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## Association of Antimicrobial Peptides (HNP1-3 And HBD3) and Adaptive Immunity Cytokines (IL10 And INF- $\gamma$ ) with Disease Extent, Bacterial Burdens and Severity in Patients With Pulmonary Tuberculosis.

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**ABSTRACT:** limited information regarding the balance between cytokines secreted from Th1/Th2 cells and their correlation with AMP before and during treatment of tuberculosis. This study aimed to investigate levels of cytokines (IL10 and INF- $\gamma$ ) and certain antimicrobial peptides (HNP1-3 and HBD3) in pulmonary tuberculosis patients (active and treated) and their correlation with the disease severity and sputum smear grade.

Sixty patients with PTB (Classified as 30 with Active PTB and 30 undertreatment PTB patient) compared to 30 apparently healthy subjects were enrolled in this case-control study. Blood samples were collected from each patient. Circulating levels of INF  $\gamma$ , IL10, HNP1–3 and HBD3 was measured using ELISA technique.

Significant higher concentration of HNP 1-3 in both patient groups in comparison to healthy, whereas, non-significant lower mean of HBD3 was observed in active patients. Decrease IL 10 with increase severity, higher mean of INF- $\gamma$  and HBD 3 were found in moderate severity cases, whereas, HNP1-3 tend to increase from mild to severe cases. Lower mean levels IL10 and HBD3 mean was found in +3 grade., whereas, lowest mean of INF- $\gamma$  and HNP1-3 was seen in +2 grade. ROC analysis showed that HNP 1-3 had higher discriminatory power indiscrimination. This study might possibly reflect the importance of the tested biomarkers in pathogenesis of TB and bacterial clearance.

## 1. INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by slow-growing intracellular pathogens *Mycobacterium tuberculosis* complex (MTBC). *M. tuberculosis* is causative agent of PTB in human, which was first discovered by Robert Koch in 1822 (1).TB predominantly classified to pulmonary or extrapulmonary TB according to infected location within host body. Clinically, TB ranges from asymptomatic and non-transmissible latent TB infection (LTBI) to highly active, transmissible TB disease(2) .

According to WHO report, in 2024, an estimated 10.7 million were infected with TB and 1.23 million died, Iraq is currently classified as a low-burden country, with approximately 9,800 TB cases in 2024. Iraq has had a dramatic decline in TB incidence, from 45 cases per 100,000 population in 2013 to 23 cases per 100,000 population in 2023 reflecting the effectiveness of its TB control strategies (3) .

Immunologically, majority of individuals exposed to the bacteria becomes infected but the progression to disease is prevented because of the effective immune response. Upon infection, alveolar macrophages and epithelial cells are the first lung cells that encounter the pathogen and produce antimicrobial peptides (AMPs). Also, infected macrophages initiate a strong inflammatory response characterized by the secretion of pro-inflammatory cytokine such as (INF- $\gamma$ ) which is a key protective cytokine in tuberculosis, primarily produced by activated type 1 helper T cells (Th1), natural killer (NK) and CD8+ T cells. However,

excessive immune response is reduced through several mechanisms like IL-10 production cells, and CD8+ T cells. However, excessive immune response is reduced through several mechanisms like IL-10 production (4)

AMPs offer several advantages in the context of TB which represents a critical element of the innate immune system because of their extensive antibacterial efficacy (5). AMPs exhibit significant activity against MTB through multiple mechanisms, including disrupting the bacterial membrane by forming pores or micelle-like structures (direct killing), inhibiting nucleic acid and protein synthesis, and interfering with cell wall synthesis (indirect killing). In addition, immunomodulatory, anti-inflammatory, and wound-healing properties, which may contribute to enhancing their therapeutic potential (6). Based on their structure and cellular origin, AMP could be classified into two main groups: alpha and beta defensins (7).  $\alpha$ -defensin peptides are primarily produced and stored in the azurophilic granules of neutrophils (8).  $\beta$ -defensin peptides primarily expressed by epithelial cells lining mucosal surface and play a synergistic role together in protecting the body from respiratory pathogens, including MTB (9).

