Detection of HSP27 and TLR4 in surgical patients

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Abstract

Background: Postoperative infection is a common healthcare-associated problem, and unfortunately, a serious complication in all surgery patients. Toll-like receptors (TLRs) are crucial in activating non-specific immunity mechanisms and integrating elements of the immune system, due to interactions between specific and non-specific responses.

Aim of the study: The purpose of this study to evaluated the level of Heat shock protein (HSP27) and Toll-like receptors (TLR4) after different type of surgery and compare with control.

Materials and methods: This study was conducted in the city of Balad, and 60 samples were collected from patients in Balad General Hospital and Primary health care centers; during August 15, 2023, to November 1, 2023 of both sexes and different age group (15 males and 45 females-aged (5 to 50) were split into two groups, patients (60 sample) and healthy groups (30 sample). The immunological parameter tested by ELISA.

Results: The study showed significant increase TLR4 in patient after surgery in compared with control group that were (222.3±88.0 and 107.2±47.3) respectively, at *p*-value (0.0004). In addition significant increase HSP-27 levels in Patient after surgery in compared with Control group that were (18.00±7.11and 12.60±5.83) respectively. Also this study showed no differences in the level of HSP27 and TLR4 according to gender.

Conclusion: The present study concluded increase TLR4 and HSP27 levels in patient after surgery as compared with healthy group. In addition, no significant differences in the mean of TLR4 and HSP27 between male and female.

Keywords: post-operative infection, HSP27, TLR4

1. Introduction

Surgery carries a high likelihood of postoperative infections due to the invasive nature of the surgery and the subsequent inflammatory response. Postoperative infections can have serious repercussions for the patient and lead to prolonged hospitalization, as well as increased hospitalization costs. A reduction of the prevalence of infections requires not only an augmentation in surgical behavior but also the implementation of a competent epidemiological and microbiological approach [1, 2].

The immune system can identify intruding pathogens through a group of receptors known as pattern-recognition receptors (PRRs). These receptors identify molecular patterns associated with pathogens (pathogen-associated molecular patterns (PAMPS)) and subsequently activate a cascade of signals to generate an immune response. The PRRs are comprised of various groups of molecules such toll-like receptors (TLRs [3]. The presence of TLRs has been confirmed in most immune cells, especially in those responsible for the recognition of pathogens, but also on the surface of neutrophils, eosinophils and lymphocytes. In addition to the cells of the immune system, TLRs are also present on the surface and inside cells that can come into contact with pathogens, such as respiratory epithelial cells, gastrointestinal tract cells, endothelial cells, skin cells, urogenital tract epithelial cells, adipocytes, and myocytes [4]. Although a role for TLR4 in the immune response to burn injury is well studied, the role of TLR4 in the inflammatory response to excisional wounds has not been well investigated (Maung *et al.*, 2005) [5].

The healing wound bed contains different inducible HSPs like HSP90, HSP70, HSP47 and HSP27 which all together bring about protein homeostasis and cell proliferation during wound healing (Laplante AF *et al*., 1998) [6]. Besides modulating inflammatory responses via the induction of HSP-reactive regulatory T cells, HSPs can directly activate the immune system through surface receptors such as toll-like receptor (TLR)2, TLR4, CD91, CD40 and CD14. Small HSPs like HSP27 support wound healing by stabilizing actin microfilaments, supporting endothelial cell migration in the wound bed, protecting sensory neuron degeneration and inhibiting stress induced apoptosis (Hirano *et al*., 2004) [7]. This function of HSP27 is governed by its MAPK mediated phosphorylation (Rouse *et al*., 1994) [8].

2. Materials and methods

2.1 Subjects

This study was conducted in the city of Balad, and 66 samples were collected from patients in Balad General Hospital and Primary health care centers ;during August 15, 2023, to November 1, 2023 of both sexes and different age group (15 males and 45 females-aged (5 to 50) were split into two groups, patients (66 sample) and healthy groups (30 sample), under medical supervision by a specialist doctor, after performing the surgical operation, before taking the bandage, and before sterilization using a cotton swab. Information was taken from each patient including: age, gender, type of operation, chronic diseases, and a questionnaire were prepared for each patient. In this study, deep wounds were excluded.

Venous blood samples (5 ml) were taken from patients, and 30 venous blood samples were taken from healthy individuals (comparison group).

2.2 Immunological test by ELISA

This ELISA kit used Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to HSP27, and TLR4 Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for HSP27, and TLR4 is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain HSP27, and TLR4 and HRP conjugated HSP27, and TLR4 antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The OD value is proportional to the concentration of HSP27, and TLR4 You can calculate the concentration of HSP27, and TLR4 in the samples by comparing the OD of the samples to the standard curve.

2.3 Statistical analysis

Data were analyzed by Minitab program system ver-17 and ANOVA test were applied. The means compaired by Duncuns Multiple Range under the level of significantly 0.05.

