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# Salivary leukotriene B4 and urinary prostaglandin E2 levels: a novel diagnostic parameters tests for scabies disease

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# **Abstract**

This study applied 120 clinical samples (40 plasma samples, 40 saliva samples, and 40 urine samples) from scabies patients and 20 healthy person samples as controls group, to determine the levels of prostaglandin E2 (PGE2) and Leukotriene B4 (LKB4) is a diagnostic parameter and evaluation its role and concentrations in the three samples and testing the best parameter that can be used to diagnose the scabies disease. This study showed that clinical samples especially urine of PGE2and saliva in LKB4 had a significant differentiation to another sample when compared to the control group. The levels of PGE2 in plasma samples were (409.9±184.3ug/ml, 29.1 St.E) and in saliva was (369±109 ug/ml, 17.3 St.E), in urine samples was (607.60±34.80ug/ml, 4.5 St.E) of patients group. While the level of PGE2 of control was (79±11.2ug/ml,3.5 St.E) in plasma and (79±11.2ug/ml,7.4St.E) in saliva samples, in urine samples was (162±43.5ug/ml,13.7St.E), there noted the concentration of PGE2 in urine was increased when compared to control results, a while the levels of LKB4 in plasma samples was (322±149ug/ml,23.7St.E) and in saliva was (939±312 ug/ml, 49St.E), in urine samples was (79±11.2ug/ml,8.5 St.E) of patients group. while the level of PGE2 of control was (179±55ug/ml,17.6St.E) in plasma and (551±56ug/ml,18St.E)in saliva samples, in urine samples was (12.8±4.5ug/ml,1.4St.E), there noted the concentration of LKB4 in saliva was increased when compared to control results, and in which can be used as laboratory samples to diagnose scabies, as they gave important differences with the other immune parameters. This study highlighted the importance of the new sample in terms of its role in diagnosed the disease compared to other clinical patient samples.

Keywords Salivary leukotriene B4, Urinary Prostaglandin E2, Scabies, Sarcoptes scabiei, Eicosanoids

# Introduction

The scabies skin scrap test is difficult to diagnose and a suspected diagnosis is mostly made based on clinical signs such as rash and severe scratching. However, it is not easy to obtain clinical samples, particularly skin scrapes and blood from patients. The enzyme or non-enzyme oxidation of arachidonic acid is generated by Eicosanoids as the signaling molecules. They are essential in various physiological and pathologic situations, such as cell growth, inflammation control, and immune response control. Eicosanoids, which consist of leukotrienes, prostaglandins, resolvins, lipoxins, eoxins, and thromboxanes, have several subfamilies (Corcuff & Harizi, 2008). The aim is to cover all aspects of the lipid roles in the functioning of cells, organs, and whole organisms and to place a specific focus on human studies (Das, 2001).

Itching is the most important symptom of scabies, and from it, we can imagine the role of lipid mediator as prostaglandin E2 and leukotriene B4 in stimulating it, and it is possible to rely on the variables that occur in it when infected with scabies, and these immune parameters are studied for the first time in this aspect, so they were approved as diagnostic samples under study.

PGE2 (352 Da) has been shown to control many aspects of inflammation and multiple functions in diverse immune cells, recognized as an important biological factor in the sixties (Phipps et al., 1991). While widely recognized as a mediator of important inflammation, the promotion and local attraction and activation of neutrophils, macrophages, or mast cells at

the local level be involved, Its capacity to encourage IL-10 suppressive induction and to directly suppress multiple proinflammatory cytokines enables its production to limit non-

Specific immunity on several molecular and cellular levels (Phipps et al., 1991) while activating, maturing and migrating dendritic cells (DCs) in the central cells during the production of Ag-specific immunity.

Prostanoids are popular but complex components of body water homeostasis management. The local lipid mediator PGE2 participates both at the exchange and the dissipation of water in recent functional and molecular studies. Four distinct G-protein-coupling receptors, called EP1-4, differentiate the intracellular signaling pathways, exercise the biological activities of PGE2. Prostaglandin (PG) E2 is a major cyclooxygenase (COX) drug, essential in human physiology and pathophysiology. The measurement of excreted urinary metabolites is the best way to measure systemic PG production in humans.

Leukotriene (LT) is critical for host immune defense (Prado et al., 2017). Lipid mediators with powerful proinflammatory activities consist of a 5-lipoxygenase-(5-LO-) of arachidonic acid(catalytic oxidation). Increased death and decreased permission of fungi, bacteria, and parasites were associated with the pharmacological or genetic inhibition of 5-LO biosynthesis in animals. LTs can also be related to pathogenesis, for example in cerebral Malaria or schistosomal granuloma, by moderating the immune system or directly cytotoxicity of the parasites. LTs can also be related to pathogenesis (Rogerio et al., 2012).