Several previous studies had investigated the level of certain cytokines and AMPs in PTB patients (Vivekanandan et al., 2023, Mohammed et al., 2026, Kumar et al., 2017 and Ali et al., 2021) (10–13). However, limited information regarding the balance between cytokines secreted from (Th1/Th2) cells and their correlation with AMPs in PTB patients. Thus, study aim to Investigate cytokines (IL10 and INF  $\gamma$ ) and AMPs (HNP1-3 and HBD3) levels in PTB patients (active and treated) compare with apparently healthy subjects and their correlation with the disease severity and sputum smear grade.

## 2. MATERIALS AND METHODS

### 2.1 Subjects and sample collection

Sixty patients with PTB (Classified as 30 with Active PTB and 30 undertreatment PTB patient) compared to 30 apparently healthy subjects were enrolled in this case-control study. This study was conducted at “Chest and Respiratory illnesses centre of Kerbala governorate, Iraq” over the period from December 2025 to March 2026. Blood samples were collected from each patient. Circulating levels of INF  $\gamma$ , IL10, HNP1–3 and HBD3 was measured using ELISA technique.

### 2.2 Diagnosis

Medical history, clinical symptoms, radiographic chest X-rays microbiological (sputum smear microscopy), molecular diagnosis using the GeneXpert device was applied to confirm PTB diagnosis. It is carried out by physician for individuals who have had chest symptoms, like a cough, for more than two to three weeks before to starting treatment for newly diagnosed active PTB. Blood samples were collected from patients at different stages of anti-tuberculosis treatment, including patients who had received treatment for less than two months and those who had received treatment for more than two months. Blood samples were also collected from newly diagnosed active PTB patients immediately after tuberculosis was confirmed and before initiation of anti-tuberculosis treatment. For microbiological diagnoses, Ziehl-Nielsen (ZN) technique was used to perform a direct microscope examination of two sputum samples (morning and evening) using acid fast bacilli (AFB). The sputum smear grading criteria for TB diagnosis were reviewed by laboratory scientists/technicians in accordance with the guidelines provided by (European Center for Disease Prevention and Control, 2018). AFS was done for all patients before starting treatment. AFB grades were classified as positive AFB if they were +1, +2, or +3, and negative AFB if there were no bacilli in the AFB. Medical information regarding each patient was provided from the healthcare institute.

**2.3 Laboratory Measurements:** Five millimetres of venous blood was drawn from each participant in this study by using a single use syringe. Serum levels were measured using a commercial enzyme –linked immunosorbent assay (ELISA) kit. As directed by the manufacturers (Elabscience@ kits-USA) for IL10, INF- $\gamma$ , HNP1-3 and (Sun long Biotech kit-China) for HBD3, serum was separated by centrifugation for 20 minutes at 2000 rpm. Catalog number: (E-EL-H6154, H0108, H2299 and QS3279Hu), Sensitivity (0.94pg/ml, 9.38pg/ml, 0.38ng/ml, 0.1ng/L), detection range (1.56-100pg/ml, 15.63-1000pg/ml, 0.63-40ng/ml, 2-50ng/L), for IL10, INF- $\gamma$ , HNP1-3 and HBD3, respectively. The standard sample serial dilution procedure is followed by the preparation of a working solution. IL10 (50, 25, 12.5, 6.25, 3.13, 1.56, and 0 pg/ml), INF- $\gamma$  (500, 250, 125, 62.5, 31.25, 250, 125, 62.5, 31.25, 15.63, and 0 pg/ml), HNP1-3 (20, 10, 5, 2.5, 1.25, 0.63, and 0 ng/mL) and HBD3 (48.0, 32.0, 16.0, 8.0, 4.0 ng/L). The ELISA assay was performed as a single measurement. Intra- and inter-assay of CV for IL10, INF- $\gamma$ , and HNP1-3 and were <10%. Whereas for HBD3 Intra-assay (<10%) and inter-assay (<12%).

**2.4 Determination of PTB severity score (PTBSS):** PTBSS designed based on previously published proposals for PTB disease spectrum(15) . Calculated PTBSS according to five important variables: presence of symptoms, cavitation detected on chest X-ray, bilateral lung involvement in chest X-ray, sputum smear AFS and GeneXpert results. Depending on the score obtained from 0 to 6 points: 0-1,2-3, and 4-5 were classified as mild, moderate, severe, respectively.

