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Galectin3 and CD16 play an important immunological role in patients infected with *Salmonella typhi*.

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ABSTRACT

Enteric fever caused by *Salmonella typhi* (*S.typhi*) is one of the most dangerous diseases in developing countries. The main aim of this work is study the immunological role of Galectin3 and CD16 in human infected with *S.typhi*. A total number of 60 outpatients infected with acute IgM and chronic IgG *S.typhi* were included in this study, serum concentration of Galectin3 and CD16 were measurement by Enzyme-linked Immune Sorbent Assay (ELISA) for each patient. The results proved that there was significant increase (P = < 0.0001***) in serum concentration of CD+16 between patients with acute *S.typhi* (IgM) (17.760 ± 0.72773 ng/ml) and healthy individuals (13.690 ± 0.36638 ng/ml). Also, there was significant increase (P = <0.0001***) in serum concentration of CD+16 between patients with chronic *S.typhi* (IgG) (20.348 ± 1.1848 ng/ml) and healthy individuals (13.690 ± 0.36638 ng/ml). Also; there was significant increase (P = 0.0011**) in serum concentration of Galectin3 between patients infected with acute *S.typhi* (IgM) (9.1485 ± 0.30444 ng/ml) and healthy individuals (7.1058 ± 0.50831 ng/ml) and there was significant increase (P value= 0.0004***) between patients infected with chronic *S.typhi* (IgG) (9.5004 ± 0.38143 ng/ml) and healthy individuals (7.1058 ± 0.50831 ng/ml). Natural killer cell (CD16) and Galectin3 have an important immunological role in patients infected with acute and chronic *S.typhi* infection. Therefore, Galectin3 and natural killer cell have synergistic immunological effect against *S.typhi* infection.

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INTRODUCTION

Salmonella Typhi (*S.typhi*) is a gram negative bacteria pathogenic bacteria, motile, produce biofilms and causes more than fourteen million acute cases of enteric fever that are responsible many thousands deaths each year especially in developing countries (Hahn and Gunn, 2020). *Salmonella typhi* chronic infections occur when *S. Typhi* persists primarily in the gallbladder by forming biofilms on cholesterol gallstone (González *et al.*, 2019). Environmental conditions ultimately dictate the key properties of the biofilms, During chronic infection, *salmonella* biofilms develop several extracellular polymeric substances which are hypothesized to prevent pathogen clearance either by shielding biofilm-associated bacteria from direct humoral attack or by modulating innate phagocyte interaction with biofilms (Aljanaby, 2018; Srinandan *et al.*, 2015; Maruzani *et al.*, 2019). Depending on human CD 16 + marker and Galectin3, CD16 is a type I trans-

membrane receptor with two extracellular Ig-like domains, the disease can be detected (Patel *et al.*, 2019). CD16 is a low-affinity IgG receptor that mediates antibody-dependent cell cytotoxicity by NK cells, is involved in antibody-dependent cell cytotoxicity, and is expressed in large granular lymphocytes (LGL) of both NK and T cell types (Gurjar *et al.*, 2017).

About 17–21% of peripheral blood lymphocytes and a much smaller fraction (5%) of bone marrow lymphocytes are CD16 expressed in almost the entire body (Vujanovic *et al.*, 2019). CD16 is also expressed in granulocytes, tissue macrophages and subsets of monocytes, eosinophils and dendritic cells at moderate levels, as well as decreased or absent expression in paroxysmal nocturnal hemoglobinuria due to structural anchor membrane protein (Cho *et al.*, 2016). Galectin-3 is a protein that binds β-galactoside and is related to lactose and N-acetyl lactosamine (Meir *et al.*, 2018). Galectin-3 is an essential protein that expresses a wide range of immunological cells such as neutrophils, macrophages, and mast cells, including the lung, stomach, colon, uterus, and ovary (Johannes *et al.*, 2018). In Iraq; there were limited studies about relationship between *S.typhi* human infection and immune response, therefor; this study aimed to study the immunological role of Galectin3 (protein) and CD16 (natural killer cell) in *S.typhi* infection to human.