The pro-inflammatory arachidonic acid (Leukotriene B4) is a mediator synthesized with myeloid cells. Synthesis is catalyzed by the use of hydrolase 5-lipoxygenase and leukotriene A4, inflammatory mediators such as endotoxin, supplementary fragment, tumor necrosis, and interleukins are increased. A vital co-factor for 5-lipoxygenase is a nuclear membrane protein, a 5-lipoxygenase active ingredient. Leukotriene B4 allows neutrophils, monocytes, and eosinophils to be recruited and activated. It also encourages the development of a variety of pro-inflammatory cytokines and mediators that indicate that tissue inflammation is increased and sustained. (Crooks, 1998)

Often known as ultra filtrated plasma (Javaid et al., 2016) is Saliva, recognized biofluids. Today nearly 1000 different proteins have been found in saliva and 19,000 unique peptide sequences (Khurshid et al., 2015). Whole mouth saliva WMS: the mix of different secretions formed in the minor and major salivary glands, fluid (GCF), gingival and serum and blood discharges, mucosal transudations, from oral wounds, desquamation pellicles, epithelial cells, acquired, bacterial products, viruses, and fungi (Khurshid, et al., 2015) saliva included in the test panel since we recently stated that saliva can be measured in leukotrienes (Gaber et al., 2007). Saliva is a non-invasive sample used in assessing immune and endocrinological variables such as secretory IgA and cortisol (Lawrence, 2002).

specificities, fostering chronic inflammation and cancerrelated immune suppression. PGE2 has been commonly shown to suppress both inborne and Ag

## **Material and Methods**

#### **Study subjects**

This study was conducted in dermatological consultations at Marjan Specialized Hospital, Imam Sadiq Hospital, Prisons and Health Centers in Babil Governorate from July 2019 to December 2019. All patients had clinical symptoms of scabies.

# Patient groups

From a total of 325 skin scraping routine KOH mount samples of scabies patients, only 40 patients were positive by a skin scraping for detection of the causative agent Sarcoptes scabiei mite under a microscope, all 40 patients group have symptoms of scabies their ages were ranged from 8-63 years.

# **Control groups**

Twenty healthy people were selected as a control group and they do not have a history of scabies.

# Samples collections

# **Blood samples**

Five ml of blood sample was obtained from each patient by vein puncture using a syringe with needle gauge 23, two ml transported to a nonheparinized blood collecting tube and then centrifuged at 1800 XG to separate serum then labeled and stored at -20°C until used, no preservative was added. The other 2 ml were transported to a heparinized (20 U of heparin / ml) vaccutainer tube then used for plasma set collection. Before preparation for the review, all samples were stored at -20°C

# Saliva samples

Saliva samples were collected according to (Durham et al., 2010), By rinsing tap water and expectorating both subjects tos prepare their teeth. Sterile 2 percent citric acid was applied to the end and sides of the tongue using a cotton-tipped applicator to stimulate saliva in the mouth. To avoid mixing induced and unstimulated saliva, the first 2 minutes of saliva is discarded. A further 5 minutes, or before 5 mL of the saliva has been obtained, of citric acid was continuously added every 30 seconds. Themes collecting saliva from the sputum into a cold conical container of 50 mL of polypropylene. Both samples were held at -20°C after sampling until the study was prepared.

# **Urine samples**

Samples of the urine have been collected and preserved at  $-20^{\circ}$  C at 500  $\mu$ g at room temperature for 5 minutes (Woo, et al., 2019).

**Note:** For detection of prostaglandin E2, indomethacin at 15  $\mu$ M, and for leukotriene B4 at 10  $\mu$ g/mL (as cyclooxygenase inhibitor) should be added immediately after collection of any biological samples collected.

## Assay detection

Prostaglandin E2 (PGE2) and leukotriene B4 (LKB4), are detected as a solid phase competitive ELISA kit, Prostaglandin E2, and leukotriene B4 detection in three human samples plasma, saliva, and urine for scabies patients and control.

#### Statistical analysis

Statistical analysis was done according to percentages to compare between samples using SPSS V.25 computer software.

#### Results and discussion

This study applied clinical samples (plasma, saliva, and urine) to determine the levels of prostaglandin E2 (PGE2) and Leukotriene B4 (LKB4). According to BMI measurement of scabies patients and control group as in Fig.1 showed a significant difference between the results of parameter and control, in plasma sample the level of PGE2 of control was (80.65±17.94ug/ml) and in the patient was (409.98±184.39 ug/ml), there noted the concentration of PGE2 is increased when compared to control results, while in the LKB4 level of control was (551.18±43.18ug /ml) while the LKB4 level of patients was (322.29±149.90), there noted the concentration of LKB4 is decreased when compared to control results.