## 2.5 Inclusion criteria and Exclusion criteria

### 2.5.1 Inclusion criteria:

1. Clinically newly diagnosed patients with active PTB and those under anti-TB therapy
2. Aged above 18 years from both sexes.
3. PTB patients were drug sensitive and resistance, however, most of them were DR-TB.

### 2.5.2 Exclusion criteria:

1. Individuals aged less than 18 years,
2. Patients with extrapulmonary TB.
3. Chronic disease (Diabetic mellitus, Hypertension patients, cardiovascular diseases) HIV-infection, cancers, any respiratory system problems.
4. Pregnant women.

**2.6 Statistical analysis:** The Statistical Package for the Social Sciences (SPSS) (version 27 software, IBM Corp., NY, USA) was used to analyze the data. Frequency, mean, and standard Error were determined using descriptive statistics. Test of normality was performed to test the normal distribution of continuous variable using Kolmogorov-Smirnov and Shapiro-Wilk statistic. Non-parametric test was used to compare the study groups and determine the presence of significant difference using Mann-Whitney U test and Kruskal Wallis Test in case of comparison between two groups and three subgroups, respectively. P-value  $\leq 0.05$  is regarded as significant difference.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

Sixty PTB patients were enrolled in this study compared to thirty healthy subjects. there were no significant differences according to sex and age, 31(64.6%) of patients were male and 29 (69.0 %) were female whereas 17(35.4%) and 13(31.0%) males and females were in healthy subject. Mean age was divided into subgroups 32(65.3%) were  $\leq 40$  years and were 28 (70.0%)  $>40$  years among PTB patient, as shown in Table 1.

**Table (1):** Demographic data between patients and healthy.

|     |                 | PTB patients | Healthy    | p-value<br>Kruskal Wallis Test |
|-----|-----------------|--------------|------------|--------------------------------|
| Sex | Male            | 31 (64.6%)   | 17 (35.4%) | 0.654                          |
|     | Female          | 29 (69.0%)   | 13 (31.0%) |                                |
| Age | $\leq 40$ years | 32 (65.3%)   | 17 (34.7%) | 0.638                          |
|     | $>40$ years     | 28 (70.0%)   | 12 (30.0%) |                                |

Significant higher level of IL-10 was found in undertreatment group followed by active PTB patients, whereas, IFN- $\gamma$  mean level showed opposite pattern. Significant higher concentration of HNP1-3 in both patient groups in comparison to healthy, whereas, non-significant lower mean of HBD3 was observed in active PTB patients, as shown in Table 2.

**Table (2):** Comparisons of cytokines and antimicrobial peptides according to study groups

|                   | Active PTB             |                  | Undertreatment         |                 | Healthy               |                  | p-value<br>Kruskal Wallis<br>Test |
|-------------------|------------------------|------------------|------------------------|-----------------|-----------------------|------------------|-----------------------------------|
|                   | Mean Rank<br>Mean± SE  | Medium<br>(IQR)  | Mean Rank<br>Mean± SE  | Medium<br>(IQR) | Mean Rank<br>Mean± SE | Medium<br>(IQR)  |                                   |
| IL10<br>(pg/ml)   | 51.87<br>(0.04±0.005)  | 0.04 (0.03)      | 56.37<br>(0.06±0.01)   | 0.04 (0.06)     | 28.27<br>(0.05±0.03)  | 0.02 (0.02)      | 0.001*                            |
| INF γ<br>(pg/ml)  | 57.58<br>(68.10±17.85) | 0.90<br>(125.56) | 50.73<br>(51.07±17.10) | 0.04<br>(90.41) | 28.18<br>(7.74±7.78)  | -0.12<br>(0.140) | 0.001*                            |
| HNP1-3<br>(ng/ml) | 64.93 (1.62±0.02)      | 1.62 (0.11)      | 46.35<br>(1.48±0.03)   | 1.50 (0.20)     | 25.22<br>(1.22±0.07)  | 1.33 (0.30)      | 0.001*                            |
| HBD3<br>(ng/L)    | 43.32<br>(0.84±0.32)   | 0.30 (0.60)      | 49.47<br>(0.89±0.24)   | 0.43 (0.45)     | 43.72<br>(1.02±0.38)  | 0.38 (0.30)      | 0.59                              |

\*Significance level at 0.05. IQR; Interquartile Range. SE; Standard Error.