MATERIALS AND METHODS

Patients

This is a case-control study performed in Al-Najaf City, Iraq. Sixty outpatients infected with *S.typhi* were included in this study, male and female age ranged 20-50 years old during April 2019 to the February 2020. Data on the following variables were recorded included; clinical variables age and gender. The authors received approval for this study from all patients and healthy individuals include: blood collection and all information.

Healthy individuals (control)

Twenty nine healthy individuals were selected in this study as control, 15 Male and 14 female, age range 20-50 years old. All healthy individuals were don't have any infections.

Collection of blood and culture

By using sterile disposable syringe; 5 ml of blood were collected from all patients and healthy individuals (control) in sterile tube and left at room temperature till being clotted, and then were centrifuged at 3000 rpm for 5min. The serum was aspirated from the tube and stored at -25C^0 until used (Majeed and

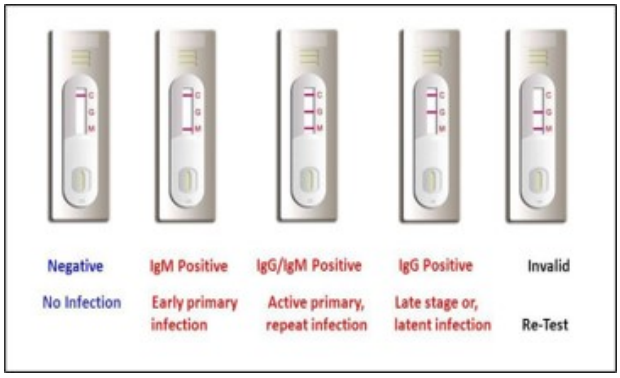

Figure 1: Positive and negative results of Salmonella typhi IgG/IgM combo rapid test according to manufacturingcompany (CTK Biotech, USA)

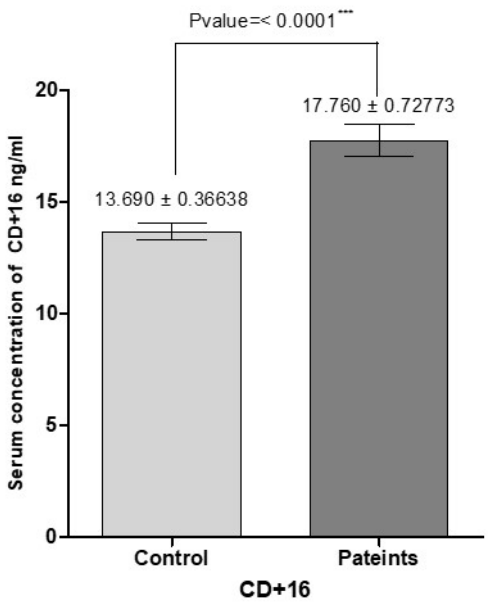

Figure 2: Serum concentration of CD16+ in patients infected with acute S.typhi(IgM) and healthy individuals

Aljanaby, 2019; Mohy *et al.*, 2019). For blood culture; 5 ml of blood were collected from all patients suspected infected with *S.typhi* by sterile syringe and added to 45 ml of brain heart infusion broth and incubated aerobically in 37C^o for 7 days with continuous shaking every day , then streaked (by sterile loop) on blood, MacConkey and SSagar agar plates then incubated aerobically in 37C^o for 24h (Collee *et al.*, 1996; Hayder and Aljanaby, 2019).

Stool collection and culture

Five gram of stool were collected from all patients in sterile container and mixed with 10 ml sterile distilled water and added to brain heart infusion broth and incubated aerobically in 37 ^{o}C for 24 hours then streaked (by sterile loop) on blood, MacConkey

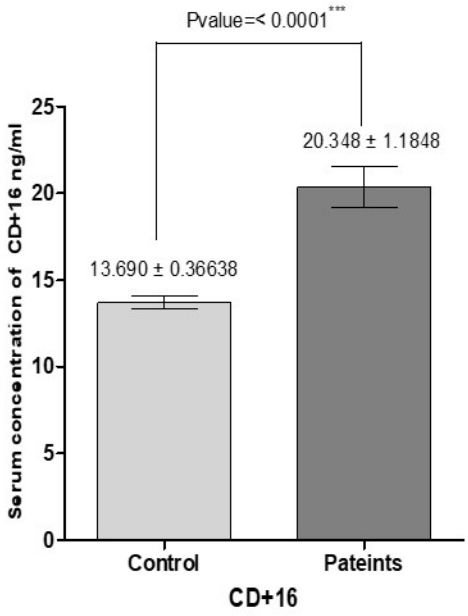

Figure 3: Serum concentration of CD+16 in patients infected with chronic S.typhi(IgG) and healthy individuals

