* IgG antibodies, while 42 cases(35.59%) were positive among healthy volunteers (control). This result was significantly higher ($P<0.001$).For anti- *Toxoplasma* IgM antibodies, only two cases among cancer patients were positive while none of healthy controls were positive (Table 1).

Table (1): Seropositivity of IgG and IgM *Toxoplasm*a antibodies among cancer patients and healthy volunteers control groups

Groups	Number examined	IgG No. +ve(%)	IgM No. +ve(%)
Cancer patients	137	82(59.85)[a]	2(1.45)
Healthy control	118	42(35.59)[b]	0(0.0)
Total	255	124(48.62)[b]	2(0.78)

In the same column, the values sharing the same letters are not significantly different from each others.

Most cancer patients were having brain cancer (78 out of 137) and the highest rate of anti-*Toxoplasma* IgG antibodies among them was 66.66%. The statistical analysis showed that the brain cancer group and the other cancer groups (lymph node, colon and urinary bladder) were significantly higher ($P<0.001$) in comparison with the control group (Table 2).For anti-*Toxoplasma* IgM antibodies, only two cases among cancer patients had positivity while none of healthy controls were positive (Table2). One of these two cases had lymph node cancer and the other one had colon cancer.

Table (2): Seropositivity ofIgG and IgM *Toxoplasma* antibodies among cancer patients and healthy volunteers control groups according to cancer type

Cancer types	Number examined	No. +veIgG (%)	No. +veIgM (%)
Brain	78	52 (66.66)[a]	0(0.00)
Lymph node	22	10 (45.45)[b]	1(4.54)
Colon	19	10 (52.63)[b]	1(5.26)
Urinary bladder	18	10 (55.55)[b]	0(0.00)
Healthy control	118	42 (35.59)[c]	0(0.00)
Total	255	124(48.62)	2(0.78)

In the same column, the values sharing the same letters are not significantly different from each others.

This study revealed that there was a significant increase ($P<0.001$) in IgG concentration in brain cancer patients and control groups seropositivity for toxoplasmosis in comparison with brain cancer patients and control groups negative for toxoplasmosis(Table 3).For GFAP concentration, there was a significant increase($P<0.001$) in brain cancer patients sero-(positivity and negativity) for toxoplasmosis and in control group seropositivity for toxoplasmosis(Table 3).

Table (3): Concentration of IgG and GFAP in brain cancer patients and control groups with and without seropositivity for toxoplasmosis

Groups	IgG mean±SD(mg/dl)	GFAP mean±SD(ng/ml)
Cancer +ve toxoplasmosis	113.06±14.08[a]	204.09±24.97[a]
Cancer -vetoxoplasmosis	50.98±16.17[b]	201.81±28.81[a]
Control +ve toxoplasmosis	87.32±15.75[c]	59.83±14.00[b]
Control -ve toxoplasmosis	48.39±12.02[b]	19.51±10.55[c]
p-value	0.001	0.001

In the same column, the values sharing the same letters are not significantly different from each others.

Results in Table4 revealed that relative risk factors of *Toxoplasma* infection were twice larger in brain cancer patients than in healthy control and this result is statistically significant (more than 1).The odd ratio to get an infection among brain cancer was 3.619 times more than in healthy ones.

Table(4): Toxoplasmosis odds ratio and risk factor in cancer patient and healthy control groups

Groups	Number of cancer patients	Number of healthy control
With toxoplasmosis	52	42
Without toxoplasmosis	26	76
Odd ratio	3.619	
Relative risk factor	2.200	

The concentration of serum cytokines was recorded in Table5.Concentration of INF-γin the serum of brain cancer patients and control groups with or without seropositivity for toxoplasmosis were 29.27±3.17 ng/ml, 15.63 ± 5.16 ng/ml, 18.34 ± 6.11 and 14.28 ± 9.01, respectively.There was a significant difference between brain patient positive for toxoplasmosis compared with other groups.The concentration of IL-4 increased in brain cancer patient group positively for toxoplasmosis (7.99±2.16 ng/ml)compared with other groups in which there was nosignificant difference among them(Table 5). Also, there was a significant difference in concentration of TNF-α in both brain patients' groups (positive or negative for toxoplasmosis) compared with control groups (positive or negative for toxoplasmosis) anda significant difference between both control groups while there was no significant difference between both cancer groups. The concentration of IL-10 showed no significant difference in all groups, while IL-17 increased in brain cancerpatient's positivity for toxoplasmosis and control group positivity for toxoplasmosis (Table5).

Table (5): Concentration of serum cytokines in brain cancer patients and control groups with or without seropositivity for toxoplasmosis

Cytokines	Brain cancer patients+ve (52)mean±SD (ng/ml)	Brain cancer patients-ve (26)mean±SD(ng/ml)	Control +ve (42)mean±SD(ng/ml)	Control -ve (76)mean±SD(ng/ml)	P-value
INF-γ	29.27±3.17[a]	15.63±5.16[b]	18.34±6.11[c]	14.28±9.01[b]	0.001
IL-4	7.99±2.16[a]	0.91±0.16[b]	1.89±0.90[b]	1.09±1.01[b]	0.001
TNF-α	49.89±9.61[a]	33.09±3.71[a]	20.08±9.09[b]	10.08±3.88[c]	0.001
IL-10	18.58±1.01[a]	14.49±1.44[a]	12.42±3.00[a]	11.13±1.48[a]	0.001
IL-17	21.75±5.05[a]	10.54±3.12[b]	15.40±1.98[a]	3.36±1.11[b]	0.001

In the same row, the values sharing the same letters are not significantly different from each others.