Nonsignificant decrease in the mean of IL10 concentration was seen with increase severity of TB and Non-significant higher mean of INF γ and HBD3 were found in moderate severity of TB cases, whereas, HNP1-3 tend to increase non-significantly from mild to severe cases, as shown in Table 3.

**Table (3):** comparisons of cytokines and antimicrobial peptides according to disease severity.

|                   | Mild                   |                  | Moderate               |                  | Sever                  |                   | p-value Kruskal<br>Wallis Test) |
|-------------------|------------------------|------------------|------------------------|------------------|------------------------|-------------------|---------------------------------|
|                   | Mean Rank<br>Mean± SE  | Medium<br>(IQR)  | Mean Rank<br>Mean± SE  | Medium<br>(IQR)  | Mean Rank<br>Mean± SE  | Medium<br>(IQR)   |                                 |
| IL 10<br>(pg/ml)  | 36.67<br>(0.07 ±0.01)  | 0.07 (0.06)      | 33.03<br>(0.05 ±0.00)  | 0.05<br>(0.03)   | 24.24<br>(0.04 ±0.00)  | 0.03 (0.02)       | 0.10                            |
| INF γ<br>(pg/ml)  | 25.89<br>(50.04±42.04) | -0.02<br>(35.07) | 31.97<br>(66.63±18.32) | 0.13<br>(121.62) | 30.38<br>(53.61±16.67) | 0.125<br>(117.16) | 0.65                            |
| HNP1-3<br>(ng/ml) | 30.94<br>(1.50±0.08)   | 1.54 (0.23)      | 29.68 (1.55±0.03)      | 1.58<br>(0.19)   | 31.48 (1.55±0.03)      | 1.59 (0.16)       | 0.93                            |
| HBD3<br>(ng/L)    | 31.56<br>(0.68±0.21)   | 0.43<br>(0.70)   | 31.68 (1.17±0.39)      | 0.44<br>(0.47)   | 28.36 (0.51±0.07)      | 0.34 (0.49)       | 0.78                            |

IQR; Interquartile Range. SE; Standard Error.

Lower significant IL-10 mean was found in +3 grade. However, non-significant lower mean was observed for HBD-3 in +3 grade, whereas, non-significant lowest mean of INF-γ and HNP1-3 was seen in +2 grade. as shown in Table 4.

**Table (4):** comparisons of cytokines and antimicrobial peptides according to smear findings.

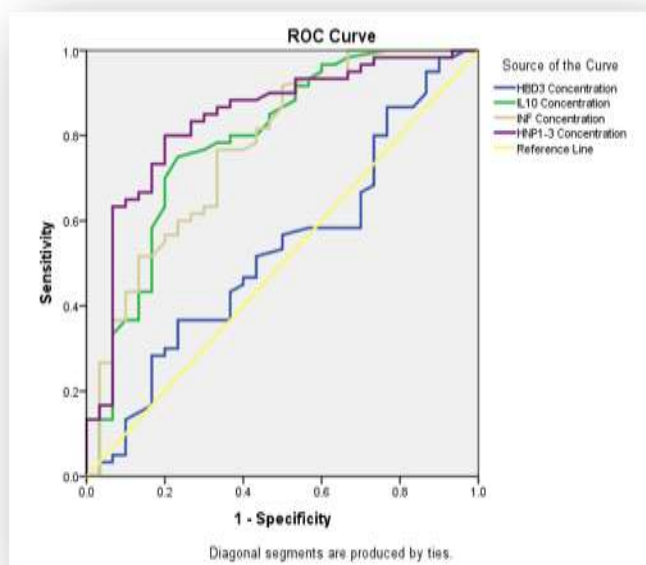
| Mean Rank                      |          |                             |              |                              |                 |                            |              |                            |              |
|--------------------------------|----------|-----------------------------|--------------|------------------------------|-----------------|----------------------------|--------------|----------------------------|--------------|
| Mean $\pm$ Standard Error (SE) |          |                             |              |                              |                 |                            |              |                            |              |
| Variable                       |          | IL10                        | Median (IQR) | INF- $\gamma$                | Median (IQR)    | HNP1-3                     | Median (IQR) | HBD3                       | Median (IQR) |
| Direct Examination             | Negative | 33.18<br>(0.08 $\pm$ 0.01)  | 0.05<br>0.07 | 29.21<br>(91.55 $\pm$ 27.50) | 0.13<br>184.19  | 25.71<br>(1.55 $\pm$ 0.03) | 1.53<br>0.18 | 29.24<br>(1.34 $\pm$ 0.57) | 0.43<br>0.65 |
|                                | +1       | 21.79<br>(0.04 $\pm$ 0.007) | 0.03<br>0.04 | 22.21<br>(29.78 $\pm$ 10.89) | -0.02<br>60.52  | 27.40<br>(1.55 $\pm$ 0.03) | 1.59<br>0.23 | 21.40<br>(0.65 $\pm$ 0.21) | 0.30<br>0.39 |
|                                | +2       | 26.57<br>(0.05 $\pm$ 0.01)  | 0.04<br>0.11 | 26.64<br>(27.92 $\pm$ 21.00) | 0.12<br>27.17   | 22.71<br>(1.51 $\pm$ 0.04) | 1.54<br>0.13 | 35.14<br>(0.87 $\pm$ 0.20) | 0.72<br>0.87 |
|                                | +3       | 13.00<br>(0.02 $\pm$ 0.006) | 0.02<br>0.02 | 29.50<br>(70.69 $\pm$ 50.87) | 33.54<br>178.63 | 25.75<br>(1.54 $\pm$ 0.06) | 1.58<br>0.24 | 18.75<br>(0.32 $\pm$ 0.05) | 0.30<br>0.20 |
| P-value Kruskal Wallis Test    |          | 0.02*                       |              | 0.47                         |                 | 0.91                       |              | 0.08*                      |              |

