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DETECTION OF SOME CYTOKINE LEVELS AND MOLECULAR ASSAY IN PSORIASIS PATIENTS

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ABSTRACT: *Psoriasis is a chronic, inflammatory skin disease in which recurring reddish patches, often covered with silvery scales, appear especially on the knees, elbow, scalp and trunk . In this study,one hundred and seventy -six samples were collected from psoriasis patients taken from Al-Hilla educational hospital , Marjan hospital. In order to detect IL-17 and IL-Fasl concentration in serum of psoriatic patients ,Also to determine genes for clumping factor A (ClfA) and fibronectin-binding protein A (FnbA) in Staphylococcus aureus (S.aureus)strains using PCR technique .The result show that , increased in IL-17 and IL-Fasl values in all patients group compared with healthy control, Of the total sixty-five S. aureus isolated , thirty –six of DNA sample were selected for PCR assay .Thirty –six DNA samples (100%)were CIfA gene positive ,thirty –three DNA sample (91.66%) were fnbA gene positive and three sample (8.33%) were negative for fnbA gene visualized on 1% agarose gel electrophoresis*.
KEYWORDS: Psoriasis, Fas-L, IL-17, S.aureus, Bacterialgene

Psoriasis is a common chronic, and inflammatory skin disease affecting about 2% of the general population but its prevalence varies among geographical areas and ethnic groups. Clinical observations have suggested a relationship between the exposure of a number of exogenous agents and the development or exacerbation of psoriasis (James *et al* ., 2005). The main type of Psoriasis is chronic plaque psoriasis (Cpp) accounting for approximately 85–90% of all cases. The Cpp is characterized by erythematous scaly plaques, usually on elbows, knees, scalp and buttocks. Plaque size can diverge from minimal to the involvement of the entire skin surface (erythrodermic psoriasis) (C. E. Griffiths *et al.*, 2007; Nestle *et al.*, 2009).

Other forms of psoriasis comprise guttate psoriasis, inverse, palmoplantar and generalized pustular psoriasis (Griffiths and Barker, 2007; Nestle *et al.*, 2009). Although the initial event triggering a psoriatic lesion is still unknown many factors have been shown to play a role in the pathogenesis of psoriasis: physical trauma, infections, stress, drugs, alcohol and smoking can all trigger an initial episode of psoriasis in individuals with genetic predisposition (Bowcock & J. G. Krueger, 2005).The body's inflammatory process is maintained in part by chemical messengers.

During a normal immune response, certain types of chemical messengers are produced by activated immune cells and sent to the site of infection. Some of these chemical messengers may cause the skin to grow at an accelerated rate (Krueger, 2005). Normally, skin cells grow, mature, and are sloughed off over a period of about a month. The body eventually sheds these cells, revealing new skin cells. In people with psoriasis, however, the immune system is overactive and the skin cells reproduce in only 3 to 4 days. The body has no way to shed the skin cells fast enough, and they accumulate on the surface, forming raised, red patches (Bowcock & J. G. Krueger, 2005). From the foregoing it is clear that the contribution of both innate and adaptive

immune responses are important in mediating the inflammatory psoriasis cascade (Gaspari, 2006) T helper 17 (Th17) cells, has now been described in murine models of autoimmune inflammation (Weaver et al., 2007). Th17 has independent differentiation and growth regulatory mechanism which play an important role in immunological and infectious disease (Weaver et al.2000) .IL-17 inhibitis IL-22 production by Th-17 cells and enhance bleomycin-induced apoptosis of airway epithelial cells (Volpe et al ., 2008) . IL-17 decreases the release of its upstream regulator IL-23 from human macrophage in vitro (Chan *et al* ., 2006). Phagocytosis of apoptotic neutrophils by human macrophage decreases release of regulator IL-23 (Boniface *et al*.,2005). Fas/Fas ligand (FasL) interaction is another reported mechanism that modulates keratinocyte apoptosis in Stevens Johnson Syndrome and Toxic epidermal necrolysis (SJS/TEN) and is also of paramount importance for CTL-mediated lysis *in vitro*. The pathophysiological study of Fas and FasL on the epidermal cells of TEN patients revealed that an augmentation of soluble FasL (sFasL) and epidermal FasL expression were observed in the sera and skin biopsy specimens from patients with TEN, respectively, suggesting that sFasL detected in the sera is derived from cleavage of a membrane-bound FasL (mFasL) on the epidermal cells of patients with TEN (Viard *et al*., 1998) in addition to the regulation of apoptosis, Fas-FasL interaction has also been shown to play a prominent role in the activation of Nuclear factor kappa –light – chain – enhancer of activated Bcells (NF-κB) (Ahn et al ., 2001 ; Xiao et al ., 2002)and the induction of inflammatory response (Peter et al ., 2007 ; Wilson et al ., 2009).

