Using real time PCR technique to detect antioxidant PRDX1 gene for proteins in mitochondria for autistic patients

Nahid Ahkzarmadhloom Turky¹, Assist. Prof Zubaidah Adnan Khudhair Aljashaami¹

¹Depart. of Biology, College of Education for Pure Science, Tikrit University, Iraq.

Corresponding author: nahda6874@gmail.com (Turky)

Abstract

In the current study, the level of gene expression of one of the genes in the genetic material of mitochondria had been measured. The gene is one of six genes belonging to the family of peroxiredoxin enzymes, PRDX1. This family of enzymes is considered responsible for the production of antioxidant enzymes. Our study had been included 35 sick children (age 2-14 years), who were compared to 14 healthy children (control group). The children were from various cities including Baghdad - Kirkuk - Salah al-Din. The level of gene expression was measured by extraction of RNA from the hair follicles of the patients and their mothers as well as for control group. We used the Real Time-PCR technique and statistical analyzes, including extraction value of Fold expression by \(2^{-\Delta\Delta C_t}}\), as well as the applying of T-test and F-value of the samples. The results showed a significant difference in the level of PRDX1 gene expression in autistic children compared with healthy children. The results showed that the level of expression of the PRDX1 gene in autistic children compared with healthy children and a significant difference of -2.32 for the T test and a level of probability \((P \leq 0.05)\), and was similar. This value was decreased with the expression of this gene in the sick children compared to their mothers with a significant difference of 2.15 for the T test and a probability level of \((P \leq 0.05)\), while no significant difference was observed between the mothers of patients and healthy mothers. In general, the F-value test for the four groups showed a significant difference in the gene expression of the PRDX1 gene by 2.46 with a probability level of \((P \leq 0.05)\).

Keywords: PRDX1 gene, mitochondria, autistic patients, proteins

How to cite this article: Mohammed AA, Ahmed SE, Ahmeid MS (2020): Procalcitonin and C-reactive protein as prognostic biomarker of infection in traumatic fractures, Ann Trop Med & Public Health; 23 (S5): 547-556. DOI: http://doi.org/10.36295/ASRO.2020.23530

Introduction:

Autism is a developmental disorder disease that usually affects children during the first three years of life. It can be caused by a disorder in the nervous system, which affects brain function, causing problems in their development. The prevalence of this disorder with associated behavioral symptoms is estimated at 1 from 500 people and the proportion of boys and girls increases by 1: 4 [7]. Previous studies had been conducted on autism and pointed to several factors may cause the Autism, however, there was no evidence about the direct cause of autism. On the other hand, some studies had indicated to the existence of genetic, environmental and social factors, although these factors are different from one child to another [1, 12]. Such a factors can be listed within: disorder in genes, chemical imbalance in the immune system, heredity by mother-to-child transmission, problem in the formation of the brain and nervous system during pregnancy [8, 12], the mothers
who came under severe psychological pressures and those who are taken medical drugs, all these factors have a role in causing the disease. Recent evidence suggests that the enzymes which prevent oxidative stress of proteins and fats are found in mitochondrial genes called Peroxiredoxins (PRDXs). The family of genes responsible for a range of enzymes that have a role in the maintenance of proteins from oxidative stress consists of six enzymes (PRDX1, PRDX2, PRDX3, PRDX4, PRDX5, and PRDX6). Those enzymes are important in the defense of antioxidants which discovered about 25 years ago with a molecular size of 30-20 kDa that reacts very quickly with H2O2, glutathione peroxidase, organic hydroperoxides, and peroxidetrate, present in mammals. Mitochondria are associated with cell proliferation, apoptosis, differentiation, and gene expression. The PRDX1 gene is a key enzyme of the 2-Cys PRDXs family and is found mainly in the cytoplasm, cytosol, nucleus, mitochondria, and peroxisomes and is associated with many cellular phenomena including cell proliferation and growth control, cell differentiation, immune responses, tumors and apoptosis. The gene responsible for encoding the enzyme PRDX1 in the human body is located in chromosome 1q34.1 and its expression ratios vary depending on changes in oxidative stress.

**Aim of the study**

The aim of this study is to examine the illiteracy effect on autism inheritance by studying the defect in the PRDX1 gene found in mitochondria, which has a role in protecting proteins from oxidative stress exposure.

**Sample collection**

Our study have included a collection of (5-3) of the head hair with their follicles for 35 children with autism and their mothers, in addition to control group samples which have included 15 natural children and their mothers. The children age was (14-2) years. Patients were obtained from Al-Noor Institute for Autism (Baghdad-Palestine Street) as well as from the Central Child Hospital (Baghdad-Iskan) and from the outpatient clinics of Kirkuk governorate. The samples were kept in plan tubes, and complete information was recorded for the patients' families including educational attainment, age, chronic diseases, hereditary diseases, smoking and also the relationship between parents. The molecular tests were conducted at the wahj al-Dana company - Baghdad from November 2018 to April 2019. The DNA was extracted by using an extraction kit equipped by (RNA extraction Direct-zol™ RNA MiniPrep, R2051, ZYMO RESEARCH / USA) company.

