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# Oxidative Stress Biomarkers and NLRP3 Polymorphism in Type 1 Diabetes

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**Abstract**

T1D is an autoimmune condition influenced by environmental and genetic factors and typified by persistent inflammation. Hyperglycemia promotes oxidative stress and the activation of multiprotein inflammatory inflammasomes such as NLRP3. We aimed to evaluate the NLRP3 rs10754558 C/G polymorphism, and oxidative stress in Egyptian T1D. There were 241 patients: 120 with T1D and 121 healthy controls. Rs10754558 was genotyped using the Artus Rotor-Gene Qiagen device's TaqMan™ SNP in a real-time polymerase chain reaction (RT-PCR). Malondialdehyde (MDA) and total antioxidant capacity (TAC) were estimated. There was no significant correlation between T1D susceptibility and the NLRP3 gene, (P>0.05). Logistic regression analysis revealed that patients with higher MDA and lower TAC had an increased risk for prediction of T1D in Univariate analyses {(OR= 2.133, 95%CI= 1.762–2.581), (OR= 0.352, 95%CI= 0.254-0.488), respectively, p<0.001} and multivariate analyses {(OR= 1.040, 95%CI= 1.029-1.051), (OR= 0.961, 95%CI= 0.944-0.978), respectively, p<0.05}. Glycosylated Hemoglobin (HbA1c) was negatively correlated with TAC among the T1D group (r=-0.313, p<0.001). We discovered that the NLRP3 gene's rs10754558 is unrelated to T1D susceptibility. Furthermore, TAC might be a useful tool for monitoring T1D progression.

**Keywords:** T1DM, NLRP3, oxidative stress, genotyping, HbA1c

## 1. Introduction

One of the biggest health issues of the twenty-first century is type 1 diabetes (T1D), an organ-specific autoimmune disease that was formerly known as juvenile diabetes or insulin-dependent diabetes. The chronic inflammation of the pancreatic β cells characterizes it. Thus, it is crucial to investigate the etiology of T1D and create innovative methods to lessen the impact of diabetic complications [1,2]. Environmental and genetic factors contribute to the complexity of T1D. Even if the exact origin of T1D is unknown, genetics greatly influences how the illness presents itself [3]. Numerous susceptibility loci have been connected to the emergence of diseases repeatedly observed in various populations [3]. The most frequent cause of genetic variations in humans is single-nucleotide polymorphisms (SNPs), which can alter a gene's function if they occur in that gene [4]. NLRP3 (NOD-like receptor protein 3) gene is approximately 32.9 kb long. It has nine exons and is found on chromosome 1q44 [5]. SNPs in the NLRP3 gene may alter how the gene functions, raising IL-1β levels and inflammasome activation. Approximately 60 SNPs have been found in the NLRP3 gene thus far. The most researched NLRP3 polymorphism site, including rs10754558 found in the 3′-untranslated region (3′-UTR) of the NLRP3 gene, is the subject of this investigation [6]. Most people now agree that NLRP3 is essential for initiating, developing, and regulating immunological and inflammatory reactions [7]. Furthermore, earlier studies have demonstrated that NLRP3 is a key mediator of several inflammatory and autoimmune diseases [7, 8]. However, data available regarding NLRP3 rs10754558 C/G SNP in T1D patients are scanty, so our team was encouraged to assess this contribution among Egyptian patients with T1D. The imbalance between the overproduction of reactive oxygen species (ROS) in cellular compartments and the biological tissues' incapacity to neutralize or detoxify these harmful substances through the activity of antioxidants is known as oxidative stress [9]. High levels of free radical species can trigger apoptosis and cellular death, which can result in the pathophysiology of several illnesses, e.g., cancer [10], renal failure [11], cardiotoxicity [12], and diabetes mellitus [13]. Furthermore, NLRP3 is activated by oxidative stress [14]. Here, we postulated that NLRP3 genetic variation and unbalanced oxidative stress could contribute to the emergence of T1D problems. Research on the connection between the NLRP3 and T1D susceptibility in Egyptian patients has not yet been conducted. Thus, the current study aimed to investigate the relationship between T1D and the common NLRP3 rs10754558 C/G SNP. Additionally, it links laboratory and clinical data and signs of oxidative stress.

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## 2. Materials and methods

### 2.1 Patients

121 healthy control participants (63 males and 58 females) aged 11.37 ± 0.29 years and 120 T1D patients (62 males and 58 females) aged 12.05 ± 0.30 years, from March 2022 to September 2022 were recruited at MUCH, the children's hospital of Mansoura University, Egypt, for the current study. The American Diabetes Association was used to diagnose diabetes based on fasting blood glucose (FBG) and glycated hemoglobin (HbA1c) readings [15]. The study excluded patients with other serious conditions, such as tuberculosis, cardiovascular, respiratory, type 2 diabetes, and gestational diabetes. All individuals' body mass indices (BMIs) were computed using the formula [weight (kg)/ (height (m))2]. The Medical Ethics Committee approved each participant (code number: MDP.22.01.96), and their parents were notified that written consent had been acquired. Every procedure was conducted under the Declaration of Helsinki.

### 2.2. Methods

#### 2.2.1. Blood test

Five milliliters of peripheral blood were used with aseptic precautions. Coagulated and non-coagulated blood samples were taken from the control group and patients following 8–10 hours of fasting. First, hematological parameters and fasting blood sugar were determined using an automated method. Turbidimetric inhibition immunoassay for hemolyzed whole blood on Cobas C 311 immunoassay analyzer was used to test for HbA1c, Roche Diagnostics (Roche Diagnostics GmbH, Sand Hofer Strasse 116, D-68,305 Mannheim, www.roche.com) provided the kits. Serum samples were centrifuged for 10 minutes at 2500 rpm and stored at -80 °C until serum creatinine levels were measured using the ARCHITECT System's kinetic alkaline picrate technique. Alanine aminotransferase (ALT) (K752, Bio Vision, Inc., USA), aspartate aminotransferase (AST) (K753, Bio Vision, Inc., USA), total bilirubin (K553, Bio Vision, Inc., USA), and albumin (K554, Bio Vision, Inc., USA) were estimated. Assays were carried out following the manufacturer's recommendations.

#### 2.2.2. Estimation of malondialdehyde (MDA)

MDA in serum was isolated as a conjugate with Thio-barbituric acid (TBA). Trichloroacetic acid (TCA) precipitated serum proteins, which were then removed by centrifugation. At 534 nm, the (MDA-TBA) complex was detected [16].