There is considerable evidence for a genetic element in psoriasis. About 40% of individuals with psoriasis have an affected first degree relative. However, patterns of hereditary transmission are complex and not completely understood (Nair *et al*.,2009; Frank et al ., 2009). Clinical isolates of *S. aureus* can produce a variety of extracellular toxins(.Balaban and Rasooly., 2000). Clumping factor A, or ClfA, is a virulence factor from *S. aureus* (*S. aureus*) that binds to fibrinogen. Inactivation of ClfA results in extreme inhibition of *S. aureus* attachment to fibrinogen- coated surfaces in vitro and ex vivo (Hair et al ., 2008). Furthermore, the role of ClfA as a virulence factor was shown in an endocarditis model, where the *clfA*-defective mutant produced about 50% less endocarditis than the parent strain (Hiramatsu et al ., 1997). ClfA also has been shown to bind to complement regulator I protein(Saravia-Otten et al ., 1997).

It is responsible for the clumping of blood plasma observed when adding *S. aureus* to human plasma. Clumping factor can be detected by the slide test (Becker et al.,2001)
Cell surface-associated protein implicated in virulence. Promotes bacterial attachment exclusively to the gamma-chain of human fibrinogen. Induces formation of bacterial clumps, which diminish the ability of group IIA phospholipase A2 to cause bacterial phospholipid hydrolysis and killing. Significantly decreases macrophage phagocytosis possibly thanks to the clumps, clumped bacteria being too large to be phagocytosed(Wolz,2000). The *fnbA* and *fnbB* genes of *S. aureus* 8325-4 encode fibronectin (Fn) binding proteins FnBPA and FnBPB (Kelly Rice.,2001) which promote dherence to host tissues. Each adhesin contains three copies of a repeated D motif that binds Fn and is a target for vaccine development Variation in Fn binding among MRSA isolates was inversely related to protease activity but not to the number of *fnb* genes or the number of D motifs. Therefore, the *fnb* locus is polymorphic in a small number of strains, but this does not contribute to variation in Fn binding Fibronectin (fn)-binding proteins play importants roles as adhesins and invasins(Terao.,2001)

METHOD

Sampling
Between October 2013 and Novmber 2014, 176 samples taken from patients suffering from psoriasis . Samples were taken from Al-Hilla educational hospital , Marjan hospital.

Detection of cytokines:
Interleukine – (IL-17) Enzyme Linked Immunosorbent Assay (IL-17) ELISA Kit (eBioscience)were used to detection the concentration of IL-17 in Psoriasis and healthy control individual

Interleukine- Human FASL Enzyme Linked Immunosorbent Assay (IL-Human FASL) ELISA Kit (eBioscience) were used to detection the concentration of IL-Fasl in Psoriasis and healthy controlindividual

Genotypic Identification
DNA Extraction
DNA of S.aureus isolates was extracted and purified using extraction and purification Kit from Geneaid Company (UK).

Primer
Primer is prepared and resolved by the manufacture instruction (Bioneer and IDT) .The primer is consist of two parts Forward and Reveres and it resolves by Nuclease free water for each primer by Scheduled amount of manufacture instruction. then take 10 ml from primer and was added to anew eppendroff tube is contain 90 ml of nuclease free water to form the stock of each primer that used for PCR technigue .

Table -2 name and sequence of primers used for PCR analysis

Genes	Primer sequence 5…….3
CIfA	5ATTGGCGTGGCTTCAGTGCT3 5CGTTTCTTCCGTAGTTGCATTTG3
fnbA	5CATAAATTGGGAGCAGCATCA3 5ATCAGCAGCTGAATTCCCATT3

PCR Protocol

PCR was carried out in 20 µl reaction volumes containing 10 ul of single primer ,5 ul of Bioneer Master Mix , 5 ul of Genomic DNA . Amplification was carried out in a thermo-cycler (Eppendorf) programmed for 10 min at 95°C; for 25cycles of 1min each at 94°C, 1 min at 55°C and 1 min at 72°C, and a final extension of 10 min at 72°C. The amplified products were electrophoresed in 1% agarose gels and then visualized by staining with ethidium bromide. Standard molecular markers were also included in each electrophoresis run.