3. Results

As shown in Table (1), A significant increase *p*-value (0.0004) in the mean of TLR4, between patient after surgery and Control Group that were (222.3±88.0 and 107.2±47.3) respectively. In addition significant increase H S P 27 levels in Patient after surgery in compared with Control group that were (18.00±7.11and 12.60±5.83) respectively.

Table 1: Comparison between Patient after surgery and healthy individual regarding the mean of TLR4 and HSP27

Studied groups	T L R 4	H S P 27
Patient after surgery (N:60)	222.3 ± 88.0	18.00 ± 7.11
Control Group (N:30)	107.2 ± 47.3	12.60 ± 5.83
p value	0.0004	0.0002

The study show significant differences *p*-value (0.0006) in the mean of TLR4, between male and female that were (217.4±56.1 and 224.0±54.4) respectively. As well as HSP27 show significant differences *p*-value (0.008) in the mean of HSP27 between male and female that were (17.70 ± 4.86 [a] and 18.09 ± 3.93) respectively.

Table 2: Relation of TLR4, and HSP27 with the gender of patient after surgery

Gender	TLR4	HSP 27
Male (n:15)	217.4 ± 56.1 [a]	17.70 ± 4.86 [a]
Female (n:45)	224.0 ± 54.4 [a]	18.09 ± 3.93 [a]
p value	0.0006	0.008

4. Discussion

Postoperative infections are common healthcare-associated problems, and, unfortunately, are serious complications in patients undergoing surgery. The result of this study show increase TLR4 in patient after surgical that may be due to the inflammation that associated with surgical are the main cause of high level of protein TLR4. This study agree with [9], that show increase TLR4 level in patient post-surgery.

The activation of the innate immune system is the body's first line of defense occurs in response to pathogens or damage-associated molecular patterns (DAMPs) via Toll-like receptors (TLRs) and Nod-like receptors (NLRs) [10]. The level of TLR4 is known to increase in inflammation and after nerve injury and contributes to the development of neuropathic pain [11]. Watkins *et al*.,2009 have found that opioid agonists act as TLR4 agonists, while opioid antagonists (naltrexone, naloxone) act as TLR4 antagonists [12].

Exposure to morphine (that is type of anesthesia) or any other μ-opioids can result in paradoxical hyperalgesia [13]. The study done by [14] suggest this phenomenon to be associated with TLR4 level as in case of normal TLR4 levels, minimal hyperalgesia signs are observed. Opioids can activate TLR4 on glial cells, by mimicking the interface of lipopolysaccharide (LPS), bind to TLR4 co-receptor MD-2 as a LPS analog, for direct activation of TLR4 [15]. Wang *et al*. have found that morphine, similar to LPS, induces TLR4 dimerization and leads to the formation of the (TLR4/MD-2)/(TLR4/MD-2) heterotetramer. TLR4 and MD-2 have been found to be crucial for morphine-induced TLR4 pathway activation, as the production of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), IL-1β and TNF-α reduces, followed by the suppression of the peripheral and central immune systems [16].

Heat shock proteins are expressed under various pathological conditions, including trauma, focal or global ischemia, hypoxia, infarction, and infections. This study found increase Heat shock proteins HPS27 after surgery that may be due to The study done by [17] show significantly increase HPS27 in patients after cardiac surgery that suggested human myocardium releases HSP27 due to inflammatory responses and cardiac functional injury. The study done by [18] found no significant differences between patients after surgical surgery and control. HSP27 readily expressed in the adenoids of children after adenotonsillectomy and may be implicated in immunologic responses [19]. Thus, extracellular Hsps are potentially involved in activation of innate immunity and in the pathogenesis of postoperative organ dysfunction [20].

The study show significant differences in the mean and stander division of TLR4 and HSP-27. This result may be due to these parameters affected by immunity of patients. Both steroid hormones had a significant effect in reducing the bacterial growth Likewise, the direct effect of the estradiol hormone on the growth of *L. reuteri* [21]. previous studies on Gram-positive bacteria, which demonstrated that different steroids reduce *in vitro* growth and increase cell leakage [22]. Many cross sectional studies for the influence of sex steroids on bacteria have proved the existence of correlation between sex hormone levels and

microbiota composition despite the inevitable interfering factors, including genetics and environment [23]. Researchers reported that lower levels of estradiol in postmenopausal women and men is accompanied by increase in *Bacteroidetes* sp. and depletion of *Lactobacillus* sp. Compared to women with higher levels of sex hormones [24]. The effect of sex steroids on bacteria *in vivo* could not be compared by their effect *in vitro* because, in some cases, hormonally related microbial shifts result from endogenous steroid-induced tissue and immunological changes rather than from steroids' direct effect on bacteria [25].

5. Conclusion

The present study concluded increase TLR4 and HSP27 levels patient after surgery as compared with healthy group. In addition, no significant differences in the mean of TLR4 and HSP27 between male and female.

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