#### 2.2.3. Estimation of total antioxidant capacity (TAC)

Combining hydrogen peroxide with a standardized solution of the Fe-EDTA complex results in a Fenton-type reaction that produces hydroxyl radicals (. OH). Thio-barbituric acid reactive substances (TBARS) are released when these reactive oxygen species decompose benzoate. The additional serum contains antioxidants that decrease the development of TBARS. The spectrophotometric measurement of this reaction's inhibition of color development is called antioxidant activity [17].

#### 2.2.4. Genetic testing and DNA extraction

Using the standard release process, genomic deoxyribonucleic acid (DNA) was extracted from blood using the Qiagen QIAamp DNA kit (Germany). The Artus Rotor-Gene Qiagen fast real-time (RT) PCR System (Applied Biosystems, Foster City, CA, USA, software 2.1.0) was used in an allelic discrimination test using a precise TaqMan probe (ID: C__26052028_10, Transversion Substitution: C/G, Catalogue no.: 4351379) to determine the allelic variation of the NLRP3 gene's SNP rs10754558 C/G. A final volume of 20 µL per reaction will be used for the PCR reaction, using 10 µL of TaqMan™ Genotyping Master Mix (Thermo Fisher Scientific as per manufacturer's protocol, Third Avenue, Waltham, MA, USA) and 20 ng of extracted DNA. The PCR procedure began with 10 minutes of 95 degrees, followed by 45 cycles of 95 degrees for 15 seconds and 60 degrees for 60 seconds.

### 2.3. Statistical analysis

Statistical Package for Social Science (IBM Corp., 2017) was used to edit, code, and tabulate the obtained data (Version 25.0 of IBM SPSS Statistics for Windows. IBM Corp., Armonk, New York). Using the Student's t-test, the statistical significance of the difference between the means of the two study groups was assessed. The chi-square test was used to examine the relationship between two qualitative variables and to see how deviations from Hardy-Weinberg equilibrium expectations varied. When the predicted count is less than five (5) in more than 20% of the cells, the Monte Carlo test was employed to examine the link between two qualitative variables. The statistical significance of the difference between more than two study group parametric variables was evaluated using the one-way ANOVA test. Logistic regression analysis was used to predict risk factors using generalized linear models. It is considered significant if the p-value is 0.05 with a 95% confidence interval (CI). Dominant, recessive, and allelic models were described in our work according to [18]. The three genotype groups would be AA, AB, and BB if the gene of interest has two haploid alleles, A and B, and A is the "risk" allele.

The SNP genotypes can be dichotomized in the following ways: Recessive: "AA" versus "AB + BB," Dominant: "AA + AB" versus "BB". The allelic model, "Allelic: "A vs B," is also used.

## 3- Results

### *3.1. Patients' characteristics, clinical data, and NLRP3 rs10754558 C/G genotype and allele frequencies*

**Table 1** displays the clinical features, NLRP3 rs10754558 C/G genotype, and allele distributions of the 120 T1D cases and the 121 healthy control subjects. No significant difference was in the patient and control groups' age, gender, or BMI changes ($p>0.05$). White blood cells, serum creatinine, and FBG were significantly lower in healthy controls than in T1D patients ($p<0.001$). The T1D group's haemoglobin levels were considerably lower than the healthy controls ($p<0.001$). The complete genotype and allele frequencies of the patients and controls were in Hardy-Weinberg equilibrium (HWE), as shown in **Table 1** ($p> 0.05$). In all genetic models, the genotype and allele frequencies of rs10754558 C/G of the NLRP3 gene did not significantly differ between T1D patients and healthy controls. We discovered that the NLRP3 gene variant rs10754558 C/G was not linked to a risk of T1D. Additionally, **Fig. 1** showed that serum MDA levels were significantly higher, and TAC levels were markedly lower ($p<0.001$) in T1D patients compared to the healthy control group.

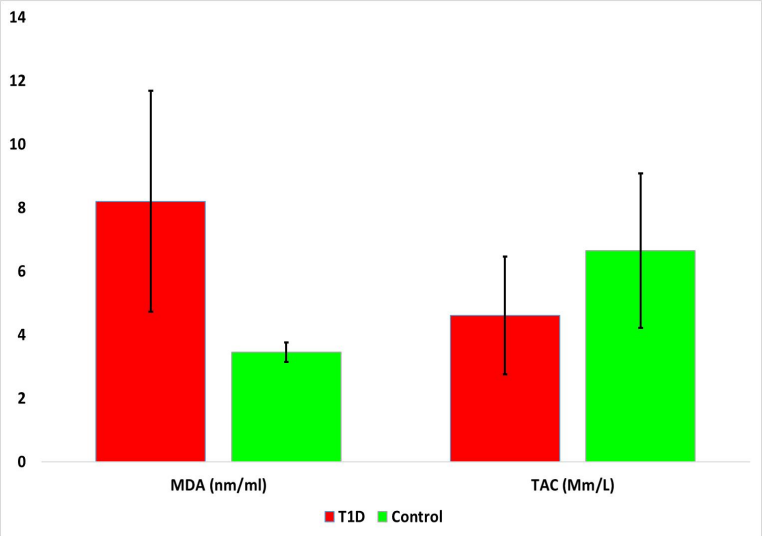


**Figure 1. Comparison of MDA and TAC among patients with T1D and control group**

### *3.2. Regression analysis of NLRP3 rs10754558 C/G genotype for prediction of T1D*

Regression analysis showed no potential genetic association between NLRP3 rs10754558 C/G and clinical and biochemical features ($P>0.05$; **Table 2**). The same table displays the polymorphism of rs10754558 with MDA and TAC, showing no evidence of any significant relationships. However, **Table 2** depicts the univariate and multivariate regression analysis for prediction of T1D susceptibility. Among all studied covariates, higher FBG, serum creatinine, MDA, and lower TAC were associated with the risk of T1D in Univariate analyses {(OR= 1.058, 95%CI= 1.038-1.078), (OR= 3.687, 95%CI= 2.663-5.105), (OR= 2.133, 95%CI= 1.762–2.581), (OR= 0.352, 95%CI= 0.254-0.488), respectively, $P<0.001$} and multivariate analyses {(OR= 1.002, 95%CI= 1.001-1.002), (OR= 2.414, 95%CI= 1.966-2.965), (OR= 1.040, 95%CI= 1.029-1.051), (OR= 0.961, 95%CI= 0.944-0.978), respectively, $P< 0.05$, **Table 2**).

