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# Tissue inhibitor of metalloproteinase-1 (TIMP-1) serum level and genetic polymorphisms associated with cutaneous leishmania infections

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ABSTRACT

*Introduction:* Cutaneous leishmaniasis is considered a parasitic contagion resulting from the flagellated parasite belonging to the genus of Leishmania. Also, cutaneous leishmaniasis is a zoonotic ailment transmitted through the bloodsucking sand-flies bite (belonging to the Phlebotomus genus). The disease's reservoirs included wild or semi-domesticated animals, in general rodents and dogs. Tissue inhibitor metalloproteinase-1 (TIMP-1) is one of the extracellular matrix proteins that have a role in vessel wall degeneration and aneurysm development. In addition, it belongs to the zinc-dependent endopeptidases family that are involved in the degradation of connective tissues proteins which are included in vascular integrity maintenance. The Genetic deviations in the TIMP-1 genes might impact their expression at the transcription level or the enzyme activity. Therefore, the present study aimed to detect the impact of TIMP-1 serum level and single nucleotide polymorphisms (SNPs) rs41454248 and rs1043428 among the cutaneous leishmaniasis patients' group compared to the control group.
*Subjects:* Seventy-five cutaneous leishmaniasis patients (39 males and 36 females) with the age mean 23.91 ± 13.14 years participated in this study, compared to the matched number, age, and gender of a healthy control group (75: 38 males and 37 females) with the age mean 22.84 ± 4.35 years. In the current study, the serum level of TIM-1 and rs41454248 and rs1043428 SNPs were studied among the cutaneous leishmaniasis patients' group compared to the control group.
*Results:* The findings of the TIMP-1 level referred to a significant decrease among the cutaneous leishmaniasis patients' group compared to the healthy control group (26339.67 ± 900.79 *vs.* 33480.25 ± 1098.63). Such, the rs41454248 SNPs findings referred that the GG genotype and *G* allele were non-significantly increased frequency percentage in cutaneous leishmaniasis patients group compared to the healthy control group (29.33 *vs.* 18.67%, OR: 1.81, $p = 0.180$; 55.0 *vs.* 47.0%, OR: 1.38, $p = 0.204$ respectively). Also, the high OR value of GG genotype and *G* allele referred to this genotype and allele might be a risk factor for cutaneous leishmaniasis. Likewise, the findings of rs1043428 SNPs appeared that the CC genotype and *C* allele were significantly increased frequency percentage in cutaneous leishmaniasis patients' group compared to the control group (37.33 *vs.* 4.0%, OR: 14.30, $p = 3.6 \times 10^{-7}$; 57.0 *vs.* 21.33, OR: 4.82, $p = 4.5 \times 10^{-10}$). Also, the high OR value of CC genotype and *C* allele referred to this genotype and allele might be risk factors for cutaneous leishmaniasis. In addition, the CG genotype appeared a non-significant increased frequency percentage in the patients' group compared to the control group and the value of OR referred to might be a risk factor for cutaneous leishmaniasis (33.33 *vs.* 25.33, OR: 1.47, $p = 0.370$). In addition, the serum level of TIMP-1 with the rs41454248 was significantly decreased in GA and AA genotypes of the patients' group compared to the control. While the level was non-significantly decreased in the GG genotype of the patients' group compared to the control group. Likewise, the level of TIMP-1 with the rs1043428 was non-significantly decreased in all genotypes (except TT genotype) of the patients' group compared to the control. Whereas, a significant decrease level was appeared in the TT genotype of the patients' group compared to the healthy control group.
*Conclusion:* The current findings demonstrated a significant association between TIMP-1 serum level and genetic polymorphisms (rs1043428 and rs41454248) among cutaneous leishmaniasis patients.

