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Research Article

Study of gene expression of the ERK1/2 gene and its relationship to PGE2 levels in the serum of patients with *Toxoplasma gondii*

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Abstract

Objective of this study, find the levels of PGE2 in serum for a group for male patients infected with *Toxoplasma gondii* and its relationship with the gene expression of the ERK gene. The patients were diagnosed with the disease by rapid IGg screening and to find the levels of IGg protein in the blood serum of a group of male patients infected with the disease. The aforementioned immune marker in the serum of healthy people as a control group. Blood samples were taken from donors flowing into the main blood bank in Najaf, and the relationship between this immune marker was studied using ELISA technology and its relationship to the gene expression of the ERK gene using real-time PCR. The total number of males was (78), including (48) patients and (30) healthy males who were not infected with the disease. The current results revealed that the serum levels of gene expression of ERK gene (pg/ml) correlated negatively and significantly with PGE (pg/ml) in patients infected with chronic Toxoplasmosis. The current study concluded that the disease is dangerous for males, and there are many complications in males when the disease worsens. It is dangerous to transfuse blood from donors without undergoing a *Toxoplasma gondii* examination, especially to people who have immunodeficiency.

1. Introduction

Toxoplasmosis is an infection of animal origin known to be caused by an obligate intracellular protozoan called *T. gondii*. Domestic cats and members of the cat family (felines) are the definitive hosts, and it is possible for mammals, birds and humans to be the intermediate hosts of the parasite [1]. *Toxoplasma gondii* is a very successful parasite. Because of its wide range, the host species it infects are wide. The ability of the parasite to adapt helped in its preservation and spread in vitro or in vivo and genetic manipulation, which led to this parasite being a biological model widely used to study biological processes in parasites that are difficult to deal with in the laboratory [2]. The metabolic pathway for PGE2 production is a major branch of the arachidonic acid cascade, which begins with the activation of cellular phospholipase A2 (cPLA2). Arachidonic acid is then presented to the cyclooxygenase enzymes, COX1 and COX2, to produce PGH2, which is further converted to PGE2 by prostaglandin E synthase [3] Cyclooxygenases (COXs) activate the rate-limiting production of prostaglandins (PGs), and the COX-2 enzyme has a role in the formation of PGs, and its final product is prostaglandin E2 (PGE2). PGE2 has many roles that contribute to cellular processes, such as inflammation [4]. (ERK) pathway is the main signalling cascade among (MAPK) signal pathways [5]. ERK 1/2 was very expressed in infected cells [6]. Regulation of COX-2 expression and subsequent PGE2 production in macrophages in response to various inflammatory and viral factors. PGE2, an inflammatory factor, is the most abundant prostaglandin found in the human body [7]. The MAPK family consists of JNK, ERK1/2, and p38.There is activation of JNK, ERK1/2, and p38 MAPKs by infection and proliferation of T. gondii tachyzoites, and this has been reported in other studies [8].

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2. Materials and Methods

The subjects

Patient and Control Collection

This study included a total of (76) people, (48) males who were confirmed to be infected with toxoplasmosis by diagnosing them with the rapid diagnostic test for toxoplasmosis by testing the blood serum IGg. Their ages ranged between (18-62) years. The subjects were examined by measuring blood serum IGg and were blood donors attending the blood bank in Najaf province. There were (30) males who were not infected with toxoplasmosis as a control group.

Blood Specimens Collection

Vein blood samples with a capacity of 5 milliliters were collected from blood donors at the blood bank using single-use syringes. The blood was placed in gelatinous tubes and left to clot for approximately 30 minutes. The serum was then separated by centrifugation at 300 rpm for 5 minutes. The samples were stored (Serum and blood) in deep freezing until used in testing to measure immune biomarkers and extract RNA from blood to determine gene expression.

Identify the reference gene

A reference gene is designated by finding the reference gene that is the most stable and genetically expressed in blood samples. The reference gene chosen is based on three factors high expression level Stable expression in all samples The expression level appeared to be similar in all samples [9].

