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ORIGINAL ARTICLE

Detection of Bacterial DNA in the Plasma of Psoriasis Patients and its Association with Serum Intestinal Fatty Acid Binding Protein Concentration

SAMAH ALI ABD[1], JINAN MJ ALSAFFAR[2], ALICE K. AGOP[1], ENTISSAR JAWAD KADHEM[2]
[1]Biotect. Dept., Coll. of Sci., Univ. of Baghdad, Iraq
[2]Ministry of Industry, Ibn Sina Research Center, Iraq
Correspondence to: Samah Ali Abd, Email: samah.abd.1985@gmail.com

ABSTRACT

Background: There is evidence of the role of microorganisms, in particular in the throat and skin in psoriasis pathogenesis. This study aimed to investigate the presence of bacterial DNA (bacDNA) in patients' plasma and its association with gut barrier integrity .

Methodology: Plasma was collected from 60 psoriasis patients in the flare up stage and 40 healthy controls. BacDNA was detected by PCR amplification using a 16S ribosomal DNA primer. The amplified DNA fragments were then sequenced to determine their bacterial origin.

Results: Bacterial DNA was detected in 51 of 60 patients and only in 4 of 40 healthy controls. BacDNA sequencing results revealed that these bacterial species originated from the gut and skin. On the other hand, intestinal fatty acid binding protein (I-FABP) was measured by ELISA technique to investigate the integrity of the intestinal barrier. Serum concentrations of I-FABP were significantly higher in psoriasis patients compared with the healthy group and were positively correlated with the presence of bacDNA in patients' plasma.

Conclusion: These findings suggest that psoriasis flare up is associated with translocation of bacteria and its metabolites into the blood stream, causing an immune system response. The specific mechanism of bacDNA in the pathogenesis of psoriasis needs to be comprehensively studied.

Keywords: psoriasis, gut barrier, I-FABP, skin-gut axes

INTRODUCTION

Psoriasis is an autoimmune, chronic inflammatory skin condition that causes skin cells to proliferate rapidly, resulting in plaques of thick skin covered in scales. According to the World Health Organization global report, psoriasis affects approximately 100 million people worldwide(1). Psoriasis share some immunological characteristics with Crohn disease. There is evidence that bacterial DNA (bactDNA) fragments can trigger an immune response in people with Crohn's disease and other conditions(2,3). Fatty acid binding protein is an intracellular protein that expressed primarily in the epithelial cells of the mucosal layer of both the small and large intestine tissue expressed primarily in mucosal epithelial cells of the small and large intestine tissue(4). There are many ways that microorganisms can interact with the human gut, but none are as effective as the mucosal layer that acts as a mechanical, biological, chemical, or immune barrier. When the intestinal barrier is compromised, bacteria and their metabolites or endotoxins can leak into the bloodstream and trigger or exacerbate systemic inflammation (5). When the intestinal mucosal injury occurs, the location of I-FABP in the mature villus epithelium facilitates its penetration into the blood (5-7). Gut dysbiosis has been linked to psoriasis pathogenesis and the development of local and systemic immune responses, as well as increased gut permeability and intestinal damage as a result of systemic inflammation (6).

MATERIALS AND METHODS

Subjects: Sixty patients with chronic plaque psoriasis, consisting of 39 females and 21 males, were included in this study at the Department of Dermatovenereology, Al-Yarmouk educational hospital in Baghdad, along with forty healthy controls, comprising of 27 females and 13 males excluded those with other immunological disease and who take biological or chemical treatment. All participant patients were chosen in the flare-up onset stage.

Blood collection: Five to ten millilitres of venous blood were collected, and transferred into EDTA tubes and gel tubes, then centrifuged at 2000 rpm for 10minutes to separate serum and plasma then stored under -20 °.

Psoriasis area severity index calculation (PASI): The psoriasis severity was determined using PASI Calculator (1.7.3), which takes into account the plaques' redness (erythema), thickness (induration), and scaling (desquamation), as well as the proportion of the affected body area. (8).