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## 1. Introduction

Cutaneous leishmaniasis is one of the parasitic hits caused by the flagellated parasite belonging to the Leishmania genus. It is considered a zoonotic disease transmitted through the bite of the bloodsucking sandflies that belong to the genus of Phlebotomus (Mokni, 2019). The disease is disseminated extensively worldwide in Africa, the Americas, Asia, and Europe (Ansari et al., 2006). The disease epidemiology is influenced by several agents such as environmental, climatical and migratory factors (Sharma et al., 2005). The identification of leishmaniasis types is mainly based on biochemical peculiarities. The Leishmania parasites are dimorphic intracellular creatures inside the phagosome of the host's immune cells, while it appears as a single-cell flagellated protozoan in the gut tissues of vector and culture (Sharma et al., 2000). There are three main clinical configurations: cutaneous leishmaniasis, mucosal leishmaniasis and visceral leishmaniasis. The clinical appearance rides on the elements related to the parasite virulence, body immune response and lesions regions (Ansari et al., 2008). Although, that each type of leishmaniasis may have specified cutaneous scores and endemic sites, the most prevalent appearances are crusted, plaques and ulcerated nodules (Mokni, 2019).

The prime pathomecha nism included in the aneurysm formation is considered to be chronic inflammation leads to hemodynamic strain at the blood vessels bifurcations (Hashimoto et al., 2006). The infiltrating macrophages and neutrophil cells excrete the matrix metalloproteinases (MMPs) which are considered as extracellular matrix proteins (ECM) resulting in vessel wall degeneration and aneurysm developments (Aoki et al., 2007). The Metalloproteinases (MMPs) belong to the zinc-dependent endopeptidases family that are involved in the connective tissues' proteins degradation such as collagens, elastin, gelatins, and proteoglycans which are included in the vascular integrity preservation (Onal et al., 2009; Maradni et al., 2013; Mackawy and Megahed, 2017). Tissue inhibitor of matrix metalloprotease-1 (TIMP-1) is the inhibitor of the MMPs, inhibits the extracellular matrix degradation that MMPs intermediated its (Wang, 2005; Robert et al., 2016; Asgari et al., 2021). TIMP-1 as well can attach to the CD63/Integrin β1 complex and participate in the drug resistance (Toricelli et al., 2013; Lee et al., 2014; Forte et al., 2017). Moreover, TIMP-1 stimulates the nuclear factor-kappa B (NF-κB) and participates in the activation of mitogen-activated protein kinase signalling-5 pathways (Li et al., 2019). The Genetic variances in the TIMP-1 genes may impact their expression at the transcription level or enzyme activity (Krex et al., 2004) The role of TIMP-1 gene variances was previously studied in many populations (Indelicato et al., 2006; Hinterseher et al., 2007; Boggio et al., 2010; Tabatabaee et al., 2018; 'Oria et al., 2021). The present study was designated to reveal the impact of the serum level and genetic polymorphisms of TIMP-1 rs1043428 and rs41454248 in cutaneous leishmaniasis patients compared to the healthy control group.

## 2. Subjects

### 2.1. Sample collection

All the blood samples were collected from the cutaneous leishmaniasis and healthy control who enrolled Baquba Teaching Hospital, Baquba city, Diyala province, Iraq. The personal approval of all participants was taken before collecting the sample. Concerning the scientific research ethics, the current study was approved according to the Helsinki declaration for Human subjects' research (World Medical Association, 2013), in addition to the approval ethics of the Ministry of Health. The samples collecting was in the period from October 2020 to January 2021.

The participants were divided into two groups, the cutaneous leishmaniasis group consist of 75 participants (39 males and 36 females) with age mean 23.91 ± 13.14 years, compared with the matched number, age, and gender of a healthy control group (75: 38 males and 37 females) with age mean 22.84 ± 4.35 years.

Venous blood (5 ml) was collected from each participant, and then divided into two parts; 2 ml of the blood in EDTA tube for genomic experiments of the TIMP-1 gene polymorphisms rs1043428 and rs41454248, 3 ml was put in a silicone gel tube, then let to coagulate for getting the serum to assess the level of TIMP-1.

The questionnaire was used to inquire about the presence of chronic and inflammatory diseases for all individuals included in the study and those with this disease were excluded from the study.

### 2.2. Genomic DNA extraction

The DNA was extracted according to the procedure of the traditional genomic DNA extraction supplied by INTRON Company, Korea. The purity of the extracted DNA reached 1.7 to 2.0, while the concentration was recorded to be 50 to 100 ng/ml that calculated by the Nanodrop.