RESULTS AND DISCUSSION

IL-17 role in Psoriasis

The results of the current study showed increased in IL-17 values in all patients group compared with healthy control , The highest concentration of IL-17 appear in age group (50-59) year was 930 pg/ml while the lower concentration appear in age group (1-9) was 82 pg /ml . The statistical analysis

revealed the presence of a significant difference between the patients and healthy control in all age group under the level of significance P<0.05. Also The statistical analysis revealed the presence of a significant difference between the patients in all IL-17 age groups but the age group of(50-59) showed high significant difference compared with in the categories

IL- 17 is a cytokine that acts as a potent mediator in delayed-type reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation, similar to Interferon gamma. IL-17 pathway plays a role in the host immune response during acute infection (Mangan *et al* ., 2006) , aberrant expression of IL-17 has been implicated in a number of autoimmune diseases and chronic inflammatory conditions , all these injuries work on increase IL-17 was one of cytokines of result from beginning of inflammation (Chen *et al* , 2006)

Table(1-1): IL-17 values in of infected with psoriasis

Age groups (Years)	IL-17 pg\ ml	IL-17 pg\ ml
	Healthy control (Mean± SD)	**Patients (Mean± SD)**
1-9	7.21± 2.11	82.31± 8.44
10-19	17.01± 2.12	320.08± 20.15
20-29	31.83± 3.44	650.15 ± 5.10
30-39	16.50± 2.12	690.30± 50.11
40-49	8.75± 1.55	730.01± 30.33
50-59	32.11± 3.09	930.23± 30.9
60-69	30.33± 2.31	820.22± 20.01

LSD=39.213

IL-Fasl role in Psoriasis

The results of the current study for IL-Fasl role in psoriasis record enhanced of IL-Fasl concentration in all age group particulary (60-69) . The results of the current study show increased in Fasl values in all patients group compared with healthy control , The highest concentration of IL-17 appear in age group (60-69) year was 666.66 pg/ml while the lower concentration appear in age group (1-9) was 47.33. The statistical analysis at the level of p<0.05 did not show any significant differences between patients in values IL-Fasl but statistical analysis showed presence of significant difference between age range (50-59)-(60-69) the statistical analysis at the level of p<0.05 did not show any significant differences between patients and healthy control in IL-Fasl values but statistical analysis showed presence of significant difference between age range (20-29)-(60-69) The Fas/FasL system has been assigned an important role in the regulation of many immune responses(van and Abbas, 19996) . Unlike Fas, which is constitutively expressed on a variety of cells in different tissues . the expression of FasL appeared to be more restricted to activated T cells, NK cells (Walker *et al* ., 1997)

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Table :(1-2) IL-Fasl values in of infected with psoriasis

Age groups (Years)	IL-FASL pg\ ml	IL-FASL pg\ ml
	Healthy control (Mean± SD)	Patients (Mean± SD)
1-9	25.01± 2.22	47.33± 5.03
10-19	23.18± 3.25	58.08± 6.23
20-29	18.10± 3.33	82.01± 6.35
30-39	31.33± 5.03	86.11± 14.75
40-49	31.21± 6.27	110.23± 10.95
50-59	20.25± 3.15	125.10± 10.01
60-69	41.11± 4.44	666.66± 113.72

LSD= 52.156

Genetic Study

In CIfA , the DNA sample were examined was 36 sample ,the positive results of these gene is 36 sample, all these result show the same size product 288 bp (fiq 1-1) . Clf A is a fibrinogen-binding surface protein of S. aureus. It account for interceeding the adhesion of S. aureus to matrix proteins (McDevitt et al., 1995). And, it prevents phagocytosis during bacterial infection (Higgins et al., 2006). It showed that clf A is a significant virulence factor in various experimental infections such as endocarditis and sepsis (Vernachio et al., 2003). In a study conduct by Higgins et al. (2006), clf A was shown to be important an as protein A as in S. aureus strains . In a study conduct(Nizami et al ., 2010) the existence of clf A gene (51.1%) have been identified in more than half of S. aureus strains isolated from the wounds sample CIfA ratio (100%) in our study was found very differ to the findings from(Nizami et al ., 2010) study.