Discussion

Many previous studies recorded a positive relationship between the seroprevalence of *T. gondii* and cancer. *Toxoplasma* has been detected in cancer patients[9-15, 18]. The results of these studies agree with the present study, which shows that the overall percentage of *Toxoplasma* infection was higher in cancer patient compared with a healthy control group. The significant finding in the present study that there was a high

percentage of positivity for *Toxoplasma* antibodies in cancer patients, especially in brain cancer patients (66.66%) than that in control volunteers group (35.59%) which may refer to the presence of an association between *Toxoplasma* infection and brain cancer. Brian cancer patient showed great susceptibility for their infection which means that brain cancer may stimulate initiation of *T. gondii* infection or that *T. gondii* may potentially increase the risk of initiation and progression of brain cancers in humans. Yazar *et al.*[9]showed significantly high percentages of positivity for *T. gondii* IgG antibodies in patients with neoplasia compared with the controls. They concluded that this relation may be due to the fact thatpatients with neoplasia are immunocompromised and neoplasia increases their susceptibility to infection with toxoplasmosis. Yuan *et al.*[11] found higher positivityrates of *T. gondii* IgG in cancer patients than in the control individuals. They concluded thatthere is a possible link between *T. gondii* infection and some kinds of cancer, especially nasopharyngeal carcinoma andrectal cancer. They suggested that cancer paves the way for toxoplasmosis based on the fact that patients with malignant neoplasia are immunocompromised, which increases their susceptibility to *T. gondii*. In contrast, the opposite could be trueas Thomas *et al.*[19]indicated that there was an association between *T. gondii* infection and brain cancers in human populations and they predicted that *T. gondii* could increase the risk of brain cancer because it is a long-lived parasite which encysts in the brain, provokes inflammation and inhibits apoptosis. Their study showed that infection with *T. gondii* was associated with a 1.8-fold increase in the risk of brain cancer. This agrees with the present findings which indicated that the infection with *T. gondii*was associated with a 2.2 fold increase in the risk of brain cancer. Thirugnanam *et al.*[13]indicated that *T. gondii* infection may have the ability to manipulate the host microRNAs and could potentially stimulate the development of brain cancer. They suggested a further research on the specific microRNA pathways affected by *Toxoplasma* in various brain cells which may lead to open new methods in the diagnosis and treatment of braincancers caused by *Toxoplasma* infection of humans. The above hypothesis needs more studies to be proven.

Serum marker profiles have been investigated frequently to discover many diseases and the ability of the immune system to magnify the appearance of disease by producing relatively great amounts of antibody in response to small amounts of disease which makes it as a natural biosensor[16, 17]. In the present study, there were increases in serum IgG and GFAP in both brain cancer patient groups and in healthy control (positive for toxoplasmosis) group. Previous studies showed that serum GFAP can be used as for diagnosis of injury in brain tissue[20, 21]. These latter authors recorded an increase in serum GFAP in patients sever with injury in their brain and this agrees with the present study. The increase in serum GFAP concentration in cancer patients indicates damage in brain tissue in case of brain cancer patients and may indicate the presence of injury in brain tissue of healthy control infected with toxoplasmosis. This may be used as a serumindicator for development of brain cancer, but this needs further studies to prove that.

Sanders *et al.*[22]indicated that attenuated *T. gondii* stimulates immunity to pancreatic cancer by manipulation of myeloid cell populations. Blanchard *et al.*[23] and Molan and Rasheed[15] proposed that the chance of reactivation of the latent infection will be high and at the same time the opportunity for the cancer to be more aggressive will also be high that when individuals are previously infected with chronic toxoplasmosis and then get the infection with any type of cancer. In contrast, when individuals are having any type of cancer and then they get the infection with *T. gondii*, toxoplasmosis will be aggressive and the opportunity for the acute infection to last longer (instead of changing into chronic one due to the active immune system) will be ideal because of the weakened immune system due to cancer itself or its immunosuppressive therapy.

Thus, the immunity may play a crucial role in regulation of *T. gondii* proliferation in the central nervous system or may play a role in its pathology. In the present study, the results showed that there was an increase in concentration of each of INF-γ, TNF-α and IL-17 in both brain cancer patient's seropositivity and healthy control seropositivity groups. Early studies indicated that IFN-γ, TNF-α, IL-1, and IL-6 may control the growth of *T. gondii* in the brain via activation of microglia[24, 25]. IFN-γ has been shown to prevent reactivation of *Toxoplasma* encephalitis in mice, while TNF-α, IL-1 and IL-6 are up-regulated in the brains of mice with chronic toxoplasmosis[23].On the other hand, IL-10 was established as a vital player in the control and regulation of immunopathology during toxoplasmosis. The importance of IL-10 represents in preventing and limiting inflammation[26, 27].In addition to IFN-γ, infection with *T. gondii* induces a variety of other cytokines by microglia ,astrocytes and neurons[28].These may promote (e.g., TNF-α) or suppress (e.g.,IL-10)the inflammatory response. These cytokines may play an important role in regulating the resistance of hosts against *T.gondii* infection in the brain. Although T cells are the predominant lymphocyte population in the brains of infected animals, B cells, NK cells[29],macrophages[30]also infiltrate into the brain after the infection.

The study that conducted by Al-Dahmoshi *et al.*[31]showed an increase in IL-17 and IL-8 in women with repeated spontaneous abortion with or without toxoplasmosis in Iraq. The authors concluded that IL-17 and IL-8 are involved in the induction of inflammation and occurring of repeated abortion.

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