### 2.3. Primer's preparation

The primers were designed by using the NCBI-primer blast online website. The designed primers were prepared as the instructions of the supplier Scientific Researcher Co. Ltd., Diwaniya, Iraq. The primers information and condition were illustrated in Table 1

### 2.4. Polymerase chain reaction (PCR) protocol

The Allele-specific primer technique in the detection of single nucleotide polymorphisms (SNPs) of TIMP gene rs41454248 and rs1043428, was followed the applied protocol illustrated by adding in each Eppendorf tube (2 Eppendorf tube for each sample of the rs41454248) 12.5 μl of master mix, 2 μl of DNA, 2 μl of primers (1 μl of the forward 1 primer and 1 μl of the common reverse primer of the first Eppendorf tube and 1μl of the froward 2 primer and the common reverse primer of the second Eppendorf tube), and complete the volume to 25 μl with free nucleases water. while in rs1043428 the protocol was by adding in each Eppendorf tube (3 tubes for each sample), 12.5 μl of master mix, 2 μl of DNA, 2 μl or primers (1μl of the forward 1 primer and 1μl of the common reverse primer of the first Eppendorf tube, 1 μl of the forward 2 primer and 1 μl of the common reverse primer of the second Eppendorf tube, and finally 1 μl of the forward 3 primer and the common reverse of the third Eppendorf tube), then complete the volume to 25 μl by adding the free nucleases water.

The protocol of thermocycler for both TIMP SNPs involved three steps; the first step was at 95 C° for 10 minutes. The second step included 95 C° for 35 seconds, 57 C° for 35 seconds, and 72 C° for 35 seconds, this step was repeated 40 times. The final step included 72 C° for 10 minutes.

The PCR amplicons were visualized through the electrophoresis of these products on agarose gel (1.5%) that was stained with the safety stain (Red Safe stain from INTRON Company, Korea) then the bands were visualized using a UV transilluminator.

**Table 1**
The primers information and condition of TIMP-1 gene polymorphisms rs41454248 and rs1043428.

| rs41454248 | Sequence (5'->3') | Product length |
|---|---|---|
| Forward primer 1 | GCCCCGCCTTTCTCCTTAG | 120 bp |
| Forward primer 2 | ACCCCGCCTTTCTCCTTAG | |
| Reverse primer | GTAGGTCTTGGTGAAGCCCC | |
| rs1043428 | | |
| Forward primer1 | CCGCCATGGAGAGTGTCTG | 168 bp |
| Forward primer2 | CGGCCATGGAGAGTGTCTG | |
| Forward primer3 | CTGCCATGGAGAGTGTCTG | |
| Reverse primer | AGTGGGACCATTCCCATCAG | |

### 2.5. Statistical analysis

The homogeneity, normality and normal distribution of the parametric data were checked before calculating the mean, standard deviation, Student's T-test by utilizing the IBM SPSS program version 26.0 (IBM Corp. Released, 2019), the probability was considered significant when it was $< 0.05$. While Pearson's chi-square was used to calculate the probability for the non-parametric data. In addition, the odd ratio, 95% confidence interval and Fisher's exact probability were calculated by WinPepi version 11.65 (Abramson, 2011) for the genotyping and alleles frequencies. Such for the genotyping and alleles frequencies calculations, an online Hardy-Weinberg calculator was used (Andrews, 2010).

## 3. Results

The current results of the seventy-five patients suffering from cutaneous leishmaniasis included thirty-nine males and thirty-six females that compared with seventy-five healthy control individuals included thirty-eight males and thirty-seven females. The parametric results were normally distributed. There was no significant difference between the age mean of the two groups (Table 2). The power of the sample (1 – β error probability) (Two-tailed) was 0.50, while the effect size was 0.5 and the α probability (Two-tailed) was 0.62 for the total number of cutaneous leishmaniasis (Table 2).

The current findings of TIMP-1 level referred to a significant decrease in cutaneous leishmaniasis patients' group compared to the control group (26339.67 ± 900.79 *vs.* 33480.25 ± 1098.63) (Table 2)

Also, the genetic variation of the TIMP-1 single nucleotide polymorphisms (SNPs) rs41454248 was investigated two alleles (*G* and *A*) were corresponding to three genotypes (GG, GA and AA), Which were genotyped by polymerase chain reaction with allele-specific primers (PCR-ASP) technique (Ye et al., 2012; Abbas et al., 2020). In this technique, if the participant has the homozygote GG genotype, it appeared a single band of the well loaded with Forward 1 primer in agarose gel. While, if the participant has the heterozygote GA genotype, it appeared two bands in agarose gel, one band of the well loaded with Forward 1 primer and the other band with the well loaded with the Forward 2 primer. In contrast, if the participant has the homozygote AA genotype, it appeared as a single band of the well loaded with Forward 2 primers in agarose gel (Fig. 1).