### *3.3. Correlation analysis between MDA, TAC, and different parameters among patients with T1D*

There was a negative connection between TAC and HbA1c, as shown in **Table 3** and **Fig. 2** (Correlation coefficient -0.313 and $p<0.001$). However, there was no discernible difference between MDA and other measures in T1D. Additionally, diabetic patients did not significantly differ between TAC and MDA.

**Table 1. General characteristics, NLRP3 rs10754558 C/G genotype, and allele frequencies were distributed in the studied type 1 diabetes (T1D) and control groups.**

| Characteristic | | T1D (N= 120) | Control (N= 121) | p | OR (95%CI) |
|---|---|---|---|---|---|
| Sex | | | | | |
| Male: n (%) | | 62 (51.7%) | 63 (52.1%) | | |
| Female: n (%) | | 58 (48.3%) | 58 (47.9%) | 0.951 | - |
| Age/year | | 12.05 ± 0.30 | 11.37 ± 0.29 | 0.116 | |
| BMI (kg/m$^2$) | | 21.10 ± 0.40 | 20.54 ± 0.39 | 0.315 | - |
| T1D duration(year) | | 5.18 ± 0.31 | - | - | - |
| FBG (mg/dL) | | 173.55 ± 6.72 | 102.80 ± 0.94 | <0.001 | - |
| HbA1c (percentage) | | 8.82 ± 0.20 | - | - | - |
| Serum creatinine (mg/dL) | | 0.67 ± 0.02 | 0.44 ± 0.01 | <0.001 | - |
| **Hemoglobin indices** | | | | | |
| Hb (g/dL) | | 11.67 ± 0.13 | 12.83 ± 0.06 | <0.001 | - |
| WBCs (K/µL) | | 9.68 ± 0.45 | 6.92 ± 0.12 | <0.001 | - |
| Platelets (K/µL) | | 319.04 ± 11.78 | 294.78 ± 6.01 | 0.558 | - |
| Neutrophil % | | 51.63 ± 16.37 | 52.52 ± 12.37 | 0.633 | - |
| Lymphocyte % | | 36.95 ± 1.34 | 36.10 ± 1.07 | 0.674 | - |
| **Liver function test** | | | | | |
| ALT (U/mL) | | 20.95 ± 0.78 | - | - | - |
| | | | | | |
| AST (U/mL) | | 24.34 ± 1.15 | - | - | - |
| Total bilirubin (mg/dL) | | 0.62 ± 0.02 | - | - | - |
| Serum albumin (mg/dL) | | 4.41 ± 0.04 | - | - | - |
| **Gene/SNP (NLRP3 rs10754558 C/G):** n (%) | | | | | |
| Genotypes | GG | 24 (20%) | 21 (17.4%) | - | Reference |
| | GC | 70 (58.3%) | 61 (50.4) | 0.991 | 1.004 (0.509 – 1.980) |
| | CC | 26 (21.7%) | 39 (32.2) | 0.169 | 0.583 (0.271 – 1.257) |
| Dominant model | GG | 24 (20%) | 21 (17.4%) | - | Reference |
| | GC+CC | 96 (80%) | 100 (82.6%) | 0.599 | 0.840 (0.439-1.608) |
| Recessive model | GG+GC | 94 (78.3%) | 82 (67.8%) | - | Reference |
| | CC | 26 (21.7%) | 39 (32.2%) | 0.066 | 0.582 (0.326 – 1.037) |
| Alleles | G | 118 (49.2%) | 103 (42.6%) | - | Reference |
| | C | 122 (50.8%) | 139 (57.4%) | 0.146 | 0.766 (0.535-1.097) |
| HWE | P | 0.067 | 0.733 | | |

Data are presented as percentages and mean ± SE; p<0.05 is significant. Test: Student t-test, Chi-Square, Mann-Whitney. N, number; logistic regression analysis BMI, body mass index; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; Hb, hemoglobin; WBCs, white blood cells; ALT, alanine aminotransferase; AST, aspartate aminotransferase; OR, odds ratio; CI, confidence interval; HWE, reference, according to NCBI data; HWE, Hardy–Weinberg equilibrium.

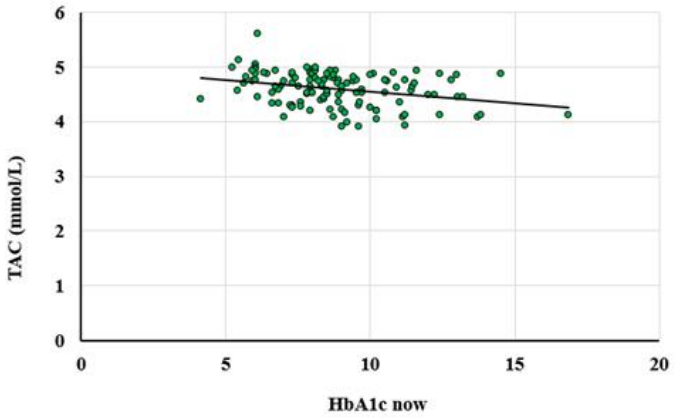


**Figure 2. Correlation between TAC and HbA1c among patients with T1D**

**Table 2. Regression analysis of NLRP3 rs10754558 C/G genotype for prediction of T1D clinical and biochemical characteristics**

