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Research Article

Human Beta Defensin 1 (hBD1) Levels in Sputum and Lysate of Mononuclear Blood Cells of Drug-sensitive and Drug-resistant Pulmonary Tuberculosis Patients Attending a Tertiary Hospital in Ibadan, Nigeria

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Abstract

Background: *Mycobacterium tuberculosis (M. tb)* that causes pulmonary tuberculosis (PTB) occupies the lungs, while human β-defensin-1 (hBD1) is expressed in all human epithelial tissues as one of the products of phagocytic leucocytes, especially at the site of microbial colonisation such as the lungs. The involvement of hBD1 in mycobacterial infection has not been extensively studied, thus there is the need to measure the levels of the hBD1 in mononuclear cell lysates and sputum of PTB patients at diagnosis. **Materials and Methods:** Ninety participants aged between 15 and 64 years were recruited as follows: 30 newly diagnosed multi-drug-resistant TB (MDR-TB) patients and 30 newly diagnosed drug-sensitive TB patients (DS-TB) from MDR-TB Treatment centre and the Medicine Outpatient Clinic at University College Hospital (UCH) Ibadan, Nigeria. Thirty (30) non-TB apparently healthy individuals served as controls. The analytical method employed for the measurement of hBD1 in the sputum and lysate was the Enzyme-linked immunosorbent assay (ELISA). The data were expressed as mean and standard deviation, and the differences between the means were established using Student (*t*) test. *P*-value ≤ 0.05 indicated statistical significance. **Results:** The mean levels of lysate and sputum hBD1 were not significantly different in newly diagnosed DS–TB patients (D_0)compared with control ($p > 0.05$). Whereas, the mean levels of lysate and sputum hBD1 were significantly higher in newly diagnosed MDR–TB patients (M_0) compared with newly diagnosed DS–TB patients (D_0)or control ($p < 0.05$). **Conclusion:** Due to the higher levels of hBD1 in the sputum and lysate of M_0 than in D_0, one might conclude that there is a relationship between chronicity of PTB and hBD1 level.

Keywords: defensins, tuberculosis, lysate

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1. Introduction

Tuberculosis (TB) is an infectious disease caused in humans by several strains of mycobacterium, especially *Mycobacterium tuberculosis (M. tb)* [1]. Globally, TB has remained a major health problem that is endemic in most developing countries of the world and responsible for ill health among millions of people. Nigeria is ranked fourth among the 22 countries with a high burden of TB in the world with a prevalence of 330 per 100,000 [2]. There is also an increasing incident of MDR-TB in Nigeria which is jeopardising the efforts of TB control [2]. Among reasons why *M. tb* is a major health problem globally is due to the occurrence of drug resistant strains of *M. tb* [3].

Resistance to TB drugs could result from inappropriate treatment with a single anti-TB drug (usually isoniazid), wrong-drug prescription leading to ineffective treatment and may also be due to the patient not taking the medication correctly, which can be due to a variety of reasons, including cost or scarcity of medicines, patient's forgetfulness, or patient stopping treatment early because they feel better [4]. None of these reasons addressed innate intracellular components of cells involved in combating *M. tb* infection.

Transmission of *M. tb* is via inhaled air and as *M. tb* reaches the pulmonary alveoli, phagocytosis by alveolar macrophages and dendritic cells (DCs) is the first event in the non-specific host–pathogen interaction [5, 6]. Alveolar macrophages and DCs recognize *M. tb* through pattern recognition receptors (PRRs), such as pathogen-associated molecular patterns (PAMPs), which trigger an intracellular signalling cascade in the alveolar macrophages, leading to phagocytic activity [7, 8].

Anti-microbial peptides (AMPs), which include defensins, have been identified as key elements in the innate host defence against infections [9]. Defensins possesses and exerts antimicrobial and cytotoxic activities against microorganisms especially during *mycobacterium* infection [10]. Apart from their antimicrobial abilities, defensins also act as chemo-attractants to immature DCs and T cells [11].

Human β-defensins (hBD) are expressed in all human epithelial tissues [12] as one of the products of phagocytic leucocytes, especially at the site of microbial colonisation [13]. Both hBD1 and hBD2 genes are also expressed in human trachea and lung and are believed to contribute to antimicrobial defence in the respiratory tract [14]. Since *M. tb* inhabit lungs and certain leucocytes where defensins are produced, there is a need to determine the levels of human defensin in the local environments (monocytes and sputum), where *M. tb* normally infect.

2. Materials and Methods

2.1. Study participants

This is a case-control study that involved the recruitment of 90 participants aged between 15 and 64 years. After obtaining a written informed consent for this study, the participants were divided into three groups that comprised of 30 multi-drug-resistant TB (MDR-TB) patients, 30 drug-sensitive TB patients (DS-TB), and 30 non-TB, apparently healthy, individuals as controls. The MDR-TB patients were diagnosed as being infected with isoniazid and rifampicin-resistant strains of *M. tb* with the aid of their clinical history, chest X-ray and GENE Xpert® test. After which they were admitted into the MDR-TB centre, University College Hospital (UCH) Ibadan, Nigeria for anti-TB treatment. As for the DS-TB patients, they were recruited from the Medicine Outpatient Clinic at UCH, Ibadan, Nigeria, by a Consultant Chest Physician after laboratory tests and chest X-rays were performed and from the clinical history presented. The study protocol was reviewed and approved by the University of Ibadan/University College Hospital Joint Institutional Research Ethics Committee.