In addition, the genotyping and alleles frequencies of TIPM-1 rs41454248 SPNs appeared that the genotypes of both groups were compatible to hardy-Weinberg equilibrium. The GG genotype and *G* allele were non-significantly increased frequency percentage in cutaneous leishmaniasis patients' group compared to healthy control group

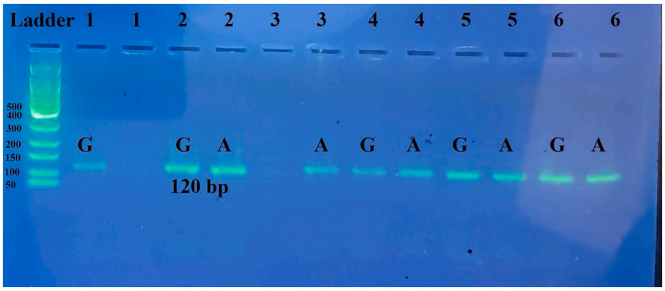


**Fig. 1.** Gel electrophoresis of TIMP-1 SNP rs41454248 PCR product on 1.5% agarose gel stained with Red Safe stain (Intron company, Korea) for 45 min. at 100 voltages. Ladder: Universal DNA Ladder (Intron company, Korea), 1 – 6: electrophoresed samples' numbers, the single band for the GG and AA genotypes (wild and mutant homozygote, respectively), the double bands for the GA genotype (mutant heterozygote).

(29.33 *vs.* 18.67, OR: 1.81, $p = 0.180$; 55.0 *vs.* 47.0, OR: 1.38, $p = 0.204$ respectively) (Table 3). Also, the high OR value of GG genotype and *G* allele referred to this genotype and allele might be a risk factor for cutaneous leishmaniasis (1.81, 1.38 respectively) (Tale 3). In addition, the frequency percentage of GA and AA genotypes were increased males' subgroup compared to females' subgroup in the patients' group (51.3 *vs.* 50.0% and 20.5 *vs.* 19.4%, respectively) (Table 4).

Such, the present findings appeared that GG genotype individuals in both groups have a non-significant increased level of TIMP-1 compared with the other genotypes (28447.27 ± 7512.66 and 34268.08 ± 9630.30 pg/ml respectively), while the AA genotype of patients group and GA genotype in the healthy control group have the lowest level (21741.67 ± 8366.97 and 32725.0 ± 7998.64 pg/ml respectively) (Table 5). In addition, the present findings appeared that there was a non-significant decrease level of TIMP-1 in the GG genotype of the patients' group compared to the control (28447.27 ± 7512.66 *vs.* 34268.08 ± 9630.30 pg/ml). Whereas, the significant decrease level of TIMP-1 appeared in GA and AA genotypes of patients group compared to the healthy control group (26934.47 ± 7184.40 *vs.* 32725.0 ± 7998.64 and 2174.67 ± 8366.97 *vs.* 34725.77 ± 9491.96 pg/ml). (Table 5).

Three alleles were detected at TIMP-1 rs1043428 SNPs (*C, G,* and *T*) which were corresponding to sex genotypes (CC, CG, GG, CT, GT, and TT). Which were genotyped by polymerase chain reaction with allele-specific primers (PCR-ASP) technique [Ye et al., 2012]. In this technique, if the participant has the homozygote CC, GG or TT genotypes appeared a single band of the well was loaded with Forward 1 primer, or Forward 2, or Forward 3 respectively in agarose gel. While, if the