| Parameter | NLRP3 rs10754558 C/G | | | P |
|---|---|---|---|---|
| | GG (N=24) | GC (N=70) | CC (N=26) | |
| Sex: N (%) | | | | |
| Male | 15 (62.5) | 36 (51.4) | 11 (42.3) | 0.360 |
| Female | 9 (37.5) | 34 (48.6) | 15 (57.7) | |
| Age | 12.08 ± 0.62 | 12.06 ± 0.43 | 11.97 ± 0.55 | 0.992 |
| BMI | 20.74 ± 0.77 | 21.22 ± 0.54 | 21.12 ± 0.87 | 0.899 |
| T1D duration | 5.39 ± 0.74 | 5.41 ± 0.38 | 4.36 ± 0.70 | 0.231 |
| FBG | 177.58 ± 14.95 | 171.73 ± 8.08 | 174.73 ± 17.76 | 0.831 |
| HbA1c | 9.17 ± 0.45 | 8.69 ± 0.27 | 8.82 ± 0.40 | 0.662 |
| Serum creatinine | 0.69 ± 0.03 | 0.66 ± 0.02 | 0.70 ± 0.04 | 0.506 |
| Hb | 11.24 ± 0.32 | 12.00 ± 0.18 | 11.17 ± 0.22 | 0.112 |
| WBCs | 11.29 ± 1.23 | 9.18 ± 0.56 | 9.53 ± 0.85 | 0.163 |
| Platelets | 337.7 ± 21.80 | 316.2 ± 15.62 | 309.5 ± 28.56 | 0.324 |
| Neutrophil | 55.56 ± 2.78 | 50.76 ± 2.14 | 50.33 ± 2.75 | 0.422 |
| Lymphocyte | 33.84 ± 2.72 | 37.62 ± 1.89 | 38.00 ± 2.51 | 0.477 |
| ALT | 21.92 ± 1.93 | 20.34 ± 1.06 | 21.69 ± 1.35 | 0.462 |
| AST | 23.67 ± 1.62 | 23.58 ± 1.69 | 27.03 ± 2.24 | 0.051 |
| Total bilirubin | 0.64 ± 0.03 | 0.60 ± 0.03 | 0.66 ± 0.04 | 0.243 |
| Serum albumin | 4.41 ± 0.08 | 4.33 ± 0.06 | 4.60 ± 0.09 | 0.056 |
| MDA | 7.73 ± 0.64 | 8.12 ± 0.36 | 8.83 ± 0.94 | 0.853 |
| TAC | 4.57 ± 0.06 | 4.58 ± 0.04 | 4.69 ± 0.05 | 0.251 |

| Regression analysis for T1D prediction | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|
| | P | OR | 95%CI | P | OR | 95%CI |
| Sex | 0.951 | 1.016 | (0.613-1.684) | - | - | - |
| Age | 0.116 | 1.064 | (0.985-1.149) | - | - | - |
| BMI | 0.314 | 1.031 | (0.972-1.093) | - | - | - |
| FBG | <0.001* | 1.058 | (1.038-1.078) | <0.001* | 1.002 | (1.001-1.002) |
| Serum Creatinine | <0.001* | 3.687 | (2.663-5.105) | <0.001* | 2.414 | (1.966-2.965) |
| MDA | <0.001* | 2.133 | (1.762-2.581) | <0.001* | 1.040 | (1.029-1.051) |
| TAC | <0.001* | 0.352 | (0.254-0.488) | <0.001* | 0.961 | (0.944-0.978) |
| NLRP3 rs10754558 (Dominant model) | 0.599 | 0.840 | (0.439-1.608) | - | - | - |

Data are presented as percentages and mean ± SE; p<0.05 is significant. Test: Student t-test, Chi-Square, Mann-Whitney. N, number; logistic regression analysis BMI, body mass index; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; Hb, hemoglobin; WBCs, white blood cells; ALT, alanine aminotransferase; AST, aspartate aminotransferase; OR odds ratio; CI, confidence interval; HWE, reference, according to NCBI data; HWE, Hardy–Weinberg equilibrium

### *3.4. Analysis of data in silico*

**Fig. 3** displays the NLRP3 gene's bioinformatics outlines. C1orf7, CIAS1, PYPAF1, and NLRP3 are some of the gene's synonyms. It traverses 33,032 bases (chr1: 247,416,077-247,449,108) along the plus strand on the long arm of chromosome 1 (1q44). Furthermore, the NLRP3 gene produced 10 transcripts [ENSG00000162711]. The rs10754558 single nucleotide variant with the MAF equal to 0.47, has alleles GC/GT and is situated on chromosome 1 at position 247448734 (GRCh38.p14) [**Data source: Ensembl.org; Human Genome assembly GRCh38.p13**]. This gene produces a pyrin-like protein with a leucine-rich repeat (LRR) motif, a nucleotide-binding site domain, and a pyrin domain. This protein interacts with the apoptosis-associated speck-like protein PYCARD/ASC, which contains a caspase recruitment domain, and is a member of the NLRP3 inflammasome complex [NCBI Gene] and comprising 1036 amino acids with a molecular mass is 118173 Daltons [Protter database]. Protein interaction networks suggested that the NLRP3 plays a critical role in the following diseases and conditions: obesity, malignant pleural mesothelioma, nucleotide-binding oligomerization domain pathway, prostaglandin signaling, Kawasaki-like disease, COVID-19, SARS-CoV-2 mitochondrial chronic oxidative stress, and endothelial

dysfunction [**Data source: STRING**]. Additionally, the mitochondria, Golgi apparatus, cytoskeleton, cytosol, and nucleus are where the cellular NLRP3 is primarily found [**Data source: Cellular compartment database**].

**Table 3. Correlation between MDA, TAC, and different parameters among patients with T1D**

| | MDA | | TAC | |
|---|---|---|---|---|
| **Parameter** | **r** | **p** | **r** | **p** |
| Age | -0.051 | 0.579 | 0.105 | 0.252 |
| BMI | -0.031 | 0.733 | 0.114 | 0.215 |
| T1DM duration | -0.001 | 0.995 | 0.157 | 0.086 |
| FBG | -0.069 | 0.455 | -0.069 | 0.454 |
| HbA1c | 0.122 | 0.183 | -0.313 | <0.001* |
| Serum creatinine | -0.089 | 0.336 | 0.108 | 0.240 |
| Hb | -0.017 | 0.857 | -0.020 | 0.824 |
| WBCs | 0.130 | 0.157 | 0.004 | 0.969 |
| Platelets | 0.121 | 0.190 | -0.010 | 0.912 |
| Neutrophil | 0.080 | 0.384 | -0.026 | 0.780 |
| Lymphocyte | -0.083 | 0.368 | 0.008 | 0.935 |
| ALT | 0.296 | 0.132 | 0.057 | 0.540 |
| AST | 0.147 | 0.109 | 0.148 | 0.107 |
| Total bilirubin | 0.056 | 0.544 | 0.085 | 0.355 |
| Serum albumin | 0.119 | 0.195 | 0.085 | 0.357 |
| TAC | -0.126 | 0.170 | - | - |

r, correlation coefficient; *: $p<0.05$ is significant; BMI, body mass index; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; Hb, hemoglobin; WBCs, white blood cells; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MDA, malondialdehyde; TAC, total antioxidant capacity.