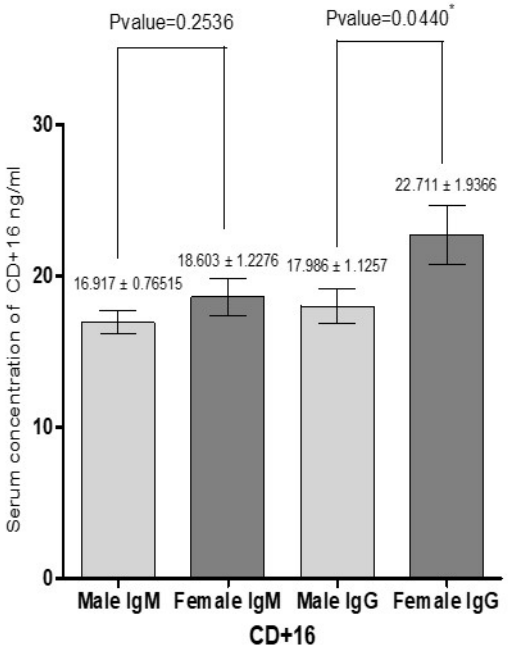

Figure 4: Serum concentration of CD+16 in patients infected with acute S.typhi(IgM) and chronic S.typhi (IgG) according to gander

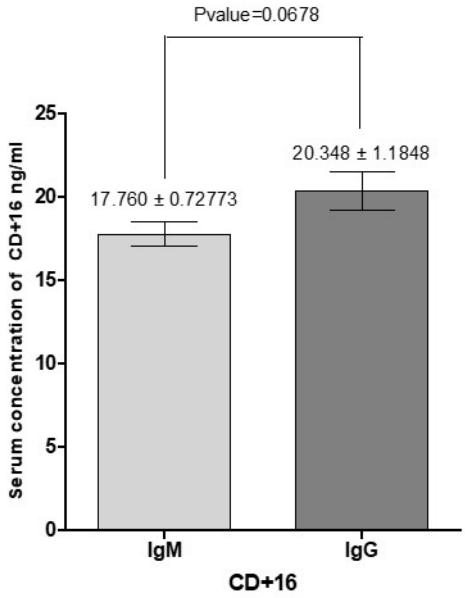

Figure 5: Serum concentration of CD+16 in patients infected with acute S.typhi(IgM) and chronic S.typhi (IgG)

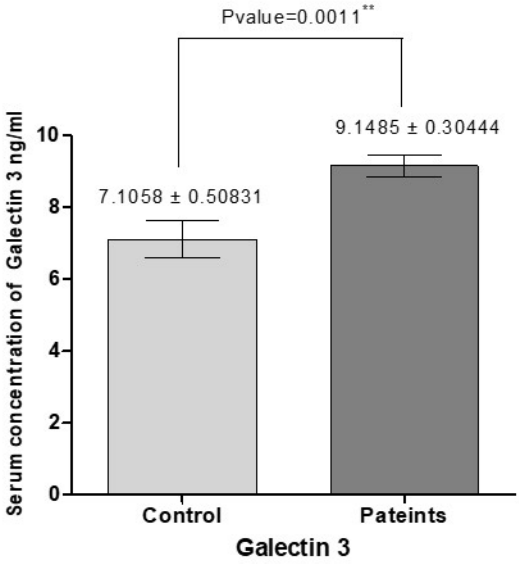

Figure 6: Serum concentration of Galectin3 in patients infected with acute S.typhi(IgM) and healthy individuals

and SSagar agar plates and incubated aerobically in 37 °C for 24h (Collee *et al.*, 1996; Al-Labban *et al.*, 2019).

Identification of *S.typhi*

Standard bacteriological methods were used to identified all *S. typhi* isolates (Collee *et al.*, 1996; Aljanaby, 2018; Aljanaby and Medhat, 2017; Aljanaby and Aljanaby, 2018) and by using Vitek2® system (BioMerieux, France).