**Table 2**
The demographic data of the studied groups.

| | | Patients group | Control group | Probability |
|---|---|---|---|---|
| Total samples size | | 75 | 75 | |
| Gender | Males (%) | 39 (52.0) | 38 (50.7) | 0.870 |
| | Females (%) | 36 (48.0) | 37 (49.3) | |
| Age (mean ± SD) | | 23.91 ± 13.14 | 22.84 ± 4.35 | 0.506 |
| Family history | Yes | 27 (36.0) | 0 (0.0) | **$9.6 \times 10^{-9}$** |
| | No | 48 (64.0) | 75 (100.0) | |
| Chronic diseases | Yes | 12 (16.0) | 0 (0.0) | **0.00003** |
| | No | 63 (84.0) | 75 (100.0) | |
| Other diseases | Yes | 1 (1.3) | 0 (0.0) | 0.316 |
| | No | 74 (98.7) | 75 (100.0) | |
| Lesions number (mean ± SD) | | 2.96 ± 2.09 | 0 | – |
| Power of samples (1 – β error probability (Two-tailed) | | | | 0.50 |
| Effect size | | | | 0.5 |
| α error probability (Two-tailed) | | | | 0.62 |
| TIMP-1 (pg/ml) | 26339.67 ± 900.79 | | 33480.25 ± 1098.63 | **0.000001** |

**Table 3**
The genotyping and alleles frequencies of TIMP-1 rs41454248 SNPs.

| Genotypes | Patients group No. (%) | Control group No. (%) | OR (95% CI) | Fisher's exact probability |
|---|---|---|---|---|
| GG | 22 (29.33) | 14 (18.67) | 1.81 (0.85–3.86) | 0.180 |
| GA | 38 (50.67) | 42 (56.0) | 0.81 (0.43–1.53) | 0.624 |
| AA | 15 (20.0) | 19 (25.33) | 0.74 (0.34–1.58) | 0.559 |
| Total | 75 (100.0) | 75 (100.0) | | |
| *P*-HWE | 0.85 | 0.28 | | |
| Allele's frequency | | | | |
| *G* | 82 (0.55) | 70 (0.47) | 1.38 (0.88–2.17) | 0.204 |
| *A* | 68 (0.45) | 80 (0.53) | 0.73 (0.46–1.14) | |

*P*-HWE: the probability of Hardy-Weinberg equilibrium, OR: odds ratio, 95% CI: 95% confidence intervals.

**Table 4**
The genotyping and alleles frequencies of TIMP-1 rs41454248 SNPs according to the gender of the studied groups.

| Genotyping | Patients group No. (%) | | Control group No. (%) | | Fisher's exact probability |
|---|---|---|---|---|---|
| | Males | Females | Males | Females | |
| GG | 11 (28.2) | 11 (30.6) | 7 (18.4) | 7 (18.9) | 1.0 |
| GA | 20 (51.3) | 18 (50.0) | 21 (55.3) | 21 (56.8) | 0.827 |
| AA | 8 (20.5) | 7 (19.4) | 10 (26.3) | 9 (24.3) | 1.0 |
| Total | 39 (100.0) | 36 (100.0) | 38 (100.0) | 37 (100.0) | |

**Table 5**
The TIMP-1 level between the TIMP-1 rs41454248 SNPs of the cutaneous leishmaniasis genotyping group compared to the genotyping of a healthy control group.

| Genotyping | TIMP-1 level (pg/ml) mean ± SD | | Probability |
|---|---|---|---|
| | Patients group | Healthy control group | |
| GG | 28447.27 ± 7512.66$^{A}$ | 34268.08 ± 9630.30$^{A}$ | 0.312 NS |
| GA | 26934.47 ± 7184.40$^{A}$ | 32725.0 ± 7998.64$^{A}$ | **0.031*** |
| AA | 21741.67 ± 8366.97$^{A}$ | 34725.77 ± 9491.96$^{A}$ | **0.001**** |

Duncan test: the identical letters indicated the non-significant difference (P > 0.05) between the genotypes of the same group.
NS: Non-significant.
* Significant at 0.05.
** Significant at 0.001.

participant has the heterozygote CG, or CT, or GT genotypes, appeared two bands in agarose gel, one band of the well loaded with Forward 1 primer and the other band with the well loaded with the Forward 2 primer for CG genotype, or in the well loaded with the Forward 1 primer and the other band in the well loaded with the Forward 3 primer for the CT genotype, or in the well loaded with the Forward 2 primer and the other band in the well loaded with the Forward 3 primer for the GT genotype (Fig. 2).