## 2. Discussion

T1D is an autoimmune disease characterized by autoreactive T lymphocytes that impair β cell activity. Glycolysis and oxidative phosphorylation in peripheral organs fuel the intracellular pathways frequently implicated in the development of T1D, hyperglycemia raises the generation of ROS and oxidative stress [19]. Erythrocytes, leukocytes, platelet function, and morphological changes are among the hematological changes that T1D patients frequently experience [20]. Angiotensin and cytokines encourage the production of polymorphonuclear and mononuclear leukocytes in a hyperglycemic state [21]. Previous research indicates leukocytosis is a common symptom in people with T1D [20]. In this regard, our results confirmed a significant rise in leukocytes among T1D patients compared to healthy individuals. These results are supportive of the findings of Adane et al. [22], in Ethiopia, Umeji et al. [23], in Nigeria, and Alam et al. [24], in Bangladesh, who found that patients with diabetes mellitus had higher mean total leukocyte counts than non-diabetic controls. The elevated oxidative stress brought on by the high levels of hyperglycemia in diabetes mellitus patients may be the source of the high leukocyte count [20].

In people with T1D, anemia is a frequent and under-recognized consequence [25]. In this aspect, our results confirmed a significant decline in hemoglobin among T1D patients compared to the control group. Diabetes-related anemia may be brought on by medicines, renal impairment, altered iron metabolism, systemic inflammation, decreased erythropoietin production, and hyperglycemia [20]. Additionally, children with T1D are more likely to suffer from kidney involvement, which can manifest as acute kidney injury, tubular damage, or diabetic kidney disease in the chronic stage [26]. Our findings revealed that T1D cases showed greater creatinine levels than controls ($p<0.001$), as one of the most common clinical features of T1D. Additionally, we discovered that serum creatinine was substantially linked to T1D, univariate and multivariate analyses support its status as an independent disease predictor ($p<0.001$). The development of T1D involves both innate and adaptive immunity. T1D is a complex illness that has both hereditary and environmental causes. One of the main components of the NOD-like receptors, the NLRP3 inflammasome, is a part of the innate immune system and is crucial to the inflammatory response in T1D [27]. According to earlier research, polymorphisms may affect how genes are expressed, and changes in the NLRP3 gene are implicated in several illnesses [28]. Numerous cellular processes connected to stress have been shown to trigger NLRP3 activation and the production of ROS. Abnormal ROS production can cause tissue damage by harming host cells and accumulating harmful lipid peroxides [29]. This is the first study that we are aware of to evaluate the severity of T1D in Egyptian patients using measurements of the NLRP3 rs10754558 genetic variation and oxidative stress indicators.