\*Significance level at 0.05. IQR; Interquartile Range. SE; Standard Error.

Regarding correlation among parameters, significant correlation between INF- $\gamma$  and HNP1-3 was observed, as shown in Table 5. HNP1-3 value had the highest discriminatory power in discrimination of PTB patients and healthy individuals, followed by IL10 and INF- $\gamma$ . The HBD3 had poor prognostic ability, as shown in Figure 1.

**Table (5):** Correlations among the tested parameters

|                                                             |                     | IL10 | INF- $\gamma$ | HNP13 | HBD3  |
|-------------------------------------------------------------|---------------------|------|---------------|-------|-------|
| IL10                                                        | Pearson Correlation | 1    | .002          | -.077 | -.019 |
|                                                             | Sig. (2-tailed)     |      | .983          | .469  | .858  |
|                                                             | N                   |      | 90            | 90    | 90    |
| INF- $\gamma$                                               | Pearson Correlation |      | 1             | .236* | 0.092 |
|                                                             | Sig. (2-tailed)     |      |               | .025  | 0.386 |
|                                                             | N                   |      |               | 90    | 90    |
| HNP1-3                                                      | Pearson Correlation |      |               | 1     | .062  |
|                                                             | Sig. (2-tailed)     |      |               |       | .564  |
|                                                             | N                   |      |               |       | 90    |
| *. Correlation is significant at the 0.05 level (2-tailed). |                     |      |               |       |       |



| Variables    | AUC   |
|--------------|-------|
| IL10         | 0.787 |
| INF $\gamma$ | 0.772 |
| HNP1-3       | 0.838 |
| HBD3         | 0.530 |

**Figure (1):** ROC curve showed sensitivity and specificity for IL10, INF- $\gamma$ , HNP1-3 and HBD3 levels.

## 3.2 DISCUSSION

According to sex and age, PTB were more frequent in males than females and more frequent in patients who were under 40 years of age, Table (1). WHO reported that PTB can affect anyone, regardless of age or sex(3) .

The tested cytokines, IL-10 and IFN- $\gamma$  had a higher level in PTB patients compared to healthy, In addition IL10 increased after initiation of anti-TB treatment in undertreatment group followed by positive new cases as shown in Table (2), Similar findings conducted by Vivekanandan et al.,(13) it has been reported IL10 production regulates one of the most important mechanisms in protecting the over immune response and tissue damage thus , IL-10 as Th2 regulatory cytokine, sequential increase during treatment may be reflect its role in regulatory scar formation there by pulmonary fibrosis prevention which is represent a complication of tuberculosis (16).