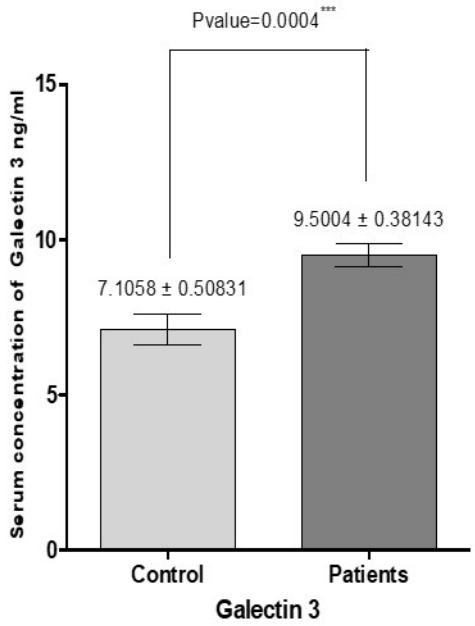

Figure 7: Serum concentration of Galectin3 in patients infected with acute S.typhi(IgG) and healthy individuals

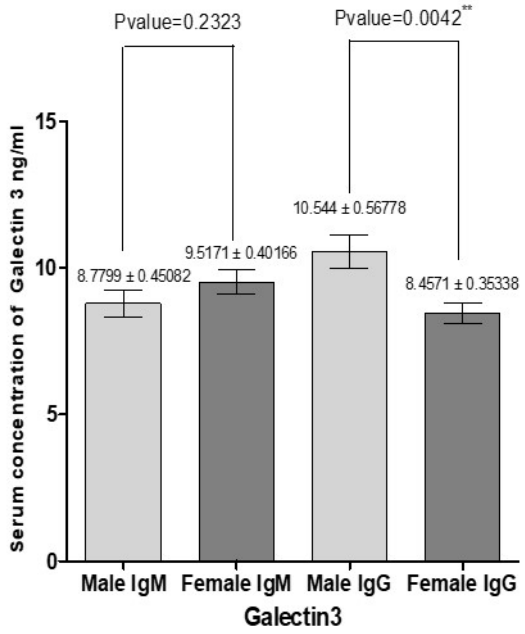

Figure 8: Serum concentration of Galectin3 in patients infected with acute S.typhi(IgM) and chronic S.typhi (IgG) according to gander

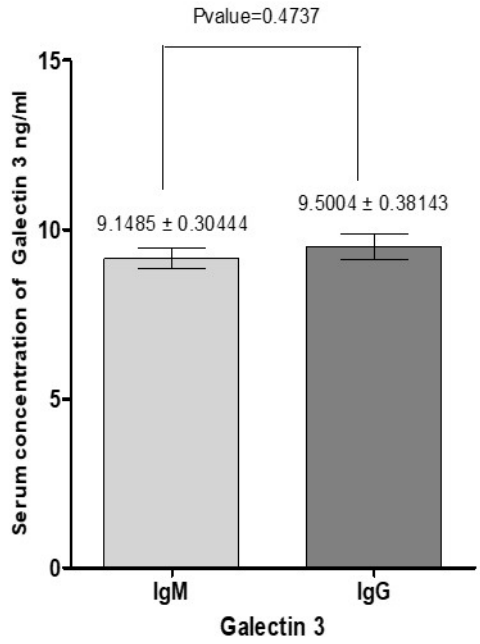

Figure 9: Serum concentration of Galectin3 in patients infected with acute S.typhi(IgM) and chronic S.typhi (IgG)

Salmonella typhi IgG/IgM combo rapid test

The following test was done according to the steps of the manufacturing company (CTK Biotech, USA) as follows:

1. One drop of patient serum was mixed well with sample diluent
2. One drop of mixture was added in the specified place in the kit and wait 15 minutes
3. The result was read according to Figure 1.