PCR amplification and analysis of sequence: bacDNA was extracted and purified from 200 µl of plasma using Bioland EasyPrep™ Genomic DNA as described in the DNA preparation protocol. A universal primer derived from highly conserved regions of 16S ribosomal DNA V3-V4 was used. Primer pair 347 F GGAGGCAGCAGTRRGGAAT and 803R CTACCRGGGTATCTAATCC amplify a 466 DNA fragment. PCR was performed using the following conditions: 94°C for 5 min, 35x (94°C for 1 min , 58° C for 40 s, 72°C for 40 s), 72°C for 10 min. Bacterial DNA quality were tested by 2% agarose gel electrophoresis, sequenced by Macrogen (Seoul, Korea) and sequences identified using the BLASTN program.

IFABPs: Five millilitres of venous blood, from all patients and control groups, were collected by vein puncture using 5 mL syringe and were transferred into EDTA tubes and stirred gently for few seconds to avoid clotting then centrifuged at 2000-3000 rpm for 15 minutes, plasma was separated and transported to Eppendorf tubes, then stored frozen at -20 ºC until assayed. Plasma IFABP levels were measured by using Human IFABPs ELISA kit (Hycult Biotech company, USA) according to the manufacturer's protocol.

Statistical analysis: The mean and standard error of the mean were calculated using IBM SPSS version 28.0. Furthermore, the probability was tested using the student T-test. The probability was calculated via Pearson's chi-square test for nonparametric data.

RESULTS

Subjects: There were no statistical significant differences between the two studied groups according to age, gender, and BMI ($p > 0.05$). The mean age of patients was 34.90 ± 1.80 years and 33.05 ± 1.91 for healthy controls. There were also no significant differences between patients regarding disease duration and PASI (table 1), as the majority of patients had the disease for more than 8 years and the PASI mean was 6.6 (ranging from 0.4 to 17). The severity was positively correlated with the disease duration.

Table 1: Clinical characteristics of psoriatic patients and the healthy control group.

Groups	Age mean ± SE (Years)	BMI mean ± SE (Kg/m^2)	Disease duration mean ± SE (Years)	PASI mean ± SE
Psoriasis Patients (n=60)	34.90 ± 1.80	28.06 ± 0.67	8.90 ± 0.98	6.66 ± 0.65
Control (n=40)	33.05 ± 1.910.	28.17 ± 0.83	-	-
probability	P > 0.05	P > 0.05	-	-

Bacterial DNA detection: PCR amplification using universal primer showed a 466 bp DNA band identical to bacterial 16S rDNA in plasma samples of 51 psoriatic patients (85%) (figure 1), but only in 4 (10%) of controls in very low concentrations. It has been demonstrated that bacDNA strongly significantly (P < 0.001) higher in psoriatic patients than in healthy control. In addition there were no significant difference between male and female in both groups as shown in table 1, and there is no positive correlation between positive bacDNA group and PASI or disease duration.

Sequencing the extracted bacDNA: The amplified bacDNA samples were sequenced and the results were aligned by the alignment tool of NCBI, BLAST, in which the obtained DNA sequences were aligned against the 16s rRNA gene. Where