Three millilitres (3 ml) of blood was drawn from the antecubital vein of each participant into lithium heparin tube and mixed with 3ml of Phosphate Buffered Saline (PBS). Lymphoprep (6ml) carefully layered on it and was at 600g for 15 mins to obtain mononuclear cells above the mixture of polymorphonuclear cells and red blood cells. Mononuclear cells obtained were washed, resuspended in Ringers solution, counted and adjusted to 0.5 x 10^6 cells/ml. Mononuclear cell lysate was obtained by freeze thaw method. Cell suspension was frozen for 15 mins at –20°C and thawed at 4°C for 30 mins. This procedure of freezing (–20°C, 15 mins) and thawing (4°C, 30 mins) was repeated to make three cycles. Microscopic examination confirmed complete disruption of mononuclear cells. Lysate was stored at –20°C until analysis.

Early-morning sputum samples were collected into sterile bottles to avoid contamination. Sputum samples were diluted in Phosphate Buffer Saline (PBS) in a ratio 1:1 dilution factor. The mixture was centrifuged and the clear supernatant was collected and stored for analysis at –20°C.

Enzyme-linked Immunosorbent Assay (ELISA) method was used for the determination of mononuclear cell lysate and sputum concentrations of hBD 1 as specified by kit manufacturer (Elabscience Biotechnology Co., Ltd, P.R.C). Student *t*-test was used to compare two mean values; *p*-value less than 0.05 was considered significant.

3. Results

As shown in Table 1, the mean levels of mononuclear cell lysate and sputum hBD1 in control, $D_0$and M_0 were compared in Table 1. The mean levels of lysate and sputum hBD1 were not significantly different in $D_0$when compared with the controls. The mean levels of WBC lysate and sputum hBD1 were significantly higher in M_0 compared with D_0 or control. However, the mean concentration of sputum hBD1 in M_0 was higher than the level in the lysate. The reverse was the case in other groups.

Table 1: Mean levels of hBD 1 in mononuclear cell lysate and sputum hBD1 levels in controls, D_0 and M_0 at diagnosis.

Variables	Control (n = 30)	D_0 (n = 30)	M_0 (n = 30)	F
WBC Lysate hBD1(pg/ml)	57.08 ± 12.71	57.58 ± 13.75	70.71 ± 21.27$^{\#+}$	3.898
Sputum hBD1(pg/ml)	19.1 ± 4.7	35.5 ± 30.7	171.4 ± 210.4$^{\#+}$	13.561$^{\#}$
$^{\#}$Significantly different when compared with controls,				
$^{\#}$Significantly different when compared with controls,				
$^{+}$Significantly different when compared with D_0				

4. Discussion

Defensins, especially β-defensins, are known as endogenous antibiotics [15] that are involved in antimycobacterial defence in the local environment, especially during the inflammation and colonisation phase of mycobacterial infection [13]. Yet, studies on the levels of hBD 1 in the lysate and sputum in TB patients is lacking, though reported in the fluids of patients having other conditions apart from TB [16–18].

Our finding of increased levels of sputum and lysate hBD1 in M_0 compared with D_0 and control might be due to chronic nature of *M. tb* infection in the M_0 group, which would have led to increased recruitment and activation of immune cells (macrophages and DCs), pro-inflammatory cytokines (TNF-α, IFN-γ, IL-1β and IL-6) and chemoattractant. Secretion of pro-inflammatory cytokines, especially TNF-α, is a contributory factor to increased inflammation in situ. This is further exacerbated by the consistent replication and imminent necrotic activity of the *M. tb* within the macrophage leading to increased leucocyte activation. This increased activation consequently cause production of large amount of hBD1 from the epithelial cells in M_0 with a view to inhibiting the mycobacterial growth in the alveolar macrophages and defending other alveolar cells from being infected.

Non-significant differences observed when the mean levels of lysate and sputum hBD1 in D_0 were compared with the control could be attributed to the early or non-severe phase of the *M. tb* infection in the patients. There is supporting evidence from the studies conducted by Ertugrul *et al* [19] and Kaltsa *et al* [20], which reported higher levels of hBD1 in chronic periodontitis patients compared with patients having gingivitis and elevated levels of hBD1 in patients with cirrhosis when compared with hepatitis patients and control.

One of the sites of β-defensins production is the epithelial cells of the lungs where it acts as defence to mycobacterial infection and furthermore inhibits mycobacterial growth. Defensins also act as chemoattractant to antigen specific T-cells and immature macrophages with the aid of IL-2 and IFN-γ that induce macrophage activation towards the development of a microbicidal granuloma via host-specific cell-mediated immune response to the end of halting the replication and spread of the infection to other parts of the body. In an earlier study, Schwander et al. [21] reported high number of poly-morphonuclear (PMN) cells and immature macrophages in BAL fluids from the lungs of TB patients. Thus, the observed elevated levels of sputum hBD1 in M_0compared with D_0 and controls could indicate that there is heightened production of hBD1 in the lungs of M_0 that probably resulted in high level of sputum hBD1 as observed by this study. hBD1 is mainly expressed by epithelial cells, tissues and salivary glands, especially in the local environment of the infection, which is in the epithelial of the lungs. This could also account for higher level of sputum hBD1 compared with monocyte lysate hBD1.

5. Conclusion

1. Since the levels sputum and lysate hBD1 were higher in TB patients than normal individuals, it shows that hBD1 might be involved in combating TB and may be used as a therapeutic target for treating TB.
2. There is an association between and TB severity and hBD1.

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