Enzyme immunoassay

These tests were achieved according to the manufacturing company (Elabscience, China) for Galectin3 and CD16 as follows:

1. 100 μL of sample or standard were added to the wells and incubated for 90 min at 37°C.
2. 100μL was added Biotinylated detection Ab working solution to each well and incubated for 60 min at 37°C.
3. Plates were aspirated and washed for 3 times.
4. 100 μL of HRP conjugate working solution was added and incubated for 30 min at 37°C. Aspirated and washed the plate for 5 times.

5. 90 μL of substrate reagent was added and incubated for 15 min at 37°C.
6. 50 μL of stop solution was added.
7. All plates were read at 450nm immediately and of the results were calculated.

Statistical analysis

In this study; Graph pad prism 6 computer software was used. Mean and standard error (SE) for each value was determined by using T- Test. P- value less than the 0.05 level of significance was considered statistically significant (Aljanaby, 2018; Adam *et al.*, 2019; Witwit *et al.*, 2019).

RESULTS AND DISCUSSION

CD16

The results of the current study proved that there was significant increase (P = < 0.0001***) in serum concentration of CD+16 between patients infected with acute *S.typhi* (IgM) (17.760 ± 0.72773 ng/ml) and healthy individuals (13.690 ± 0.36638 ng/ml) (Figure 2). Also, there was significant increase (P = <0.0001***) in serum concentration of CD+16 between patients infected with chronic *S.typhi* (IgG) (20.348 ± 1.1848 ng/ml) and healthy individuals (13.690 ± 0.36638 ng/ml) (Figure 3). According to gender, the results of the present study demonstrated that there was no significant differences (P = 0.2536) between male (16.917 ± 0.76515 ng/ml) and female (18.603 ± 1.2276 ng/ml) infected with acute *S.typhi* (IgM). While, there was significant increase (P = 0.0440*) between male (17.986 ± 1.1257 ng/ml) and female (22.711 ± 1.9366 ng/ml) infected with chronic *S.typhi* (IgG) (Figure 4). On the other hand, the current study proved that there was no significant differences (P value= 0.0678) in serum concentration of CD+16 in patients infected with chronic *S.typhi* (IgM) (17.760 ± 0.72773 ng/ml) as compare with serum concentration of CD+16 in patients infected with chronic *S.typhi* (IgG) (20.348 ± 1.1848 ng/ml) (Figure 5).

Galectin3

The results of the present study demonstrated that there was significant increase (P = 0.0011**) in serum concentration of Galectin3 between patients infected with acute *S.typhi* (IgM) (9.1485 ± 0.30444 ng/ml) and healthy individuals (7.1058 ± 0.50831 ng/ml) (Figure 6) and, there was significant increase (P value= 0.0004***) between patients infected with chronic *S.typhi* (IgG) (9.5004 ± 0.38143 ng/ml) and healthy individuals (7.1058 ± 0.50831 ng/ml) (Figure 7). According to gender, the results demonstrated that there was no significant differences (P = 0.2323) between male (8.7799 ± 0.45082 ng/ml) and female (9.5171 ± 0.40166 ng/ml) infected with acute *S.typhi* (IgM). While, there was significant increase (P = 0.0042**) between male (10.544 ± 0.56778 ng/ml) and female (8.4571 ± 0.35338 ng/ml) infected with chronic *S.typhi* (IgG) (Figure 8). On the other hand, the current study proved that there was no significant differences (P value= 0.4737) in patients infected with chronic *S.typhi* (IgM) (9.1485 ± 0.30444 ng/ml) as compare with serum concentration of Galectin3 in patients infected with chronic *S.typhi* (IgG) (9.5004 ± 0.38143 ng/ml) (Figure 9).