Regarding IFN- $\gamma$  similar findings to Hussain et al., (12) .The concept of elevation IFN- $\gamma$  levels reflects strong immune stimulation due to mycobacterial activity rather than protective immunity alone (17) .A previous study reported that healing effect on granuloma is associated with reduced number of local and circulating IFN- $\gamma$  producing activated T cells which reflect the negative association of IFN- $\gamma$  and anti-TB therapy (18) . Thus, these findings support that IFN- $\gamma$  could be used as a reliable biomarker for active TB diagnosis in clinical practice, particularly in high-burden settings and monitoring of anti-TB treatment e(19) .

Regarding AMPs, Higher HNP1-3 levels in PTB patient, as shown in Table (2). similar to Kumar *et al.*, findings (11) . It was reported previously that HNP1-3 play important role in pathogenesis of PTB and that low levels of HNP1-3 and neutrophils have been associated with the development of multidrug-resistant TB (20) .It was documented HNP1-3 released during the early phases of antibacterial defence, plays an important role in initially limiting intracellular infectious agents. In later stages, it does so indirectly by modulating the secretion of anti-inflammatory and pro-inflammatory cytokines current results agreed with the dynamics changes of AMPs during TB infections (21,22) . Also, reported HNP1-3 has the ability to inhibit the growth of MTB in many in vitro and in vivo models. It is shown to decrease the intracellular load of MTB by more than 90% in macrophages infected with this organism in a laboratory setting. Decreased HNP1-3 reflects successful treatment supporting the hypothesis stated that successful treatment of tuberculosis may results in a trend towards normalization of circulating AMPs in PTB (11) .

limited information about the role of HBD3 in TB pathogenesis, (23). Regarding decrease HBD3 in active patients compared healthy was report by Ali et al., may suggest its association with progression of TB in infected patients (10). Also, it was documented that exposure to air pollution particulate can cause deregulation of alveolar type II epithelial cells to express HBD3 when infected with MTB which was associated with increased intracellular growth of the bacteria (24) .

It has been found previously that lower IL-10 level is non-significantly associated with increase TB severity, thus, appropriate IL-10 activation is critical in maintaining a balance with IFN and in regulating outcome of TB. Mechanism of disease progression may be due to less effective balance of IL-10 in the presence of elevated proinflammatory chemokines, this would exacerbate increased disease related pathology in the host, rather than provide an appropriate signal for host T cell and macrophage activation (25) .

Mohammed et al., reported that INF- $\gamma$  in TB can correlate with clinical severity and increase bacillary load due to its increase between mild and severe cases indicating intensified Th1-mediated immune activation with advancing disease. Thus, an increase in Th1 immune stimulation induced by the continued stimulation of mycobacterial antigen and thereby leading to an increase in effective host defense as well as increase in severity of inflammation (26).

Regarding severity results Kumar et al., found no association between HNP1-3 and severity of disease (27) . This might be related to the source of these AMP or their site of production. However, it is reported that alpha defensins concentration may be a useful marker of disease severity and deterioration of pulmonary function(28) . It is demonstrated that the level of defensins expression is influenced by tissue damage and endogenous factors such as degree of maturity of the immune system microenvironment, and total regulatory effects on the target cell not only on properties of an individual peptide (29)

the study found that there was an initial rapid expression of HBD-3 by respiratory epithelium during progressive infection that correlate with temporary control of bacterial proliferation, whereas, the HBD-3 expression was decreased during later progressive phase. In latent infection, HBD-1 was expressed continuously, but it was suppressed after reactivation of the disease (24)

The lower IL-10 level takes longer time to conversion (estimated time is 96 days), Thus, IL10 considered as a marker of the immune response of Th1 to MTB infection (30). In contrast to current study, Kumar et al., found that INF- $\gamma$  exhibit positive association with smear grades. INF- $\gamma$  is a proinflammatory cytokine and its elevation represent a hallmark of PTB disease (37).

Regarding HNP1-3, previous study found that HNP1-3 concentration was negatively correlated with the duration of MTB in sputum, while it was positively correlated with neutrophils suggesting higher HNP1-3 are associated with more pathogen clearance (20). It was documented that during the early phase of infection in murine model (which represent the time of efficient control of bacillary growth), there was high production of beta defensins. In contrast, during the progressive phase, there was a striking decrease in the production of defensins, which again suggests that these antimicrobial peptides participate in the arrest of bacillary growth (32,33). This study might possibly reflect the importance of the tested biomarker in bacterial clearance since there were approximately gradual decrease in their mean of concentration within +2 and +3 grade. Larger study with larger sample size is required (prior and after treatment) to assess their association precisely.