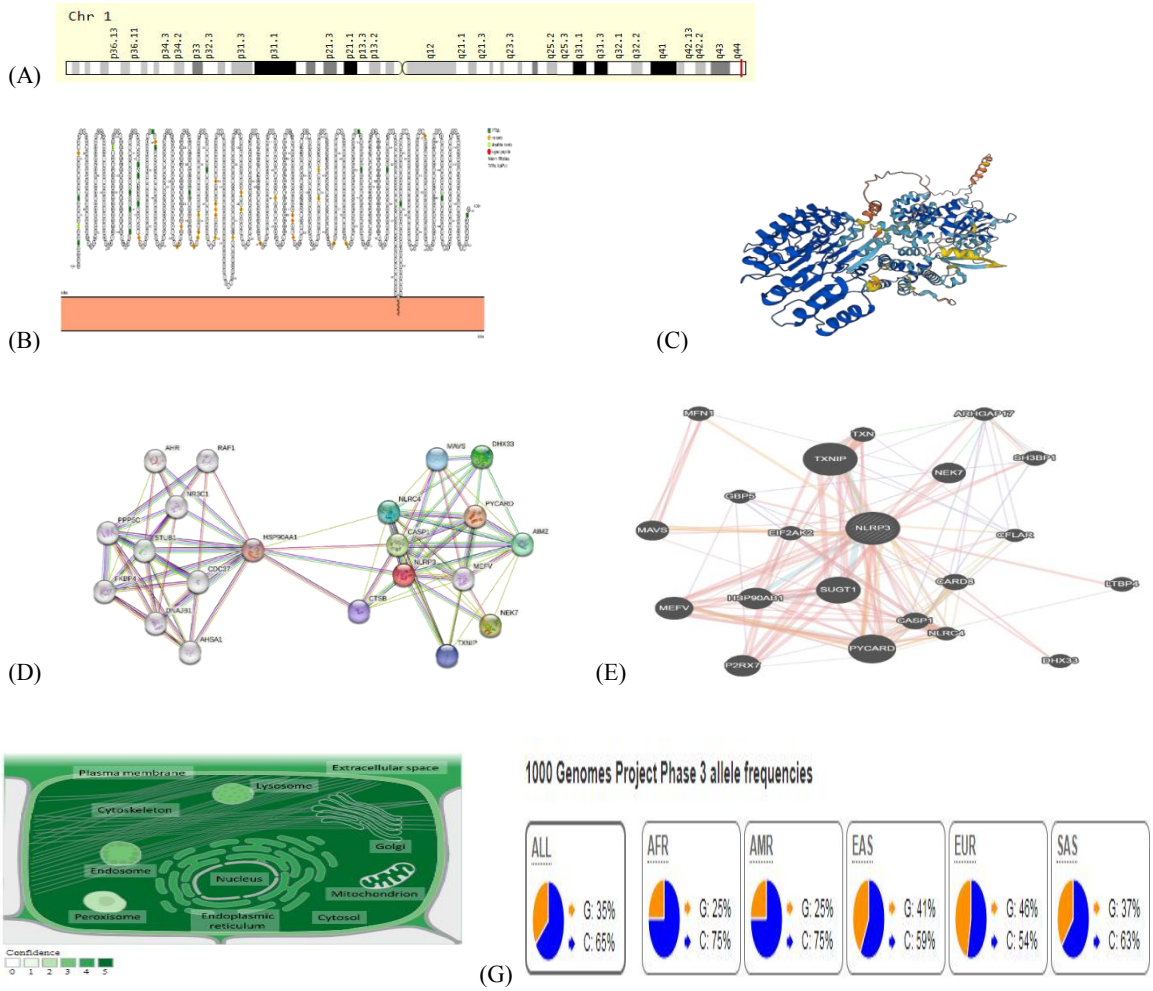


**Figure 3. Computational bioinformatic analysis of the NLRP3 gene. A.** Chromosomal localization of NLRP3 gene. The NLRP3 gene is situated within chromosome 1q44 and transverses 33,032 bases (chr1: 247,416,077-247,449,108) along the plus strand. B. The molecular structure of amino acid residues of NLRP3 protein. The amino acid residues of NLRP3 protein show 1036 amino acids. C. Tertiary structure of the NLRP3 protein. D. NLRP-3 Protein-protein interaction using STRING database. E. NLRP3 physical, genetic interaction. F. Subcellular localization of the NLRP3 (compartment database). G. Allele frequency of NLRP3 rs10754558 in different ethnicities. [Data source: HGNC: 16400; NCBI Gene: 114548; Ensembl: ENSG00000162711; OMIM®: 606416; UniProt: Q96P20].

Since SNPs are the most prevalent and studied types of genetic alteration, particular SNPs have been investigated as potential targets for disease diagnosis, prognosis, and treatment [30]. It has been shown that NLRP3 rs10754558 gene polymorphism may make people more susceptible to inflammatory conditions and a predisposition to autoimmune disorders as in Latin Americans [5, 31]. Other studies also indicated that NLRP3 SNPs have nothing to do with the risk of autoimmune diseases as in populations of Europeans, Arabs, or Asians according to the subgroup analysis of ethnicity [5]. To date, the NLRP3 rs10754558 polymorphism in T1D has only been the subject of a small number of research in the literature, so we have studied this SNP in our populations. An increased risk of type 2 diabetes mellitus relates to the NLRP3 rs10754558 genetic variation [32].

In a prior investigation, Rs10754558 SNP was strongly associated with a decreased risk of developing T1D compared to the healthy controls [33]. Conversely, our results revealed no significant connection between the NLRP3 genotype/allele frequencies in different genetic models and T1D susceptibility among the studied Egyptian population. Both genetic and environmental factors, including racial and ethnic diversity, the length of the disease, differences in sample size, and the studied age, may be responsible for these differences between our genetic findings and earlier research.

As chronic hyperglycemia progresses, prooxidants and the antioxidant defense system become out of balance, leading to oxidative stress and the accumulation of hazardous byproducts of free radical oxidation, which eventually play a critical role in diabetic complications [13, 34]. Compared to healthy controls, people with T1D have higher levels of oxidative stress, which may play a major role in the development of diabetic nephropathy, one of the consequences of the disease [35]. Increased MDA, a breakdown product of lipid peroxidation, and decreased TAC point to oxidative stress in the systemic and vascular systems [36]. TAC, a measure of antioxidant activity, and MDA, a marker of oxidative stress, were both examined in this investigation. When comparing T1D patients to healthy volunteers, the results revealed a statistically significant drop in serum TAC levels and a substantial rise in serum MDA levels. We also found that TAC and MDA were significantly associated with T1D risk in both univariate and multivariate analyses, and they may be utilized as independent predictors of the disease. This was consistent with previous findings that demonstrated elevated MDA levels in plasma in experimental models of T1D and the plasma of diabetic children and adolescents compared to controls [36]. Furthermore, the plasma TAC in children with diabetes was significantly lower than controls [37].

Chronic hyperglycemia, as measured by HbA1c, is a well-recognized predictor of macrovascular complications [38]. Our investigation showed TAC and HbA1c, not blood glucose levels, were statistically significantly correlated among T1D patients. Similar findings by Francescato et al. demonstrated a positive correlation between oxidative stress and HbA1c [39]. In the Valabhji et al. study, a rise in HbA1c and the duration of diabetes were linked to a decrease in TAC in T1D individuals [40]. Unlike previous studies, TAC did not correlate to the diabetic group's duration of diabetes, serum glucose levels, or HbA1c levels [41, 42].

Overall, we can confirm that T1D promotes higher oxidative stress, and that the oxidant/antioxidant balance will alter. Free radicals are now known to damage proteins and DNA. Thus, in the tissues and physiological fluids of T1D, we should expect increased lipid peroxidation and protein degradation, including hemoglobin, DNA, and protein. Accordingly, in the present study, persistent hyperglycemia, a drop in TAC levels, and a continuous rise in MDA levels may result in oxidative conditions that promote the earlier onset of related disorders. In T1D, oxidative stress indicators like TAC could be a helpful tool for tracking the development of problems. To successfully treat and prevent further complications in children with diabetes, it may be beneficial to evaluate oxidative stress and its relationship to the long-term glycaemic status, as shown by the HbA1c level. These findings are preliminary, and more research on a large cohort is necessary to determine the possible involvement of oxidative markers in T1D.

Regarding the associations between the selected SNP and T1D general characteristics, rs10754558 C/G of the NLRP3 did not show any significant links with the disease's clinical, and biochemical features, and with measured MDA and TAC. There are some restrictions we can't get around. First, a small number of our samples, and more validation in a wider population, as well as in other nations and ethnic groups, is necessary. Second, the fact that one SNP might not be completely representative of the entire gene could help to explain why there were no relationships between the chosen SNP and susceptibility to T1D. Furthermore, T1D is a multigenic illness, thus the impact of a single gene may be minimal. Future research may consider using more SNPs from the NLRP3 gene and larger sample sizes. Another benefit of our study was that all patients were treated at the same Endocrinology and Diabetes unit with the same follow-up procedures, unambiguous diagnostic standards, and phenotypic classification. Additionally, the genetic homogeneity of the group from which all patients and controls came limited any potential bias resulting from population structure. However, additional research in other populations is required to confirm the role of this mutation in genetic vulnerability.

## 3. Conclusions

Our results demonstrated that NLRP3 rs10754558 C/G polymorphism may not be related to T1D susceptibility. Additionally, oxidative stress status was shown to be unbalanced in Egyptian T1D patients, with serum TAC levels falling and MDA levels rising. A link between TAC and HbA1c among T1D patients was also seen. In this respect, measuring the amount of TAC in T1D may be a different approach for the early identification of diabetes. In addition, MDA and TAC were considered independent predictors of T1D development. However, additional study is necessary to verify our findings.