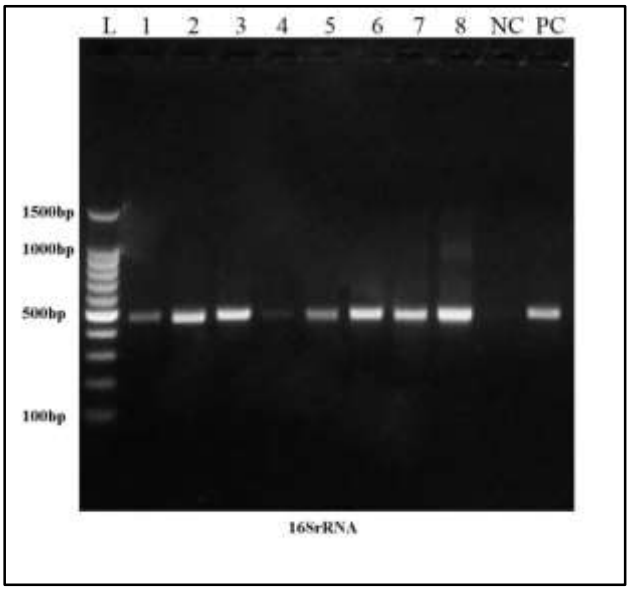

Figure 1: PCR products (agarose gel: 2%, voltages:70, time: 70min), L: DNA ladder; lane 1-8 positive patients samples show 466bp band of bacterial DNA 16s RNA gene; NC: negative control (stock solution except DNA); PC: positive control (Streptococcus pyogenes DNA).

multiple bacterial species received the same top score, all of the species were included in the results. Sequences that had less than 98% similarity to BLAST database hits were deemed poor quality and were eliminated from this study. The result of 16s rRNA sequencing revealed the species of bacteria that found in positive samples of patients as shown in (table 3).

Table 2: Bacterial DNA detection in psoriatic patients and healthy control.

bacDNA	I-FABP mean ± SE (pg/ml)		Probability
	Psoriasis patients	Healthy control	
Positive	280.11 ± 17.96	171.62 ± 12.91	P < 0.001
Negative	277.88 ± 6.49	134.44 ± 5.97	P < 0.001
Total	278.21 ± 6.08	138.16 ± 5.77	P < 0.001

Table3: bacterial species detected in psoriasis patients

Bacterial species	Frequency %
Salmonella enterica	87
Corynebacterium sp.	50
Uncultured bacterium clone	10
Uncultured Enterobacteriaceae bacterium	60
Escherichia coli	35
Klebsiella sp.	10
Citrobacter sedlakii	10
Pantoea sp.	10
Pseudomonas sp.	25
Pseudomonas stutzeri	10
Enterobacter cloacae	10

As the result showed, the most frequented bacteria were Salmonella enterica, followed by uncultured Enterobacteriaceae bacterium, Corynebacterium sp., Escherichia coli, Pseudomonas sp., Klebsiella sp. , Enterobacter cloacae, Citrobacter sedlakii, Pseudomonas sp., Pantoea sp. And Pseudomonas stutzeri.

I-FABP: The result of determination of serum level I-FABP shows significant increase (P < 0.001) in serum I-FABP concentration in psoriatic patients (278.21 ± 6.08 pg/ml) compared to its concentration in healthy controls groups (138.16 ± 5.77 pg/ml), and there was slight increased observed in the I-FABP levels in the groups who detected bacDNA in their blood rather than those without bacDNA in both groups as listed in table 4.

DISCUSSION

Our results is in line with a study of Munz et al. which showed that the bacDNA found in all of psoriasis patients and none of the healthy controls (9). In addition our result agreed with a Japanese study which found that the value of the bacterial 16S rDNA in monocytes significantly increased in psoriatic patients compared with those in the controls (10). We suggested that the presence of bacDNA in patients' plasma may be results from dysfunction of skin-gut axis barrier and/or faulty immune response as DNA specific for species normally present in the skin- gut axis is detected in the bloodstream.

There were just few articles about bacDNA in blood of psoriasis patients have done. Munz et al. reported the bacDNA in psoriatic patients and the identified species in patients were streptococci and staphylococci sp.(9). Another study of Ramírez-Boscá et al. identified bacterial DNA in psoriasis patients, the source of bacterial DNA were belonged to

Table 4: intestinal fatty acid binding protein concentration mean in pictogram.