Calculating Gene Expression (Gene Fold)

There are two ways to analyze qPCR data the first is absolute quantification and the second is relative. Absolute quantification quantifies the amount of introduced genes based on the standard curve established by Levack and Schmittgen. Whereas, relative quantification determines changes in gene expression for a sample of reference genes that are calculated with the Pfaffl equation [9]. sometimes the relative amount of genes between the two treatment groups is more important than the DNA RNA molecular numbers. Therefore, relative quantification is performed over a wide range.
Gene expression was determined by the Pfaffl equation RQ = 2-(ΔΔCT)
Gene fold was determined by 1 summing the average CT value (CT cycle threshold) from the real-time PCR for each triplicate sample 2. Calculate the ΔCT value for the samples in the following way
ΔCT = CT (gene of interest) CT (reference gene)
ΔCT expresses the difference in CT values for the target gene or the reference gene for a given sample. It is important for normalizing the target gene, which is not affected by the experiment
The value of ΔΔCT was determined according to the equation
ΔΔ CT = ΔCT (treated sample) - ΔCT (untreated sample (control)
After completing the calculation of ΔΔ CT for the samples, the final equation was taken to determine gene expression (fold change) : Gene expression fold RQ = 2-(ΔΔCT)

Statistical Analysis

Statistical analysis was performed with computer software (Chart Publisher version 6) and we obtained the mean value and standard error (SE) for all values. Where P values with statistical significance less than 0.05 were taken into account.

3. Results

The present study has indicated that there is a significantly level of serum PG in the patients with Toxoplasmosis disease, compared to the healthy controls. It is clear that PG has been increased in the serums of patients with Toxoplasmosis as the mean concentration was (7.800 ± 1.215 pg/ml) in comparison with the mean concentration of the control group (5.277 ± 0.2186 pg/ml) (P=<0.001), as in Figure 1
The current study revealed that the relationship between the patients infected with *Toxoplasmosis* and control group according the RT-PCR for gene expression was a significant increase (P<0.05) in patients with *Toxoplasmosis* (11.47 ± 0.4660) in compared with control group (1.209 ± 0.3795), as seen in Figure 2.
The current results revealed that the serum levels of gene expression of ERK gene (pg/ml) correlated negatively and significantly with PGE (pg/ml) in patients infected with chronic Toxoplasmosis (R^2 = 0. 0492), as seen in Figure 3.

4. Discussion

Toxoplasmosis is an infection of animal origin known to be caused by an obligate intracellular protozoan called *T. gondii*. Domestic cats and members of the cat family (felines) are the definitive hosts, and it is possible for mammals, birds and humans to be the intermediate hosts of the parasite [1]. *T. gondii* relies on a specialized set of structural and secretory organelles to invade host cells. When it infects the definitive host, *Toxoplasma gondii* reproduces sexually in the intestinal epithelium, producing eggs that are excreted in feces and spread to various locations. The parasite has a facultative, heterotrophic life cycle [10]. Prostaglandins are lipid mediators that regulate many functions during inflammation such as cytokine production [11]. The current study demonstrated an increase in the level of PG in the blood serum