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In FnbA, the DNA sample were examined was 36 sample, the positive results of these gene is 33 sample , all these result show the same size product 128 bp (fig1-2) Fnb A has been reported in a high proportion among the Staphylococcal strains isolated from various clinical infections (higher than 95%) (Peacock et al., 2000; Rice et al., 2001). As previously reported, fnb A adhesin gene was found to be very frequent in Staphylococcal strains. In a study conducted by Arciola et al. (2005) the significant virulence factors fibronectin (fnb A) of S. aureus strains isolated from orthopaedic surgical infections were examined. In their study, Prevalence of the fnb A genes were almost detected in all surgical samples (98%) (Arciola et al., 2005). In a study conductv (Nizami et al ., 2010) Fnb A ratio (97.7%) . Fnb A ratio (91.6%) in our study was found very similar to the findings from Arciola *et al.(2005)*

Figure (1-1) gel electrophpresis of PCR amplification of CIfA , M is ladder ., lane 1,2,3,4,5,6,7,8,9,10,11,12 represent *S. aureus* isolates all of them are positive for CIfA gene (288) bp

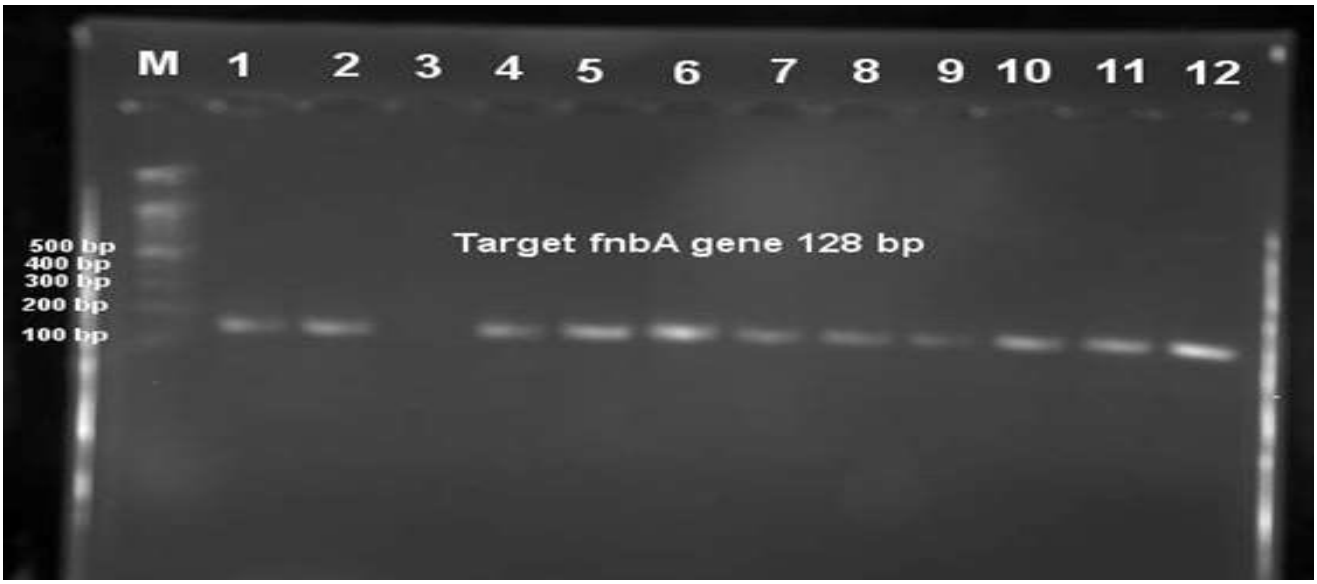

Figure (1-2) gel electrophpresis of PCR amplification of fnbA, M is ladder ., lane 1,2,3,4,5,6,7,8,9,10,11,12 represent *S. aureus* isolates all of them are positive for fnbA gene (128) bp

Conclusion:

1-There is a significant increase in the level of cytokines IL-17 and IL-fasl in patients compare with controls

2-Molecular study showed that CifA gene is the most presence in bacteria *S.aureus* compared to other genes studied

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