Groups	bacDNA frequency (%)				Probability
	Patients group		Control group		
	Positive	Negative	Positive	Negative	
Males	19 (37.3)	3 (33.3)	0 (0.0)	13 (100.0)	P < 0.001
Females	32 (62.7)	6 (66.7)	4 (14.8)	23 (85.2)	P < 0.001
Total	51 (85.0)	9 (15.0)	4 (10.0)	36 (90.0)	P < 0.001
Probability	P > 0.05	P > 0.05			

Escherichia coli and some gut flora(11).

The most common source of bactDNA, according to sequencing results was Salmonella enterica which is considered a colon normal flora. The origin of the remaining bacterial species' identified genomic fragments corresponded to the species of human gut microbiota usually seen in the intestinal lumen. As a result, we hypothesized the bacDNA identified in psoriasis patients may have originated in the intestinal lumen. This hypothesis has corroborated by the fact that intestinal permeability has been shown to be enhanced due to gut barrier dysfunction in psoriatic patients, which was supported by our result of I-FABP that considered as an intestinal integrity marker. Conjointly these findings conceived the role of bactDNA translocation in flare-up plaque psoriasis. Another identified species were Pseudomonas stutzeri and Corynebacterium

sp. which are skin originated, that enhances the possibility of translocated the bacteria from skin to blood stream due to skin barrier dysfunction leading to immune response. These findings conceived the role of bacteria of skin-gut axis and its metabolites translocation resulted in flare-up plaque psoriasis.

Our findings about I-FABP agreed with a study in Poland which revealed that psoriasis patient have higher I-FABP level in their serum than healthy controls and it shows a significant positive correlation between I-FABP and PASI (12). Another study of Sikora being through with our results showed increased concentrations of I-FABP in psoriatic patients compared with healthy controls (12). In our investigation, the determination of I-FABP concentration was utilized to determine the integrity of the gut and its correlation with the presence of bacDNA in the blood of patients. I-FABP is solely and abundantly produced as an intracellular protein in intestinal epithelial cells. Increased I-FABP serum levels indicate intestinal epithelial cell damage and bariatric malfunction (13, 14). I-FABP determination has been utilized as a biomarker of intestinal barrier dysfunction in cases of acute mesenteric ischemia (15), necrotizing enterocolitis (16), small bowel strangulation (17), celiac disease (18), Crohn's disease (19), and psoriasis (12). According to contemporary research, Psoriasis pathogenesis is increasingly linked to the gut-skin axis (20, 21). A study demonstrating that medical therapy of bacteria overgrowth problem in the small intestine may reduce psoriasis (22) reveals the microbiome's apparent participation in the etiology of psoriasis. In addition, Tan et al. (2018) discovered a decrease in the amount of Akkermansia muciniphila, a short fatty acid-binding protein and mucin degrading bacteria, in the stomachs of psoriatic patients, which could explain the elevated level of I-FABP (23). Furthermore, alterations in microbiota composition may be one of the causes of impaired intestinal barrier function and elevated serum I-FABP levels in psoriatic individuals (20, 21). Consequently, disruption of the gut microbiome increases the likelihood of a pro-inflammatory environment, as innate and adaptive immune cells infiltrate the lamina propria and stimulate the release of IFN-γ, TNF-α, IL-17, and IL-1β, resulting in epithelial cell destruction and extra-intestinal symptoms (24). Changes in the microbial composition may lead to increased gut permeability, causing immunological activation by the transfer of microbial antigens and their metabolites into the systemic circulation (13). In addition, bacDNA translocation from the intestinal lumen of patients has been described, highlighting the importance of gut microbial composition in disease flare-ups (25).

CONCLUSION

These emerging information give rise to the growing hypothesis that the dysfunction of skin and intestinal barrier leads to leakage of bacteria to blood stream causes systemic immune response that flare up psoriasis considering our finding that bacDNA present in 85% of patients blood. It is necessary to assess the specific function of bacteria in order to improve the balance therapeutically with probiotics, antimicrobials, or even topical microbiota transplantation.

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