Regarding correlation among parameters, Positive non-significant correlation between IL10 and INF- $\gamma$  was documented by Guimarães *et al.*, it is demonstrated an equilibrium in the levels of cytokines produced by the Th1 (INF- $\gamma$ ) and Th2 (IL10) lymphocyte in patients to still able to maintain the stability between the disease and the immune response(34). Negative non-significant correlation between IL10 and HNP1-3 may reflect immune modulation by AMPs. It has been documented that HNP1-3 increases the production of pro-inflammatory cytokines while decreasing the production of anti-inflammatory cytokines (IL-10) by monocytes (35).

Positive significant correlation between INF- $\gamma$  and HNP1-3 may reflect immune modulation by AMPs. It has been documented that HNP1-3 increases the production of pro-inflammatory cytokines (35). Moreover, at the infection site, neutrophils arrive and release HNP1-3 leading to macrophages activation, which subsequently secrete proinflammatory cytokines thereby improving phagocytosis (9) . Additionally, HBDs are mainly expressed by epithelial cells which enhance mycobactericidal activity. It is expression in response to the induction by pro-inflammatory cytokines and has demonstrated mycobactericidal activity in infected macrophages (36).

Positive nonsignificant correlation was observed in current study between HNP1-3 and HBD-3. Although different cellular sources and regulatory pathways have been implicated in the expression of AMPs during TB infection, HNP1-3 is predominantly released by activated macrophage while HBD-3 induced from epithelial cells in response to inflammatory cytokines and microbial stimulation (37).

This study showed higher discriminatory power for HNP1-3, Figure (1). A Study conducted by Kathamuthu et al., showed that plasma levels of HNP1-3 had good diagnostic values in discriminating individuals with tuberculosis lymphadenitis from latent tuberculosis and healthy individuals (38). In addition, Mohammed et al., showed that IFN- $\gamma$  had a diagnostic value in PTB patients (12). Mensah et al., showed that IL10 had effective diagnostic performance and known for its immunoregulatory effect as an anti-inflammatory cytokine that inhibits the actions of pro-inflammatory cytokines (39,40). Ali et al., documented good diagnostic value (AUC for HBD 3=0.77)(10). Differences in the results of ROC analysis among the latter studies and the current study could be attributed to the differences in the used ELISA kits and differences in number of patients enrolled in each study.

#### 4. LIMITATIONS

Relatively small sample size within each PTB subgroups (30) represents a limitation of the present study. case-control study design limits the ability to establish temporal or causal relationships between the investigated biomarkers and disease progression or bacterial burden. In addition, potential confounding factors and heterogeneity in treatment regimens may have influenced the observed biomarker levels. Therefore, larger longitudinal studies, including assessments prior and after anti-tuberculosis treatment, are needed to validate these findings and to determine whether IL-10, IFN- $\gamma$ , HNP1-3, and HBD3 may have potential clinical diagnostic or prognostic applications.

#### 5. CONCLUSION

Significant higher level of IL-10 was found in undertreatment group followed by active PTB patients, whereas, IFN- $\gamma$  mean level showed opposite pattern. Significant higher concentration of HNP1-3 in both patient groups in comparison to healthy, whereas, non-significant lower mean of HBD3 was observed in active PTB patients. Decrease IL10 with increase TB severity, higher mean of IFN  $\gamma$  and HBD3 were found in moderate severity cases, whereas, HNP1-3 tend to increase from mild to severe cases. Lower mean levels interleukin HBD3 mean was found in +3 grade., whereas, lowest mean of IFN- $\gamma$  and HNP1-3 was seen in +2 grade. ROC analysis showed that HNP1-3 had higher discriminatory power. This study might possibly reflect the importance of the tested biomarker in pathogenesis of TB and bacterial clearance.

#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

#### ETHICS STATEMENT

The protocol for this study was approved by the Ethics Committee of Applied Medical Sciences, Department of Clinical Laboratories /University of Karbala.

#### DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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