In the saliva sample the level of PGE2 of control was (163.65±32.39ug/ml) and in the patient was (369.99±109.90ug/ml), there noted the concentration of PGE2 is increased when compared to control results, while in the LKB4 level of control was (12.84±3.60ug /ml) while the LKB4 level of patients was (939.50±312.21), there noted the concentration of LKB4 is increased when compared to control results.

In the urine sample the level of PGE2 of control was (178.17±44.54ug/ml) and in the patient was (607.60±34.80ug/ml), there noted the concentration of PGE2 is increased when compared to control results, while in the LKB4 level of control was (675.24±137.10ug /ml) while the LKB4 level of patients was (110.73±54.34ug/ml), there noted the concentration of LKB4 is decreased when compared to control results.

The correlation between the results of scabies patient of PGE2, LKB4 in plasma, saliva, and urine samples showed differences in concentrations between immune parameters and on the type of laboratory specimens, and perhaps the role of traction mites has a great role in modifying the effectiveness of the immune system when infection, as well as the ability to restrict the role of skin immune cells in the process of defense against infection by mites. These direct and inverse changes among this parameter can be shown in Fig.2

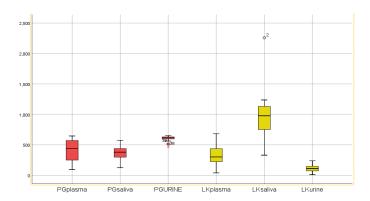


Fig.1 Distribution of the parameter measurement to patients and control groups

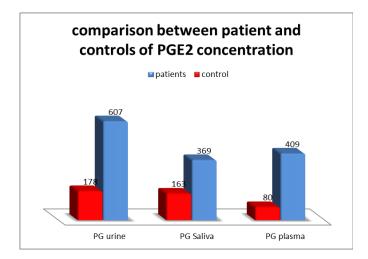


Fig. 2 The correlation between immunologic parameters among scabies patients and control of PGE2

A highly significant correlation was recorded between all clinical samples of the study group (P < 0.01), the PGE2 concentration in plasma samples has a positive correlation with PGE2 in Saliva and urine samples and LKB4 in saliva samples. While the LKB4 concentration in plasma samples has a reverse correlation with all samples test except the LKB4 in urine samples which have a positive correlation.

The PGE2 concentration in saliva and urine samples, the LKB4 in saliva has a positive correlation with all samples test except the LKB4 in plasma and urine which have a reverse correlation. Finally, the LKB4 in urine samples have a positive correlation with LKB4 in plasma and another samples test have a reverse correlation showed in Fig.3

The explanation of the reverse correlation back to the results of the LKB4 in plasma and urine samples was detected in low concentration when compared to the control group. The immune reactive LKB4 level in plasma and urine samples that affected by scabies descending effect while in saliva samples observed significantly ascending effect, the low concentration of LKB4 in plasma samples may be due to its rapidly metabolized to non-immune reactive with and /beta-oxidized metabolites, and the possibility of its release from

activated polymorph nuclear granulocytes (PMN) cells during blood sampling makes reliable measurements of circulating LTB4 extremely difficult.

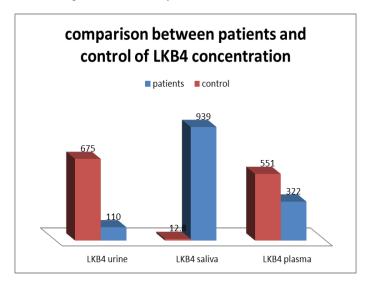


Fig.3 The correlation between immunologic parameters among scabies patients and control of LKB4

(Mikrobiologie et al, 1985). The findings published indicate that leukocoria B4 is stable within the plasma, its biological activity has not been modulated by prolonged incubation. In comparison, leukotriene B4 has been converted to compounds with lower biological activity by human polymorphic nuclear granulocytes (oxidation), and leukotrienes are difficult to reliably quantify in the blood, because of their rapid metabolism and excretion (Eun. et al, 2011) also reported Leukotriene B4 (LTB4) is an important chemoattractant in polymorphonuclear neutrophil (PMN) and a significant PMN priming drug. Leukotriene B4 is quickly transformed into inactive products.