CD+16

Unlike CD8 + T cells, NK cells recognize abnormal cells through a given set of germline-encoded receptors, such as the inhibitory receptors KIR and NKG2A and the activating receptors NKG2D, DNAM-1, and NKp30. NK cells respond to cells that exhibit an incompatible repertoire or decreased levels of MHC class I molecules, allowing for identification of certain cancer cells that may resist CD8 + T cell responses. Low inhibitory ligands expression combined with high levels of activating ligands on target cells results in NK cell action (Koch *et al.*, 2013; Pahl and Cerwenka, 2017). Natural killer (NK) cells are endogenous cytotoxic lymphoid cells which are in the first line of defense against virally infected cells and cancer cells (Spits *et al.*, 2016). In Nigeria; (Ajibola *et al.*, 2018) demonstrated that the concentration of CD+16 in patients infected with acute *S.typhi* was (18.061 ng/ml), moreover, this result is in agreement with result of study by (Wasihun *et al.*, 2015) proved that the mean concentration of CD+16 was (15.922 ng/ml), while the result of this study is not agreement with the study of Crump *et al.* (2015) proved that the level of CD+16 inpatients with *S. typhi* was (8.732 ng/ml). The study in Ethiopia by (Deksissa and Gebremedhin, 2019), they showed that the serum concentration of CD+16 in patients infected with chronic *S.typhi* was (14.585 ng/ml). CD16 is a potent cytotoxicity receptor that can be manipulated by therapeutic bispecific antibodies on human natural killer (NK) cells. Until now, the effects of CD16-mediated activation on NK cell effector functions beyond classical antibody-dependent cytotoxicity have remained poorly explained (Pahl *et al.*, 2018). The immune system is known to be involved in the development and progression of enteric fever. Immune infiltration of different immune cells in enteric fever has been shown to be related to metastasis and prognosis (Smythies *et al.*, 2005). Additionally, the circulating immune cells may represent the local immune response in the infection, thus providing potentially important

information about the progression of disease in infection. Natural killer (NK) cells are considered to be interesting targets for translational and clinical studies as an important subset of immune cells, whose behavior is triggered by an evolving and delicate balance between activating and inhibitory signals obtained by cell surface receptors (Cui *et al.*, 2019).

Galectin3

One of the most important human protein is Galectin-3 is encoded in humans by the gene LGALS3. Galectin-3 is a member of the lectin family which has identified 14 mammalian galectins. Galectin-3 is approximately 30 kDa and, like all galectins, contains a carbohydrate-recognition-binding domain (CRD) of approximately 130 amino acids that allow β-galactosides to be specifically bonded. Galectin-3 is also a member of the family of beta-galactoside-binding proteins, which plays a significant role in cell-cell adhesion, cell-matrix interactions, macrophage activation, angiogenesis, metastasis, apoptosis. Galectin-3 is encoded on chromosome 14, locus q21–q22 by a single gene, LGALS3 (Hönig *et al.*, 2018; Bänfer *et al.*, 2018). The result of this study is in agreement with the study by Sauteur *et al.* (2013) they proved that concentration of Galectin 3 in patients infected with acute *S.typhi* higher than healthy individuals. On the other side, the result of (Gayet *et al.*, 2017) demonstrated that concentration of Galectin 3 in patients infected with acute *S.typhi* was (22.785 ng/ml), this result is not in agreement the result of the current study. Also,there is a study by Gal-Mor *et al.* (2014) and his co-authors shoed that the value of Galectin 3 in patients infected with chronic *S.typhi* was (6.021 ng/ml) and this result is not in agreement with present study result. In 2016, the study by (Gal-Mor *et al.*, 2014; Marzel *et al.*, 2016) showed that Galactin3 concentration in the infection of acute *S.typhi* in males was little than females, another study by Johnson *et al.* (2017) showed that the Galactin3 concentration in the infection of acute is higher rate with acute *S.typhi* between females, the previous studies are in agreement with existing study result. Galectin-3 is commonly expressed in epithelial and immune cells, and its expression is associated with severe and metastasized cancer. Galectin3 has a variety of biological phenomena including cell growth, adhesion, differentiation, angiogenesis, and apoptosis. Recent research has found that galectin3 is associated with several stages of invasion and metastasis, such as angiogenesis, cell-matrix interaction, blood flow dissemination and extravasation (Zhao *et al.*, 2009). Galectin3, a 31 kDa member of the β-galactoside-binding proteins, is an intracellular and extracellular lectin that interacts with intracellular glycoproteins, molecules of the cell surface and extracellular proteins. (Zhao *et al.*, 2009; Takenaka *et al.*, 2002).

CONCLUSION

Natural killer cell (CD16) and Galectin3 have an important immunological role in patients infected with acute and chronic *S.typhi* infection. Therefore, Galectin3 and natural killer cell have synergistic immunological effect against *S.typhi* infection.

Funding Support

None.

Conflict of Interest

None.

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