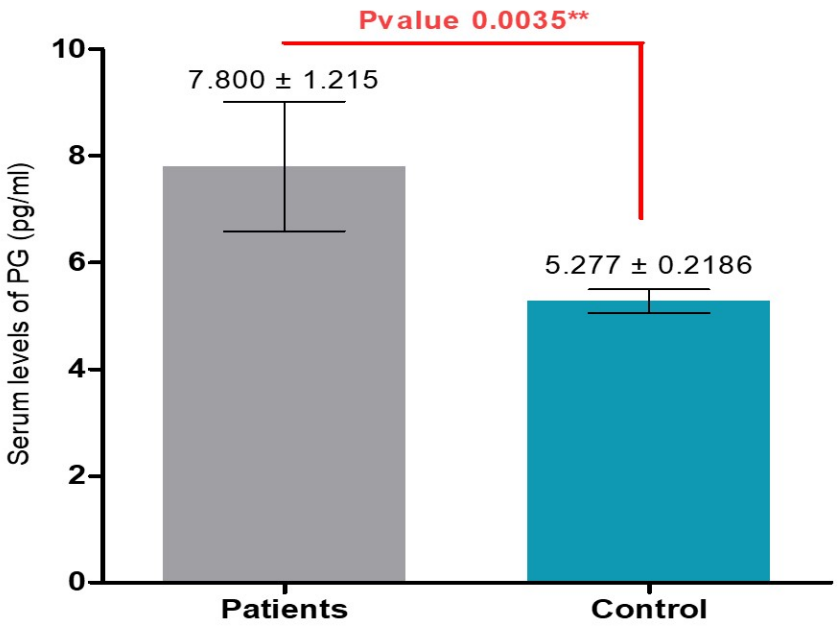

Figure 1: Mean serum level of PG in patients and healthy controls

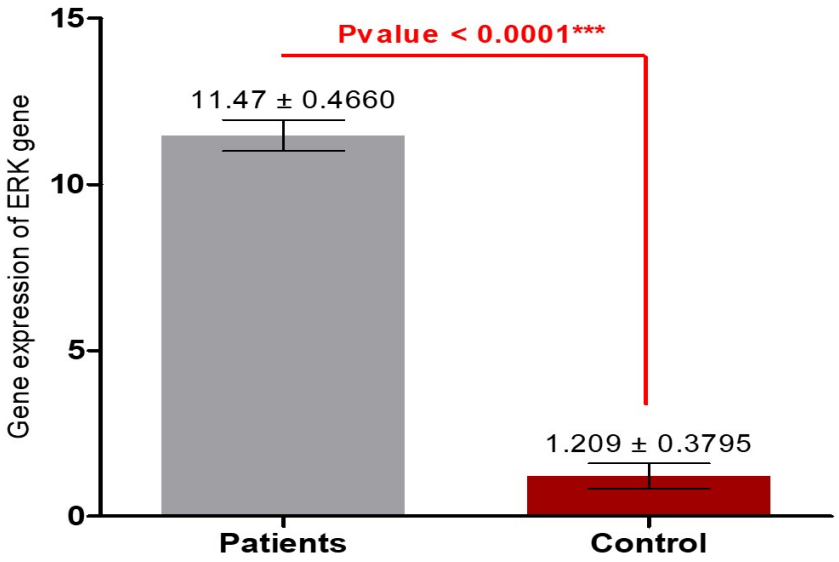

Figure 2: Serum concentration of gene expression in healthy individuals and patients infected with Toxoplasmosis

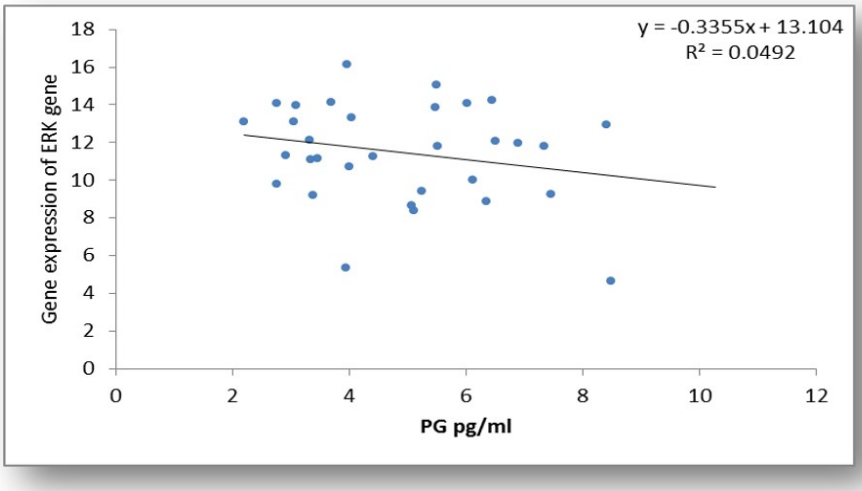

Figure 3: Correlation between gene expression Levels and PGE (pg/ml) in patients infected with Toxoplasmosis

of patients infected with toxoplasmosis compared to healthy controls. ERK1/2 phosphorylated protein, which is a key molecule in the ERK-MAPK pathway, The MAPK family consists of JNK, ERK1/2, and p38.There is activation of JNK, ERK1/2, and p38 MAPKs by infection and proliferation of *(T. gondii tachyzoites)*, this is also mentioned in another study [12]. The current study demonstrated that there was an approximately 11-fold increase in the gene expression of the gene (ERK). The current results revealed that the serum levels of gene expression correlated negatively and significantly with PGE2 in patients infected with chronic Toxoplasmosis. PGE2 works to modulate inflammation and is a powerful activator of signaling pathways. Increased gene expression resulting from stimulation of the MAPK pathway leads to a defect in the production of prostaglandins, which are produced by the COX2 enzyme, which may lead to decreased secretion in those affected. Iinfection of human monocytes by *T. gondii* ressults in an increasee in MAPK activity, ther eby implicating (MAPK phosphorylation) in signal transduction in cases of infection with th is parasite. Cyclooxygenase -2 (COX-2) is the enzyme which catalyzes the production of prostaglandins (PGEs) from ar achidonic acid at infllammation sites.

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