Undetectable LKB4 content in urine samples. This happens as it is completely absorbed and not transferred into the excretion system through its metabolic processes. Moreover, the blood ratio LKB4 in this study is low so that there is little or no LKB4 in the urine (Perestrelo, et al, 2015) reported it is important to note that there is no previous study of measuring LKB4 in urinary samples for patients with scabies, to be accepted for a test of comparison, but that the findings suggest a low level of endogenous LTB4 and potential interference from other endogenous metabolites in urine.

# Correlation between clinical samples by using PGE2, LKB4 as parameters

The choosing of the appropriate clinical sample that is easy to obtain and can be saved for long periods and can be dealt with in the laboratory as a diagnostic sample, It's easy to find parameters to diagnose scabies. The results of this study showed measuring the effectiveness of each sample by comparing it with the rest of the samples.

According to BMI / SPSS V.25 statistical analysis, the regression curve estimated the relation between PGE2 and

LKB4 in plasma samples showed the reverse correlation, when the PGE2 increased the LKB4 level could be decreased. Logistic regression provides a logistic curve, limited to 0 to 1 value. Logistic regression is similar to linear regression, but the curve is designed using the normal "odds", not the probability logarithm of the target variable in logistic regression the independent value was control of the dependent value when compare between two variables. In this study, the PGE2 of plasma samples in curve estimation was odds better than LKB4 of plasma samples, as in Fig.4

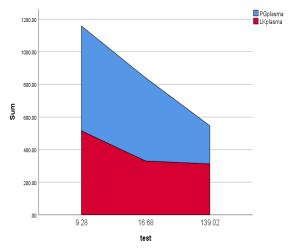


Fig.4 showed the estimation of the relation between PGE2 and LKB4 in plasma samples by logistic regression

In the saliva samples the concentration of PGE2 and LKB4 statistically estimated by logistic regression, Fig.5 showed the two parameters have a direct correlation with odd value to LKB4 concentration in saliva samples.

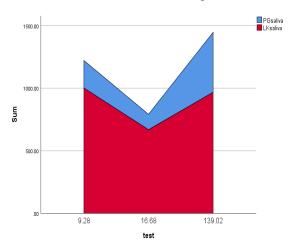


Fig. 5 showed the estimation of the relation between PGE2 and LKB4 in saliva samples by logistic regression

Fig.6 showed the odds value tend to PGE2 concentration rather than LKB4 when estimated the correlation by regression curve estimation in urine samples because the LKB4 was a very low concentration when detected in this study, also, the correlation between two this parameter was a reverse correlation.

All significant differences for parameters in this study give important indicators for their role in giving a positive indication of the total clinical samples that are affected by scabies. These changes in the immune lipid mediator show their role in the effectiveness of the immune system and the mechanisms followed against the disease.

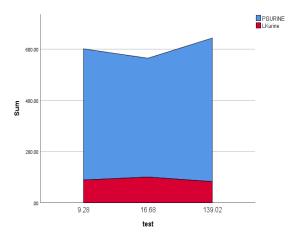


Fig.6 showed the estimation of the relation between PGE2 and LKB4 in urine samples by logistic regression

This study showed that clinical samples, especially urine of PGE2and saliva in LKB4, can be used as laboratory samples to diagnose scabies, as they gave important differences with the immune parameters adopted in this study, as Table 1. and Fig.7 showed the importance of new specimen in terms of their role in highlighting a significant difference between another specimen.

Table 1. showed ROC-Analysis /Area under the curve to highlight the difference between.PGE2 in urine and LKB4 in saliva samples from others specimen.

| Parameter   | %    | Parameter   | %   |
|-------------|------|-------------|-----|
| PGE2 plasma | 68   | LKB4 plasma | 9   |
| PGE2 saliva | 65   | LKB4 saliva | 100 |
| PGE2 urine  | 76.9 | LKB4 urine  | 0   |

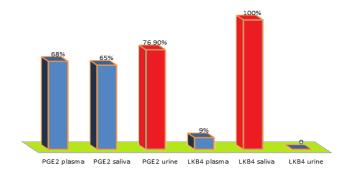


Fig. 7 The distribution of parameters in the area under the curve (AUC) and the best closest to one (1=100%)

Receiver Operating Characteristic or ROC analysis makes it possible to analyze sensitivity and specificity at several points simultaneously, by using several pairs of sensitivity and specificity this technique estimated the precision of a given measure. The ROC-Analyzed analysis shows all the sensitivities vs. 1-specificity in chosen interruption points by putting each pair in the ROC space. An inherent precision of the diagnostic test is a parameter for the region below the curve (AUC). Since the area under a ROC curve determines the utility of an overall test, where a wider area means a successful test, the areas under a ROC curve are used to compare the utility of the tests. (Zhu. et al, 2010)

Table 2. showed the ROC test for PGE2 levels in plasma, saliva, and urine and detection which tests are most useful to show the moral significance for scabies patients.