**Measuring purity and concentration:**

The DNA concentration and purity were assessed using a Nano Drop by taking 1μl of the genomic RNA placed in the sample area of the instrument. The device would then give the concentration of ng / μl and the purity (260/280) to the RNA accurately, quickly and with less use the amount of RNA was then reduced to a concentration of 20ng / μl for use in the qRt-PCR.

**Convert RNA to cDNA**

RNA to cDNA conversion was performed using a special kit (PrimeScript™ RT reagent Kit (Perfect Real Time) / Takara / USA).
Specific Primers Sequence used for PCR amplification:
The specific primers described below are designed to determine the specific sequence of genotypes in Table (1) according to the NCBI gene bank, which was supplied by IDT-Canada.

### Table (1): sequence of primers

<table>
<thead>
<tr>
<th>Primer</th>
<th>direction</th>
<th>Sequence (5'→3')</th>
<th>GC (%)</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference gene GAPDH</td>
<td>Forward</td>
<td>AGGTCATCCCTGAGC TGAA</td>
<td>55.5</td>
<td>52.6</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CTGCTTCACCACCTT CTTGAT</td>
<td>55.1</td>
<td>47.6</td>
</tr>
<tr>
<td>PRDX1</td>
<td>Forward</td>
<td>CTC TTG ACT TCA CCT TTG TG</td>
<td>51.1</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TCA CTT GGC AGT TGA GTT TC</td>
<td>53.1</td>
<td>45</td>
</tr>
</tbody>
</table>

**Primers Dilution:**
The primers were lyophilized, they dissolved in the free DdH2O to give a final concentration of 100 pmol/µl as stock solution and keep a stock at -20 to prepare 10 pmol/µl concentration as work primer suspended, 10 µl of the stock solution in 90 µl of the free DdH2O water to reach a final volume 100 µl, was investigated by IDT (Integrated DNA Technologies company, Canada).

**Performing RT-PCR**
- **Instrument:** Sacace
- **Origin:** Italy

**KAPA SYBR® FAST qPCR Master Mix (2X) Kit**
KAPA SYBR FAST qPCR Master Mix (2X) is designed for high-performance real-time PCR. The kit contains a novel DNA polymerase-engineered via a process of molecular evolution-resulting in a unique enzyme specifically designed for real-time quantitative PCR (qPCR) using SYBR Green I dye chemistry. KAPA SYBR FAST DNA Polymerase has been engineered to perform optimally in stringent qPCR reaction conditions, exhibiting dramatic improvements in signal-to-noise ratio (fluorescence), and quantification cycle (Cq), linearity, and sensitivity. The KAPA SYBR FAST DNA Polymerase and proprietary buffer system improves the amplification efficiency of difficult targets, including both GC- and ATrich templates.

KAPA SYBR FAST qPCR Master Mix (2X) Kits are a ready-to-use cocktail containing all components for the amplification and detection of DNA in qPCR. The KAPA SYBR FAST qPCR Kit is supplied as a 2X master mix with integrated antibody-mediated hot start, SYBR Green I fluorescent dye, MgCl2,dNTPs, and stabilizers.
Protocol:
Any existing qPCR assay performed efficiently using standard cycling conditions may be converted to a fast qPCR assay with KAPA SYBR FAST qPCR Kits. Typically, minimal re-optimization of reaction parameters is required.

Master Mix Preparation:
1. Ensure all reaction components are properly thawed and mixed.
2. Prepare a master mix containing the appropriate volume of all reaction components common to all or a subset of reactions to be performed.
3. Always include a No Template Control (NTC) to allow for detection of contamination of reaction components.
4. Calculate the required volume of each component based on the following tables.

Result and discussion:

Measuring the purity and concentration of RNA samples
After obtaining suitable amounts of RNA from all samples and to investigate the validity of the RNA, Nano Drop (QuantumFluorometer) was used at wavelengths 260 and 280 to calculate its concentration and purity values. RNA concentrations were nanogram / micro liters ranged from (89.8-0.36). The purity reading ranged from (2.09-0.3). The purity reading and concentration were taken and the concentration of the samples was reduced to 20 ng / µl for use in the reaction mixture.