In addition, the genotyping and alleles frequencies of TIPM-1 rs1043428 SPNs appeared that the genotypes of both groups were compatible to hardy-Weinberg equilibrium (Table 6). The CC genotype and *C* allele were significantly increased frequency percentage in cutaneous leishmaniasis patients group compared to healthy control group (37.33 *vs.* 4.0, OR: 14.30, $p = 3.6 \times 10^{-7}$; 57 *vs.* 21.33, OR: 4.82, $p = 4.5 \times 10^{-10}$ respectively) (Table 6). Also, the high OR value of CC genotype and *C* allele referred to this genotype and allele might be a risk factor for

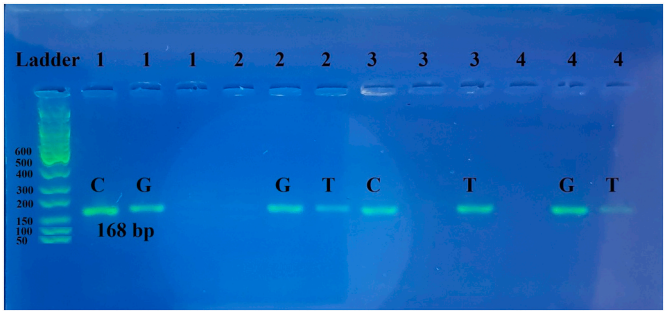


**Fig. 2.** Gel electrophoresis of TIMP-1 SNP rs1043428 PCR product on 1.5% agarose gel stained with Red Safe stain (Intron company, Korea) for 45 min. at 100 voltages. Ladder: Universal DNA Ladder (Intron company, Korea), 1–4: electrophoresed samples' numbers, the single band for the CC, GG and AA genotypes (wild and mutant homozygote, respectively), the double bands for the CG, CT and GT genotype (mutant heterozygote).

**Table 6**
The genotyping and alleles frequencies of TIMP-1 rs1043428 SNPs in cutaneous leishmaniasis patients' group compared to the control group.

| Genotypes | Patients group No. (%) | Control group No. (%) | OR (95% CI) | Fisher's exact probability |
|---|---|---|---|---|
| CC | 28 (37.33) | 3 (4.0) | 14.30 (4.15-49.30) | **$3.6 \times 10^{-7}$** |
| CG | 25 (33.33) | 19 (25.33) | 1.47 (0.73 – 2.98) | 0.370 |
| GG | 8 (10.67) | 16 (21.33) | 0.44 (0.18–1.10) | 0.118 |
| CT | 4 (5.33) | 7 (9.33) | 0.55 (0.15–1.94) | 0.533 |
| GT | 8 (10.67) | 28 (37.33) | 0.20 (0.08–0.48) | **$2.2 \times 10^{-4}$** |
| TT | 2 (2.67) | 2 (26.67) | 1.0 (0.14–7.19) | 1.0 |
| Total | 75 (100.0) | 75 (100.0) | | |
| *P*-HWE | 0.08 | 0.10 | | |
| Allele's frequency | | | | |
| *C* | 85 (0.57) | 32 (21.33) | 4.82 (2.91–7.99) | **$4.5 \times 10^{-10}$** |
| *G* | 49 (0.32) | 79 (52.67) | 0.44 (0.27–0.70) | **$6.8 \times 10^{-4}$** |
| *T* | 16 (11.0( | 39 (26.0) | 0.34 (0.18–0.64) | **$9.1 \times 10^{-4}$** |

cutaneous leishmaniasis (14.30, 4.82 respectively) (Table 6). Such, the CG genotype showed a non-significant increased frequency percentage in the cutaneous leishmaniasis group compared to the healthy control group, and the value of OR referred to might be a risk factor for cutaneous leishmaniasis (33.33 *vs.* 25.33, OR: 1.47, $p = 0.370$). While the GT genotype appeared a significant decrease in frequency percentage in the cutaneous leishmaniasis patients' group compared to the healthy control group (10.67 *vs.* 37.33, OR: 0.55, $p = 2.2 \times 10^{-4}$) (Table 6). In addition, the *G* and *T* alleles appeared a significantly decreased frequency percentage in the cutaneous leishmaniasis patients group compared to the healthy control group (33 *vs.* 52.67, OR: 0.44, $p = 6.8 \times 10^{-4}$ and 11.0 *vs.* 26.0, OR: 0.34, $p = 9.1 \times 10^{-4}$) (Table 6). The OR value of *G* and *T* alleles referred to these alleles might have a protective role from infected with cutaneous leishmaniasis.

In addition, the frequency percentage of the CG and CT genotypes were increased in males' subgroup compared to females' subgroup in the patients' group (35.9 *vs.* 30.6%, and 10.3 *vs.* 0.0%, respectively) (Table 7). While the frequency percentage of CC genotype was decreased in males' subgroup compared to females' subgroup in the patients' group (30.8 *vs.* 44.4%) (Table 7).

*P*-HWE: the probability of Hardy-Weinberg equilibrium, OR: odds ratio, 95% CI: 95% confidence intervals.

**Table 7**
The genotyping and alleles frequencies of TIMP-1 rs1043428 SNPs according to the gender of the studied groups.

| Genotyping | Patients group No. (%) | | Control group No. (%) | | Fisher's exact probability |
|---|---|---|---|---|---|
| | Males | Females | Males | Females | |
| CC | 12 (30.8) | 16 (44.4) | 2 (5.3) | 1 (2.7) | 0.576 |
| CG | 14 (35.9) | 11 (30.6) | 11 (28.9) | 8 (21.6) | 1.0 |
| GG | 4 (10.3) | 4 (11.1) | 9 (23.7) | 7 (18.9) | 1.0 |
| CT | 4 (10.3) | 0 (0.0) | 0 (0.0) | 7 (18.9) | $3.0 \times 10^{-3}$ |
| GT | 4 (10.3) | 4 (11.1) | 15 (39.5) | 13 (35.1) | 1.0 |
| TT | 1 (2.6) | 1 (2.8) | 1 (2.6) | 1 (2.7) | 1.0 |
| Total | 39 (100.0) | 36 (100.0) | 38 (100.0) | 37 (100.0) | |

Such, the present findings appeared that GG genotype participants in the cutaneous leishmaniasis group and TT participants in the healthy control group have a non-significant increased level of TIMP-1 compared with the other genotypes (30757.50 ± 7915.12 and 40220.0 ± 2651.65 pg/ml respectively). while, the TT genotype of the patients' group and CT genotype in the healthy control group have the lowest level (13795.0 ± 13859.29 and 29652.14 ± 10600.65 pg/ml respectively) (Table 8). In addition, the present findings appeared that there was a non-significant increased level of TIMP-1 in the GG genotype of the patients' group compared to the control (30757.50 ± 7915.12 vs. 29730.0 ± 4554.24 pg/ml). Whereas, the significant decrease level of TIMP-1 was appeared in the TT genotype of the patients' group compared to the healthy control group (13795.0 ± 13859.29 vs. 40220.0 ± 2651.65 pg/ml). (Table 8).

**Table 8**
The TIMP-1 level between the TIMP-1 rs1043428 SNPs of the cutaneous leishmaniasis genotyping compared to the genotyping of a healthy control group.

| Genotyping | TIMP-1 level (pg/ml) mean ± SD | | Probability |
|---|---|---|---|
| | Patients group | Healthy control group | |
| CC | 24770.0 ± 7075.81$^{A}$ | 33911.67 ± 4889.87$^{A}$ | 0.758 NS |
| CG | 28677.0 ± 8256.63$^{A}$ | 34090.83 ± 7964.14$^{A}$ | 0.728 NS |
| GG | 30757.50 ± 7915.12$^{A}$ | 29730.0 ± 4554.24$^{A}$ | 1.0 NS |
| CT | 23920.0 ± 3993.02$^{A}$ | 29652.14 ± 10600.65$^{A}$ | 0.991 NS |
| GT | 24457.50 ± 3942.51$^{A}$ | 35043.15 ± 9605.72$^{A}$ | 0.052 NS |
| TT | 13795.0 ± 13859.29$^{A}$ | 40220.0 ± 2651.65$^{A}$ | **0.050**$^{*}$ |

Duncan test: the identical letters indicated the non-significant difference ($P > 0.05$) between the genotypes of the same group.
NS: Non-significant.
$^{*}$ Significant at 0.05.

## 4. Discussion

The present findings of TIMP-1 serum level were incompatible with previous studies referred to the plasma level of tissue inhibitors of metalloproteinase-1 (TIMP-1) was shown to be related with several diseases such as arterial stiffness, diastolic dysfunction and greater systolic in hypertensive patients, autoimmune lymphoproliferative syndrome and Dianzani's autoimmune lymphoproliferative disease (Lindsay et al., 2002; Sekton, 2010; Flamant et al., 2007; Peeters et al., 2017; Tabatabaee et al., 2018). TIMPs also are natural inhibitors of the MMPs activity and thereby restrict the breakdown of ECM. By inhibiting the activity of MMP, it contributes to the tissue remodeling operation of the ECM. The balance between MMPs and TIMPs functions a crucial role in maintaining the integrity of the healthy tissues. The equilibrium disturbance of MMPs and TIMPs is noted in different pathological situations, including rheumatoid arthritis (RA), cancer, and periodontitis (Chaudhary et al., 2010; Lambert et al., 2004).

Also, the present results of TIMP-1 serum level agreed with the previous study referred that the level of TIMP-1 was detected highly significant in the patients' group compared to the healthy control (Ansari et al., 2008). This increased level of TIMP-1 was very necessary to reduce the risk of MMPs on tissue damage because the TIMPs play the main role as anti-MMPs and reduce the activity of its (Castés et al., 1984; Maretti-Mira et al., 2011). In addition, TIMP-1 has another role in other parasitic infections caused by the protozoa or helminths (Geurts et al., 2012) such as malaria caused by *Plasmodium* spp. (Kappe et al., 2010; Shikani et al., 2012), trypanosomiasis caused by the genus *Trypanosoma* (Kristensson et al., 2010), Toxoplasmosis caused by the intracellular parasite *Toxoplasma gondii* (Abbas et al., 2019; Salloom et al., 2011). The MMPs and MMPs inhibitor (TIMPs) have a role in the pathogenesis mechanism through their function as regulator and effector of the immune response (Bruschi and Pinto, 2013; Clark et al., 2011).

Regarding the findings of TIMP-1 rs41454248 and rs1043428 gene polymorphisms were studied for the first time.

## 5. Conclusion

From the present findings, we can conclude that the TIMP-1 serum level was significantly decreased in the patients' group compared to the control group. Regarding the genotyping and alleles frequencies findings, it showed that the GG genotype and *G* allele of rs41454248 were non-significantly increased frequency percentages in the patients' group compared to the control group. In addition, the high OR value of GG genotype and *G* allele referred to this genotype and allele might be a risk factor for cutaneous leishmaniasis. While in rs1043428, the findings showed that the CC genotype and *C* allele were significantly increased frequency percentage in the patients' group compared to the control group. Also, the high OR value of CC genotype and *C* allele referred to this genotype and allele might be risk factors for cutaneous leishmaniasis. In addition, the CG genotype showed a non-significant increased frequency percentage in the patients' group compared to the control group and the value of OR referred to might be a risk factor for cutaneous leishmaniasis. While, the GT genotype appeared a significant decrease in frequency percentage in the patients' group compared to the control group. In addition, the *G* and *T* alleles appeared a significantly decreased frequency percentage in the patients' group compared to the control group. The OR value of *G* and *T* alleles referred to these alleles might have a protective role from infected with cutaneous leishmaniasis.

Also, the level of TIMP-1 with the rs41454248 was significantly decreased in the GA genotype of the patients' group compared to the control. While the level was non-significantly decreased in the GG and AA genotypes of the patients' group compared to the control group. In addition, the level of TIMP-1 with the rs1043428 was a non-significantly increase in the GG genotype of the patients' group compared to the control. Whereas, a significant decrease level was appeared in the TT genotype of the patients' group compared to the healthy control group.

## CRediT authorship contribution statement

**Eman Salman Khamaes:** Conceptualization, Methodology, Software, Data curation, Writing – original draft, Visualization, Investigation, Supervision, Validation, Writing – review & editing. **Nagham Y. Al-Bayati:** Conceptualization, Methodology, Software, Data curation, Writing – original draft, Visualization, Investigation, Supervision, Validation, Writing – review & editing. **Ali Hafedh Abbas:** Conceptualization, Methodology, Software, Data curation, Writing – original draft, Visualization, Investigation, Supervision, Validation, Writing – review & editing.

## Declaration of Competing Interest

The authors have declared no conflict of interest.

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