The area under the curve shows that the PGE2 in the sample of the urine derivatives occupies a larger area than the rest of the ROC curve, (AUC) of (plasma 0.688), (saliva 0.656) and (urine 0.769), samples of urine firstly and then comes a sample of plasma and then the sample of saliva finally when the area is closer to one, The test was a Considered the most accurate. According to the significant value between the three samples the PGE2 in urine samples (P < 0.008) was more significant.

The ROC curve detection of the urine samples is more significant samples of PGE2 (showed Fig.7) from another two samples which may be used as diagnostic samples in scabies diagnosis.

Table 2. Showed ROC-Analysis /Area under the Curve for PGE2 in plasma, saliva, and urine samples

| ROC-Analysis /Area Under the Curve |      |            |      |                                       |       |  |  |
|------------------------------------|------|------------|------|---------------------------------------|-------|--|--|
|                                    |      |            |      | Asymptotic 95%<br>Confidence Interval |       |  |  |
| Test Result                        |      |            |      | Lower                                 | Upper |  |  |
| Variable(s)                        | AUC  | Std. Error | Sig. | Bound                                 | Bound |  |  |
| PGE2<br>plasma                     | .688 | .068       | .062 | .554                                  | .822  |  |  |
| PGE2 saliva                        | .656 | .096       | .122 | .468                                  | .844  |  |  |
| PGE2 urine                         | .769 | .075       | .008 | .621                                  | .917  |  |  |
| Confidence interval= 0.5           |      |            |      |                                       |       |  |  |

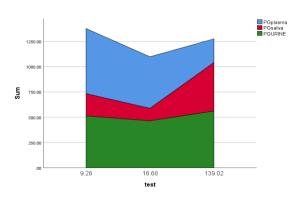


Fig. 8 ROC curve for PGE2 in three samples plasma, saliva, and urine respectively

The LKB4 under the area curve shows that the sample of the saliva derivatives occupies a complete area than the rest of the ROC curve, the area under the curve is 1, as in Table 3, and Fig. 8, (AUC) of (plasma 0.090), ( saliva 1.00) and (urine 0.00), that's mean the saliva samples is an accurate test in detection the LKB4 from another two samples, the significant value between three samples of the LKB4 was a highly significant value ( $P\!<\!0.000$ ).

The ROC curve detection of the saliva samples is more significant samples of LKB4 from another two samples which may be used as diagnostic samples in scabies diagnosis. Thus, our data suggest that the total amounts of PGE2/LTB4 may be good indicators for scabies disease.

Table 3. showed ROC-Analysis /Area under the curve for LKB4 in plasma, saliva, and urine samples

| ROC – Analysis /Area Under the Curve |            |            |      |                                       |       |  |  |
|--------------------------------------|------------|------------|------|---------------------------------------|-------|--|--|
|                                      |            |            |      | Asymptotic 95%<br>Confidence Interval |       |  |  |
| Test Result                          |            |            |      | Lower                                 | Upper |  |  |
| Variable(s)                          | AUC        | Std. Error | Sig. | Bound                                 | Bound |  |  |
| LKB4 plasma                          | .090       | .038       | .000 | .016                                  | .164  |  |  |
| LKB4 saliva                          | 1.000      | .000       | .000 | 1.000                                 | 1.000 |  |  |
| LKB4 urine                           | .000       | .000       | .000 | .000                                  | .000  |  |  |
| Confidence int                       | erval= 0.5 | •          |      |                                       |       |  |  |

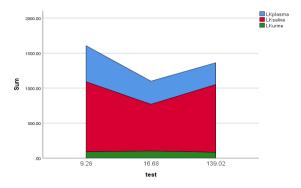


Fig.9 ROC curve for LKB4 in three samples plasma, saliva, and urine respectively.

# Conclusion

The possibility of used the prostaglandin E2 (PGE2) and Leukotriene B4 (LKB4) as parameters to scabies diagnosis.

The prostaglandin E2was elevated in three clinical samples when compared with control samples. The LKB4 elevated in saliva sample in high significant while decreased in plasma and urine samples.

There are highly significant differences of the PGE2 in the urine sample and LKB4 in a saliva sample, to another sample, therefore this document is new and preliminary can be used to diagnosed the scabies disease depended on this inflammatory lipid mediator.

#### **Conflict of Interest**

The author hereby declares no conflict of interest.

# **Consent for publication**

The author declares that the work has consent for publication.

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#### **Ethical Considerations**

The study was approved by the institutional ethical committee.

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