## 4. Conflicts of interest

The authors declared that there is no conflict of interest concerning the research, authorship, and publication of this article

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**7. References**

[1] Yahaya, T., Obaroh, I., Magaji, U., Obadiah, C., Anyebe, D., Shemishere, U. Genetic and Epigenetic Etiologies of Type 1 Diabetes Mellitus. I.J.D.O.16 (2): 127 -137, 2024. https://doi.org/10.18502/ijdo.v16i2.15712.

[2] Clark, M., Kroger, C.J., Tisch, R.M. Type 1 Diabetes: A Chronic Anti-Self-Inflammatory Response. Front. Immunol. 22 :8: 1898, 2017. https://doi.org/10.3389/fimmu.2017.01898. eCollection 2017. PMID: 29312356

[3] Xu, L. et al. Polymorphisms of the NLRC4 Gene are Associated with the Onset Age, Positive Rate of GADA, and 2-h Postprandial C-Peptide in Patients with Type 1 Diabetes. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 13:811–8, 2020. https://doi.org/10.2147/DMSO.S244882

[4] Abd El Azeem, R.A., Zedan, M.M., Saad, E.A., Mutawi, T.M.& Attia, Z.R. Single-nucleotide polymorphisms (SNPs) of antioxidant enzymes SOD2 and GSTP1 genes and SLE risk and severity in an Egyptian pediatric population. Clin. Biochem. 88:37–42, 2021. https://doi.org/10.1016/j.clinbiochem.2020.11.010.

[5] Wu, Z., Wu, S, Liang, T. Association of NLRP3 rs35829419 and rs10754558 Polymorphisms With Risks of Autoimmune Diseases: A Systematic Review and Meta-Analysis Front. Genet. 12: 690860, 2021. PMID: 34367252. https://doi.org/10.3389/fgene.2021.690860.

[6] Hitomi, Y. et al. Associations of functional NLRP3 polymorphisms with susceptibility to food-induced anaphylaxis and aspirin-induced asthma. J. Allergy Clin. Immunol. 124(4):779-85.e6, 2009. https://doi.org/10.1016/j.jaci.2009.07.044.

[7] Hirai, K., Kimura, T., Suzuki, Y., Shimoshikiryo, T., Shirai, T., Itoh, K. Gene Polymorphisms of NLRP3 Associated With Plasma Levels of 4β-Hydroxycholesterol, an Endogenous Marker of CYP3A Activity, in Patients With Asthma. Clin. Pharmacol. 116(1): 147-154, 2024. https://doi.org/10.1002/cpt.3254. Epub 2024 Mar 14. PMID: 38482940.

[8] Ren, W., Sun, Y., Zhao, L., Shi, X. NLRP3 inflammasome and its role in autoimmune diseases: A promising therapeutic targetBiomed. Pharmacother. 175: 116679, 2024. https://doi.org/10.1016/j.biopha.2024.116679. PMID: 38701567.

[9] Anber, N.H., Ahmed Shahin, H.E., Badawy, H.K., Oraby, E.A., Mohammed, S.A., Shaaban, E.I.A., et al. Potential Impact of SOD2 (rs4880; p.Val16Ala) Variant with the Susceptibility for Childhood Bronchial Asthma Biochem. Genet. 24, 2024. doi: 10.1007/s10528-024-10742-4. Online ahead of print. PMID: 38522064

[10] El Sadda, R.R., Elshahawy, Z.R.& Saad, E.A. Biochemical and pathophysiological improvements in rats with thioacetamide induced-hepatocellular carcinoma using aspirin plus vitamin C. BMC Cancer. 23: 175, 2023. https://doi.org/10.1186/s12885-023-10644-5.

[11] Habib, S.A.H., Alalawy, A.I., Saad, E.A.& El-Sadda, R.R. Biochemical and histopathological evaluations of chronic renal failure rats treated with pluripotent human stem cells. Braz. J. Pharm. Sci. 58:e20089, 2022. https://doi.org/10.1590/s2175-97902022e20089.

[12] Shosha, M.I., El-Ablack, F.Z.& Saad, E.A. Glycine protects against doxorubicin-induced heart toxicity in mice. Amino Acids. 55:679–93, 2023. https://doi.org/10.1007/s00726-023-03261-w.

[13] Bhatti, J.S. et al. Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. Free Radic. Biol. Med. 184:114-34 (2022). https://doi.org/10.1016/j.freeradbiomed.2022.03.019.

[14] Sharma, A. et al. Oxidative stress and NLRP3-Inflammasome activity as significant drivers of diabetic cardiovascular complications: therapeutic implications. Frontiers in Physiology. 9:114, 2018. https://doi.org/10.3389/fphys.2018.00114.

[15] Donaghue, K.C. et al. ISPAD Clinical Practice Consensus Guidelines 2018: Microvascular and macrovascular complications in children and adolescents. Pediatric Diabetes. 19:262-74, 2018. https://doi.org/10.1111/pedi.12742.

[16] Shah, J.K.& Walker's, A.M. Quantitative determination of MDA. Biochem. Biophys. Acta. 11:207-11, 1989.

[17] Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S.& Cosic, V. Method for the measurement of antioxidant activity in human fluids. J. Clin. Pathol. 54(5):356-61, 2001. https://doi.org/10.1136/jcp.54.5.356.

[18] Zhao, F., Song, M., Wang, Y.& Wang, W. Genetic model. J. Cell Mol. Med. 20(4):765, 2016. https://doi.org/10.1111/jcmm.12751.

[19] Argyropoulos, T., et al. Stress Hyperglycemia in Children and Adolescents as a Prognostic Indicator for the Development of Type 1 Diabetes Mellitus. Front. Pediatr. 9:670976, 2021. https://doi.org/10.3389/fped.2021.670976.

[20] Getawa, S. & Adane, T. Hematological abnormalities among adults with type 1 diabetes at the University of Gondar Comprehensive Specialized Hospital. SAGE Open Med.10:20503121221094212, 2022. https://doi.org/10.1177/20503121221094212.

[21] Chung, F.M., Tsai, J.C., Chang, D.M., Shin, S.J.& Lee, Y.J. Peripheral total and differential leukocyte count in diabetic nephropathy: the relationship of plasma leptin to leukocytosis. Diabetes Care. 28(7):1710-7, 2005. https://doi.org/10.2337/diacare.28.7.1710.