Results of RT-PCR
The present study examined the level of difference in gene expression of the PRDX1 gene contained within the mitochondrial genetic material of the six genes of the Peroxiredoxins family, which is responsible for the production of a number of enzymes with a regulated role for the level of free oxygen radicals and reactive oxygen species ROS, thus protecting cells from oxidative stress using the technique of quantitative polymerase reaction time qRt-PCR for both autistic children and their mothers, their gene expression results, their control samples and their mothers, and compared the results with the reference gene (GAPDH), as shown in the figures (1)and(2) below.
Results of PRDX1

The results of measuring the level of gene expression of PRDX1 gene showed a significant deviation in the amount of gene expression among sick children compared to healthy children as the difference of expression in sick children by 0.576 compared to healthy children 5.9 with a significant difference for the test T by -2.32 and a probability level of (P≤0.05), as shown in table (2) and figure (3).
Table (2): Amount of expression difference and T test for PRDX1 gene in autistic patients and their mothers and control samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Nu.S</th>
<th>PRDX1</th>
<th>T-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch.Patients</td>
<td>35</td>
<td>0.576</td>
<td>-2.32*</td>
</tr>
<tr>
<td>Ch.Healthy</td>
<td>14</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>M.Patients</td>
<td>35</td>
<td>2.10</td>
<td>-1.13ns</td>
</tr>
<tr>
<td>M.Healthy</td>
<td>15</td>
<td>4.96</td>
<td></td>
</tr>
<tr>
<td>M.Patients</td>
<td>35</td>
<td>2.10</td>
<td>2.15*</td>
</tr>
<tr>
<td>Ch.Patients</td>
<td>35</td>
<td>0.576</td>
<td></td>
</tr>
</tbody>
</table>

(P≤0.05) * Not significant ns

Fig (3): Level of gene expression of PRDX1 gene in both sick and healthy children.

On the other hand, there were no significant differences in the gene expression of this gene in each of the patients' mothers compared to the healthy mothers, which may indicate a weak illiteracy role in the transfer of functional disorder of this gene from mothers to their children according to table (2) and figure (4).
The lack of expression of the PRDX1 gene in sick children may lead to significant problems during the process of formation of the structure of the nervous system in the fetal stage of the child with the presence and high oxidative voltage of embryonic cells, and because the PRDX1 gene encodes a number of enzymes that have an important role in the process of cell differentiation. In particular, neuronal spinal cord formation in the embryonic stage of the child by reducing the level of oxidative stress as well as regulating the metabolic pathway of a group of protein elements that play an important role in the transfer of cellular signals between cells during the process of cell differentiation [6]. As in the synergistic role of PRDX1 enzyme with GDE2 (Glycero phosphodiester phosphodiesterase 2) which controls the encoding of six types of membrane transport proteins which have a very important role in the differentiation of spinal cord neurons [11],[9].

Studies have pointed to the significant role played by increased oxidative stress of cells and autism when increased in fetal stage in sick children [4],[2],[7]. This is corroborated by the current results where there was a significant difference of T test by 2.15 for the probability level (P≤0.05) and increased gene expression of PRDX1 gene in the mothers of patients compared to their children where the amount of expression difference in mothers was 2.10 compared to their children 0.576 and this may indicate a weak expression of this gene in autistic children during The stage of embryonic development as shown in table (2) and figure (5).

Fig (4): Level of gene expression of PRDX1 gene in both mothers of patients and healthy mothers.
Fig (5): Level of gene expression of PRDX1 gene in both mothers of patients and sick children.

When comparing the expression level of this gene in both sick children and their mothers as well as healthy and their mothers together, a significant difference was observed for the F-test by 2.46 and the probability level of ($P \leq 0.05$). This indicates the role that expression of this gene plays in causing the disease in general with high value. The mean gene expression of healthy children increased by 5.9 compared to the rest of the groups, as shown in Table (3) and Figure (6).

<table>
<thead>
<tr>
<th>Factor</th>
<th>MP. PRDX1</th>
<th>MH. PRDX1</th>
<th>ChP. PRDX1</th>
<th>ChH. PRDX1</th>
</tr>
</thead>
<tbody>
<tr>
<td>N.</td>
<td>35</td>
<td>15</td>
<td>35</td>
<td>14</td>
</tr>
<tr>
<td>Mean</td>
<td>2.10</td>
<td>4.96</td>
<td>0.576</td>
<td>5.9</td>
</tr>
<tr>
<td>StDev</td>
<td>4.173</td>
<td>9.76</td>
<td>0.737</td>
<td>15.53</td>
</tr>
<tr>
<td>F-Value</td>
<td>2.46*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3): The mean, standard deviation and F-va value of PRDX1 expression in autistic children, their mothers, control samples and their mothers.

($P \leq 0.05$)  *
Fig (6): Mean expression of PRDX1 gene in both autistic children and their mothers and control samples and their mothers.

Conclusions:-
1. Oxidative stress has an important role in causing cellular variations that lead to the onset of autism symptoms, especially in the embryonic and nervous system stages.
2. The PRDX1 gene is of great importance in showing autism in children as it is important in controlling a number of enzymes important for spinal cord formation in the embryonic stage.
3. Mitochondria play an important role in many enzymatic regulations, cell signaling, and cell differentiation processes.

References:


