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Interleukin-18 in Lichen Planus: A clinical, genetic and biochemical study

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Running title: IL-18 in LP.

ABSTRACT

Background: Lichen planus (LP) represents aninflammatory mucocutaneous disorderof unclear aetiology.Interleukin-18 (IL-18) is a pleiotropic immune-regulatory cytokine. IL-18 serum level and single nucleotide polymorphism (SNP) were reported to be associated with many immune-inflammatory disorders, but little is recognisedregarding their role in LP. Objectives: to investigate IL-18 gene polymorphism (607C/A and 137G/C) and IL-18 serum level in a trial of LP patients in Egyptian population and to correlate their results with the clinical aspects of LP in the studied cases. Methods:This case control study included 38 LP patients and 19 controls. IL-18 serum levels by ELISA and IL-18(607C/A) and (137G/C)SNPsbyPCR-RFLPtechnique were done.Results: IL-18 serum level had significant high concentrationsin LP patients than controls (p=0.03). IL-18 607 C/A genotypes CA and AA, and allele A were significantly observed in LP patients than controls increasing the risk of developing LP by 7, 13 and 3 times (P=0.04, OR=6.9) (P=0.02, OR=13.0) (P=0.02, OR=2.5), respectively. However, IL-18 137 G/C genotypes and alleles distribution showed non-significant differences among the studied groups. Both IL-18 607 C/A and IL-18 137 G/C genotypes showed non-significant associations with serum ranks of IL-18 in LP patients.Study limitations: The small number of the studied subjects. Conclusions: IL-18 may play an active role in the disease processof LP and its targeting may have appraising effects in clinical application in LP management.IL-18 607 C/A gene polymorphism might contribute to development of LP in Egyptian population.

Keywords:

Lichen planus; Interleukin-18; Single nucleotide polymorphism.

Introduction

LP is an inflammatory muco-cutaneous disease. Clinically, it presents as pruritic, flat-topped papules and/or plaques, violaceous in colour and covered by Wickham striae. These lesions commonly arise on the wrists, ankles and lower back.[1]

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The cause of LP is multifactorial including geneticalong with environmentalaspects. The exact aetiology of LP is not well known but it is thought to be an autoimmune disease. CD8+ T lymphocytes and many cytokines (e.g. TNF) are attached to keratinocytes in the basal cell layer leading to their damage.[2]
IL-18previouslynamed interferon (IFN)-γ-inducing factor. It is an 18-kDa immune-controllingpleiotropic cytokine related to IL-1 family.[3] IL-18 is similar to IL-1 in its structure and receptor utilization as well as cytokine processing.[4] IL-18 was secreted in an inactive precursor (pro IL-18) then it is cleaved into its active form (IL-18)by caspase-1.[5] Pro-IL-18 was detected in numerous cells, including keratinocytes, macrophages and dendritic cells.[6]

The IL-18 receptor presentsinnumerous cell lines including natural killer (NK), T and B cells, as well as macrophages, basophils, neutrophils, endothelial cells, melanocytes, keratinocytes and fibroblasts.[6-10]

IL-18 gene is located at the chromosome 11q22.2 to 22.3. Two SNPs were identified in its promoter at positions -137G/C and -607C/Athat could have an impact on IL18 gene activity.[11]

IL-18 in Inflammatory and Autoimmune Cutaneous Diseases:
IL-18 has beenconcerned in several inflammatory skin diseases, such as psoriasis[12], atopic dermatitis[13], urticarial[14], alopecia areata[15], graft-versus-host disease[16], cutaneous lupus erythematosus.[17]Also, IL-18 SNPs were reported in some inflammatory skin diseases.[18] However, little is recognised regarding the role of IL-18 and its SNPs in LP.

IL-18 up-regulates both Fas ligand (FasL) and perforin-dependent cytotoxic effector functions of T cells and natural killer (NK) cells. Fas binds to FasL on the keratinocyte surfaces inducing their apoptosis.[19]Additionally, IL-18 has a vital role in the Th1 cell regulation and differentiation, which induces production of many pro-inflammatory cytokines, such as TNF-α and IFN-γ. Both TNF-α and IFN-γ induce keratinocytes apoptosis which may contribute in pathogenesis of LP.[18,20]

The current study aimed to assess IL-18 gene polymorphism (607C/A and 137G/C) and IL-18 serum levels in a sample of LP patients in Egyptian population and to correlate their results with the clinical aspects of LP in the studied cases.

Patients and Methods

Our case-control study included 38 patients having LP, in addition to 19 age and sex matched apparently healthy volunteers (the control group). They were selected from Dermatology Outpatient Clinic, University Hospital during the period from August 2019 to March 2020.Cases of LP were diagnosed clinically by two expert dermatologists and confirmed by histopathological examination.
The study was approved by Committee of Human Rights of Research at our University. An informed written consent was signed by every participant or his/her parent (patient ≤18) before initiation of the study.

We included LP patients from both sexes and having different clinical LP types. All included cases had no history of receiving any systemic (past 6 weeks) or topical (past 2 weeks) treatment for LP. Any

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participant having any dermatological and /or systemic immune-inflammatory disorders rather than LP (as psoriasis, vitiligo and alopecia areata) was excluded.

Methodology

The investigated cases were subjected to complete history. Dermatological examination was done to determine site of LP lesions and identify its type (classic - linear - annular - hypertrophic - pigmented - follicular - palmoplantar - actinic - generalized).

Six ml peripheral blood sample was collected from eachparticipantby venepuncture. 3ml was used to get serum by centrifugation at 1000 g for 3-5 minutes. The separated serum was immediately stored at −80°C till time of analysis. Measurement of IL-18 serum level was done by ELISA according to the manufacturer's instructions(NeoBioscience Technology Co., Ltd, Shenzhen, People's Republic of China). The other 3ml was stored at -20°C in tubes containing ethylene diamine tetra acetic acid (EDTA) for molecular testing of polymorphism by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique.

The DNA was extracted from blood sample using Gene JET™ Whole Blood Genomic DNA Purification Mini Kit (THERMO SCIENTIFIC, EU/Lithuania). The SNPs at positions -137 (rs187238) and -607 (rs1946518) in human IL-18 gene were identified by PCR-RFLP technique. PCR primer pairs are: -607C/A: 5' TGTTGCAGAAAGTGTAAAAATTAGTA 3'(sense) and 5'TTACTTTTCAGTGGAACAGGAG3'(anti-sense) ; -137G/C: 5' ATGTAATATCACTATTTTCATGAGA 3'(sense) and 5' CTTTCTTAAAGTCAGAAAGAGAT3' (anti-sense). The total volume of PCR was 20 µl contained approximately 50 ng DNA, 12.5 pmol of each primer, 0.1 mM of each dNTP, 1 × PCR buffer (50 mM KCl,10 mM Tris-HCl, and 0.1% Triton X-100, pH 9.0,) and 1 U Taq polymerase. The concentration of MgCl2 was 1 mM. The PCR profile consisted of an initial melting step of 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 61°C (for −607C/A) or 54°C (for -137 G/C) for 40 s and 72°C for 45 s, and a final extension step of 72°C for 10 min. Restriction enzymes RsaI and DpnI (THERMO SCIENTIFIC, EU/Lithuania) was used to distinguish the −607C/A (121, 96, 25 bp) and −137G/C (108, 83, 25 bp) genotypes, respectively. PCR product and enzyme were well mixed and incubated at 37ºC for 1.5 hrs. The pattern of the digestion products migration on 3% agarose gel allowed identification of the 137 (G/C) and the 140 (C/G) genotypes.

Statistical analysis

The collected data were processed, coded and introduced to the computer to undergo analysis by SPSS version 20 (USA). Statistics were calculated in terms of percentage, mean, median, range and standard deviation (SD). Student's t-test was used for comparison of quantitative variables between two groups of normally distributed data, while Mann Whitney's test was used for comparison of quantitative variables between two groups of not normally distributed data. Chi-Squared (x^2) was used to study association between qualitative variables. Pearson's correlation (r) was used to show correlation between two continuous normally distributed variables. P- value of < 0.05 was considered statistically significant.

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Results

Personal and clinical data of studied subjects

The age of the selected cases ranged from 8 to 67 years. They were 21 males (55.3%) and 17 females (44.7%). Regarding the control group, they were 8 males (42.1%) and 11 female (57.9%), and their age ranged from 9 to 70 years. Non-significant differences were observed between the two studied groups regarding their age (P=0.36) and gender (P= 0.35)**(Table 1)**.

Regarding the clinical data of LP cases, our results revealed that duration of LP ranged from 20 days to 120 months and its age of onset was from 8 to 67 years. The most prevalent type of LP was the hypertrophicLP (11 cases, 28.9%) followed by classic LP (10 cases, 26.3%). The nail affection was reported in 8 cases (21.1%), the oral affection was found in 15 cases (39.5%), and itching was present in 32 patients (84.2%)and was severe in most (63.2%) of the studied cases. Of 38 LP patients, 8 cases (21.1%) had koebnerization, 2 (5.3%) cases had positive LP family history and 11 cases (28.9 %) were smoker.Concerning triggering factors for LP in our studied cases, we observed emotional stress in 13 (34.11%), dental amalgam in 10 (26.32%), sun exposure in 2 (5.3%) and drug intakes including oral hypoglycemic and antihypertensive agents, and NSAIDs in 10 (26.3%) cases.

IL-18 serum levels among the studied groups

IL-18 serum concentrations were significantly elevated LP patients than controls (p=0.03) **(Table 1)**, and had significant positive correlations with age (r=0.41, p=0.01) and with age of LP onset (r=0.38, p=0.02) in the studied LP patients (**Figure 1a, 1b).** Also,high IL-18 serum levels were associated significantly with nail affection (p=0.009) and presence of itching (p=0.05) **(Figure 1c, 1d).**

IL-18(607C/A) and (137G/C) genotypes and alleles destitution in the studied groups

IL-18 607 C/A SNP**(Figure 2)**showed that AA genotype and CA genotype were significantly reported in LP patients than controls increasing the risk of developing LP by 7 and 13 times (P=0.04, OR=6.9) (P=0.02, OR=13.0), respectively. Also, IL-18 607 A allele wassignificantly demonstrated in LP patients than controls increasing the risk of developing LP by about 3 times (P=0.02, OR=2.5). However, IL-18 137 G/C genotypes and alleles distribution showed non-significant differences among the studied groups (P=0.36 and P=0.49), respectively **(Table 2)**.

In the studied LP patients, both IL-18 607 C/A and IL-18 137 G/C genotypes showed non-significant associations with IL-18 serum levels **(Table 3)** or with any of the other studied parameters **(data not shown).**

Discussion

In this study, we investigated, for the first time, both IL-18 serum concentration and IL-18 gene SNPs at position −137 G/C and −607 C/A simultaneously in a section of Egyptian population having LP. We

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observed a significant increase in circulating IL-18 levels in LP patients than their matched peers. Also, we demonstrated that IL-18 607 genotypes CA and AA, andA allele were significantly reported in LP patients than controls increasing the risk of developing LP by about 7, 13 and 3 times, respectively.

Regarding IL-18 serum, levels our finding was in agreement with**Krasowska et al.**[21] who studied 57 Polish patients having LP and reported a significant higher IL-18 serum levels among LP than controls. Additionally, in OLP,significant higher IL-18 levels werereportedin serum[22-24]and in saliva**.**[22]Furthermore, IL-18 was associated with many inflammatory skin disease such as psoriasis[12], atopic dermatitis[13], and graft-versus-host disease.[16]

IL-18 regulates Th1 cell functions,[18]and up-regulates FasL and perforin-dependent cytotoxic effector of T and NKcells inducing keratinocytes apoptosis,[19]which may contribute in LP pathogensis.[20]

It was described that IL-18 binding protein, soluble IL-18 receptors, natural IL-18 inhibitor and anti-IL-18 receptor monoclonal antibodies are IL-18 targeting agents. These agents have been developed for treatment of some inflammatory diseases.[25,26]

Therefore, we suggested that increased IL-18 concentrations may have an active role in LP development, and controlling its action could be a promising method for treatment of LP patients.

IL-18 acts on both mast cells and T cells producing IL-3 and IL-4, which might have an essential role in accumulation of mast cells.[27-29]Additionally,IL-18, IL-4 and certain other factors might stimulate mast cells and other inflammatory cells to induce itching.[13]In line with these data, we observed that high IL-18 serum levels were associated with presence of itching in the studied LP cases.

Furthermore, In this study, there was significant positive correlations of IL-18 serum levels with age of the studied patients and age of LP onset. As well, a significant association with nail affection was observed. Therefore, we suggested that increased IL-18 concentrations were not only associated with LP development, but it may also affect its phenotypic state.

In the same context, we investigated IL-18 SNPs at position −137 G/C and −607 C/A (38 LP cases versus and 19 controls). Our results showed that IL-18 607 AA and CA genotypes,and A allele were significantly associated with LP patients increasing the risk of developing LP by about 7, 13 and 3 times, respectively. In agreement with this result, **Liu and Fu**[30]studied 79 Chinese with LP. They reported that IL-18 607 AA genotype and A allele were significantly reported in LP patients. However, the authors reported a non-significant difference between their studied groups regarding IL-18 607 CA genotype**.** This result can be explained by different sample size and different studied population.

Concerning IL-18 137 G/C SNP, as previously reported,[30] our result showed that there were non-significant differences between LP patients and controls regarding IL-18 137 G/C genotypes and alleles distribution. Therefore, we suggested that IL-18 607 C/A, but not 137 G/C may participate in LP development in Egyptian population.

However, inOLP**, Negi et al.**[24]studied 70 Indian cases. They found that IL-18 137 GG genotypes and G allele were significantly higher in OLP cases, but IL-18 at position 607 C/A showed non-significant differences. This inconsistent results can be explained by different genetic background of the investigated

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individuals as well as different environmental factors.Additionally, we studied LP cases but**Negi et al.**[24] studied OLP, that may have different pathogenic mechanisms.

IL-18 expression is regulated by the IL-18 promoter gene via two SNPs, at positions -137 and -607 in the promoter region. These promoter regions are predictable to be the binding sites for cyclic adenosine monophosphate response element-binding protein and human histone H4 gene-specific transcription factor-1. The differences in transcription factor binding have an impact on the IL-18 gene expression and eventually influence the production of IFN-γ.[11]Herein, we observed non- significant associations between IL-18- 607 C/A and 137 G/C genotypes with IL-18 circulating levels in the studied LP patients. Similarly, **Negi et al.**[24]reported non-significant differences between serum levels of IL-18 and different genotypes of IL-18−137 G/C and −607 C/A in OLP cases. It was suggested that IL-18 blood levels were not controlled by gene polymorphism only and other yet unidentified factors might have possibly synergistic effects.[24]

In the present study, we also found that IL-18 607 C/A and 137 G/C genotypes had no effects on any of the studied data, that may be explained by the demonstrated non-significant difference of serum IL-18 levels among these different IL-18 genotypes in our studied LP patients.

Conclusions

Our study might provide an essentialevidence to understand the LP pathogenesis. IL-18 could play a role in the disease processof LP. IL-18 targeting may have considering effects in LP management program.IL-18 607 C/A gene polymorphism might contribute to development of LP in Egyptian population. The relationship between IL-18 gene polymorphisms and LP could provide more evidences for genetic basis of LP.

Study limitations

The small number of the studied subjects was the main limitation of the current study.

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Table1: Comparison between LP patients and controls regarding their personal data and **:** IL-18 serum levels.

	The studied groups			
	Cases N = 38	**Control N = 19**	**Test**	**P value**
Sex [No (%)]				
– *Male*	21 (55.3%)	8 (42.1%)	X^2	
– *Female*	17 (44.7%)	11 (57.9%)	0.88	0.35
Age (years)				
– *Mean ±SD*	38.61±17.30	34.63±16.24	**U**	
– *Median*	44.5	35	0.92	0.36
– *Range*	8 – 67	9 – 70		
IL-18 serum level (pg/ml)				
– *Mean ±SD*	40.48±47.82	11.63±17.88	**U**	
– *Median*	13.61	4.43	3.10	0.03
– *Range*	1.36 – 149.35	1.71 – 61.73		

N: Numbers SD: Standard deviation pg: pictogram ml: Milliliter
IL-18: interleukin-18 U: Mann Whitney U test X2: Chi square test.

Table 2: IL-18(607C/A and 137G/C) genotypes and alleles among the studied groups.

	The studied groups					
	Cases N = 38	**Control N = 19**	**Test**	**P value**	**Odds ratio**	**95% CI**
IL-18(607C/A) Genotypes			FE			
– ***CC***	2 (5.3%)	6 (31.6%)	-----		Ref (1)	--------
– ***CA***	23 (60.5%)	10 (52.6%)	5.41	0.04	6.9	1.18 – 40.27
– ***AA***	13 (34.2%)	3 (15.8%)	7.2	0.02	13.0	1.7 – 99.38
Alleles	N = 76	N = 38	X^2			
– ***C***	27 (35.5%)	22 (57.9%)	5.17	0.02	Ref (1)	--------

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– ***A***	49 (64.5%)	16 (42.1%)			2.5	1.12 – 5.54
IL-18(137G/C) Genotypes			**X2**			
– ***GG***	**13 (34.2%)**	**4 (21.1%)**	**-----**		**Ref (1)**	**--------**
– ***GC***	**23 (60.5%)**	**14 (73.7%)**	**1.07**	**0.36**	**0.51**	**0.14 – 1.86**
– ***CC***	**2 (5.3%)**	**1 (5.3%)**	**0.13**	**1.0**	**0.62**	**0.04 – 8.6**
Alleles	**N = 76**	**N = 38**	**X2**			
– ***G***	**49 (64.5%)**	**22 (57.9%)**	**0.47**	**0.49**	**Ref (1)**	**---------**
– ***C***	**27 (35.5%)**	**16 (42.1%)**			**0.76**	**0.34 – 1.68**

IL-18: interleukin-18 N: Numbers FE: Fisher's Exact test.
Ref: Reference category CI: Confidence interval.

Table 3: IL-18 serum levels in relation to IL-18 (607C/A and 137G/C) genotypes among the studied LP patients.

	IL-18 serum level among cases N= 38	**Test**	**P value**
	Median (range)	**U**	
IL-18(607C/A)			
– ***CC (n=2)***	104.99 (60.64 –149.35)	1.60	0.11
– ***CA (n=23)***	26.44 (2.05 – 149.05)	1.70	
– ***AA (n=13)***	4.02 (1.36 – 146.3)	1.07	
IL-18(137G/C)			
– ***GG (n = 13)***	32.71(2.05 – 146.3)	0.67	0.50
– ***GC (n = 23)***	13.25 (1.36 – 149.35)	0.08	
– ***CC (n=2)***	65.80 (2.95 – 128.66)	0.10	

IL: Interleukin N: Number U : Mann Whitney U test

Figure legends:

Figure (1):a. Correlation between IL-18 serum level and age LP cases.

b. Correlation between IL-18 serum level and age of LP onset.

c. IL -18 serum levels in relation to nail affection in LP patients.

d. IL -18 serum levels in relation to itching in LP patients.

Figure (2): a. PCR – RFLP analysis for IL- 18 (607C/A) promotor polymorphism.

b. PCR – RFLP analysis for IL- 18 (137G/C) promotor polymorphism.

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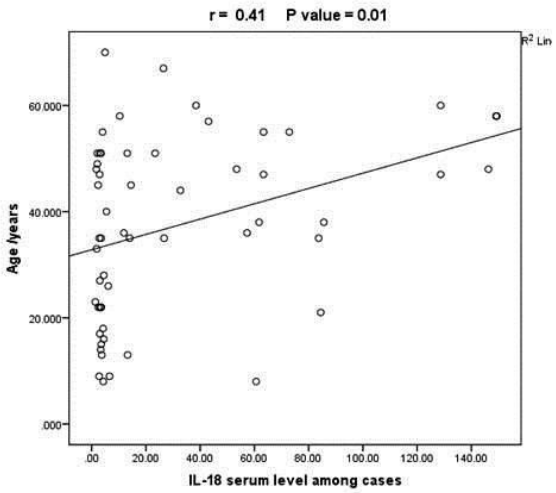
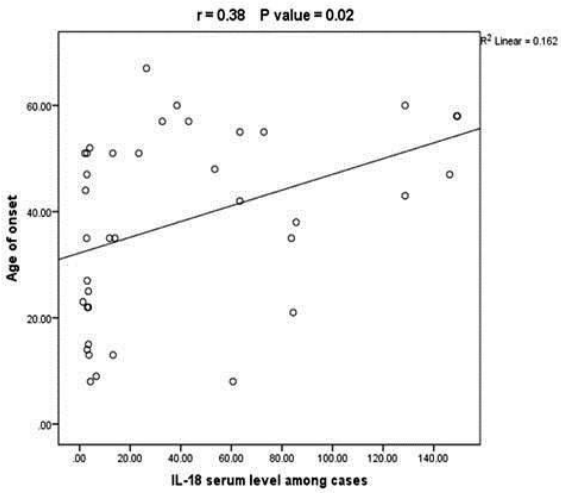
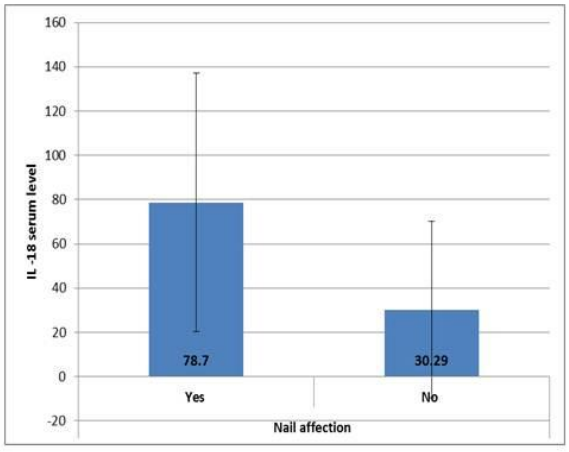
Figure (1)



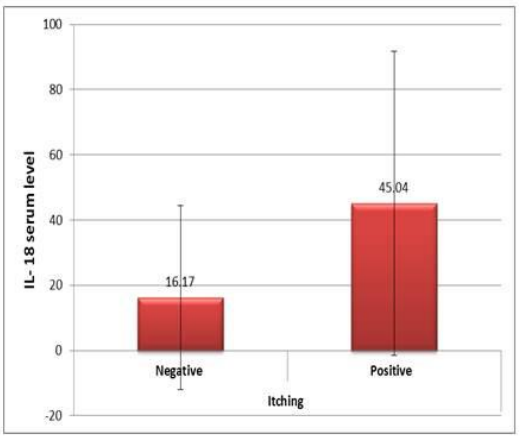
(1a)



(1b)



(1c)



(1d)

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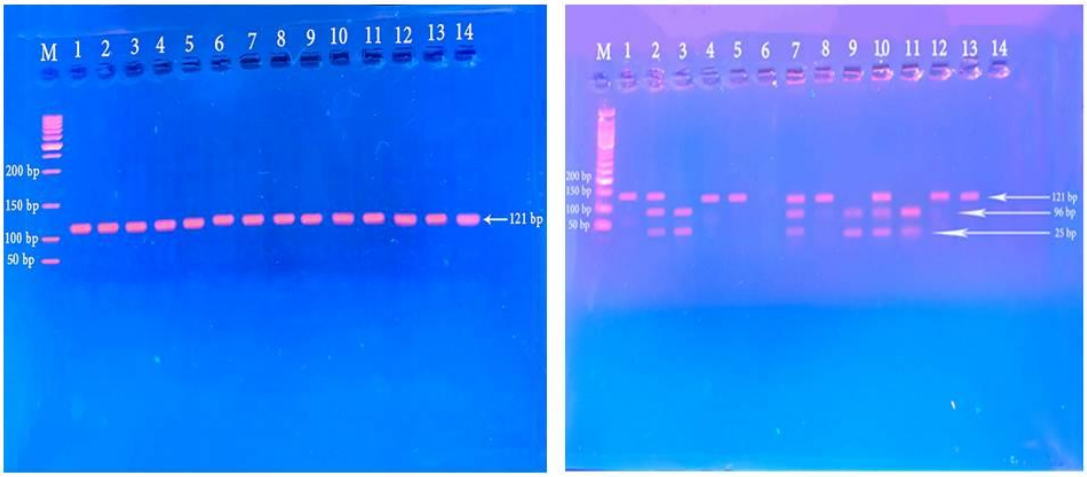
Figure (2)



(2a) (2b)