[22] Adane, T., Asrie, F., Getaneh, Z.& Getawa, S. White blood cells and platelet profiles of diabetic patients at University of Gondar specialized referral hospital: A comparative cross-sectional study. J Clin. Lab. Anal. 35(6):e23808, 2021. https://doi.org/10.1002/jcla.23808.

[23] Umeji, L. et al. Haematological profile of diabetes and non-diabetes patients in Abuja, Nigeria. IJRSI. 6(5):2321-705, 2019. https://www.semanticscholar.org.
[24] Alam, J. et al. A comparative analysis of biochemical and hematological parameters in diabetic and non-diabetic adults. Adv. Med. Sci. 2(1):1–9, 2015. https://scholar.google.com.
[25] Grossman, C., Dovrish, Z., Koren-Morag, N., Bornstein, G.& Leibowitz, A. Diabetes mellitus with normal renal function is associated with anemia. Diabetes Metab. Res. Rev. 30(4):291-6, 2014. https://doi.org/10.1002/dmrr.2491.
[26] Rivetti, G., Hursh, B.E., Del Giudice, M.E.& Marzuillo, P. Acute and chronic kidney complications in children with type 1 diabetes mellitus. Pediatr. Nephrol. 38(5):1449-58, 2023. https://doi.org/10.1007/s00467-022-05689-w.
[27] Zhang, Y., Yang, W., Li, W.& Zhao, Y. NLRP3 Inflammasome: Checkpoint Connecting Innate and Adaptive Immunity in Autoimmune Diseases. Front. Immunol. 12:732933, 2021. https://doi.org/10.3389/fimmu.2021.732933.
[28] Sun, X. et al. rs3806265 and rs4612666 of the NLRP3 Gene Are Associated With the Titer of Glutamic Acid Decarboxylase Antibody in Type 1 Diabetes. Front Endocrinol (Lausanne). 13:835054, 2022. https://doi.org/10.3389/fendo.2022. 835054
[29] Lage, S.L., et al. Persistent Oxidative Stress and Inflammasome Activation in CD14high CD16- Monocytes From COVID-19 Patients. Front. Immunol. 12:799558, 2022. https://doi.org/10.3389/fimmu.2021.799558.
[30] Lu. Q., Lao, X., Gan, J., Du, P., Zhou, Y., Nong, W., et al. Impact of NLRP3 gene polymorphisms (rs10754558 and rs10733113) on HPV infection and cervical cancer in southern Chinese population. Infect Agent Cancer. 18(1): 64, 2023. doi: 10.1186/s13027-023-005294. PMID: 37885032
[31] Abolfathi, M., Bagheri, N., Iranparast, S., Soltani, A., Sanaei, A., Rahimian, G., et al. Associations of a NLRP3 rs10754558 Polymorphism with Helicobacter pylori-Infected Patients with Gastritis and Peptic Ulcer Disease. Jundishapur. J. Microbiol. 12(5): e88231, 2019. https://doi.org/10.5812/jjm.88231
[32] Bai, L. et al. Association of two common SNPs in NLRP3 with risk of type 2 diabetes mellitus and their interaction with environmental factors. Int. J. Clin. Exp. Pathol. 9(10):10499-506, 2016. www.ijcep.com/ISSN:1936-2625/IJCEP0037524.
[33] Pontillo, A. Two SNPs in NLRP3 Gene Are Involved in the Predisposition to Type-1 Diabetes and Celiac Disease in a Pediatric Population From Northeast Brazil. Autoimmun. 43(8):583–9, 2010. https://doi.org/10.3109/08916930903540432.
[34] Ametov, A.S., Alimova, G.U. Diabetes mellitus and oxidative stress. Endocrinology. (1-2): 47-50, 2014.
[35] Elhenawy, Y.I., Thabet, R.A., Fereig, Y. A., Hegazy, M. G.A., El-Morsy, S.A.M. Oxidative Stress in Children and Adolescents with Diabetic Nephropathy. *QJM: An International Journal of Medicine*. 117(2): hcae175.851, 2024. https://doi.org/10.1093/qjmed/hcae175.851.
[36] Boarescu, P.M., Boarescu, I., Pop, R.M., Roşian, Ş.H., Bocşan, I.C., Rus, V., et al. Evaluation of Oxidative Stress Biomarkers, Pro-Inflammatory Cytokines, and Histological Changes in Experimental Hypertension, Dyslipidemia, and Type 1 Diabetes Mellitus. Int. J. Mol. Sci. 23(3):1438, 2022. https://doi.org/10.3390/ijms23031438.
[37]Stambouli-Guerriche, A.B., Mokhtari-Soulimane, N., Merzouk, H.& Bendedouche, A. Elevation of oxidative stress markers in type 1 diabetic children. J. Diab. Endocrinol. 6(2):5–11, 2015. https://doi.org/10.5897/JDE2014.0083.
[38] Morandi, A., Corradi, M., Orsi, S., Piona, C., Zusi, C., Costantini, S., Marigliano, M., Maffeis, C. Oxidative stress in youth with type 1 diabetes: Not only a matter of gender, age, and glycemic control. Diabetes Res Clin Pract. 179(4): 109007, 2021. DOI: 10.1016/j.diabres.2021.109007. Epub 2021 Aug 16. PMID: 34411622
[39] Francescato, M.P., Stel, G., Geat, M.& Cauci, S. Oxidative stress in patients with type 1 diabetes mellitus: is it affected by a single bout of prolonged exercise? PLoS ONE. 9(6): e99062, 2014. https://doi: 10.1371/journal.pone.0099062.
[40] Valabhji J., McColl A.J., Richmond W., Schachter M., Rubens M.B., Elkeles R.S. Total antioxidant status and coronary artery calcification in type 1 diabetes. Diabetes Care 24(9):1608-13, 2001. https://doi.org/10.2337/diacare.24.9.1608.
[41] Dordevic, G., Duric, S., Apostolskit, S., Dordevic, V.& Zivkovic, M. Total antioxidant blood capacity in patients with type 2 diabetes mellitus and distal symmetrical polyneuropathy. Vojnosanit Pregl. 65(9):663-9, 2008. https://doi.org/10.2298/vsp0809663d.
[42] Vessby, J., Basu, S,, Mohsen, R., Berne, C., Vessby, B. Oxidative stress and antioxidant status in type 1 diabetes mellitus. J. Intern. Med. 251(1):69-76, 2002. https://doi.org/10.1046/j.13652796.2002.00927.x.PMID: 11851867