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ORIGINAL ARTICLE

Evaluating Heme-Oxygenase-1 and Bilirubin Concentration from the Recovered and Glomerulonephritis Patients from Lady Reading Hospital (LRH) District Peshawar, Pakistan

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ABSTRACT

Purpose: Different studies suggest the defending role of bilirubin and "heme-oxygenase-1(HO-1)" in inflammatory illnesses, but there are limited studies that evaluate these two in recovered patients.

Methods: Therefore, the current study evaluates the bilirubin and HO-1 in patients who suffered from glomerulonephritis and recovered (6 months ago) from the glomerulonephritis and control group.

Findings &Practical Implication: After obtaining the informed consent, sample of the urine and blood were collected from the Lady Reading Hospital (LRH) in district Peshawar, Pakistan. After analysis, it was established that HO-1 and bilirubin levels were found to be greater in participants with kidney infections (HO-1:3.220 and bilirubin: 5.536) compared to the control group (HO-1:1.402 and bilirubin: 2.637). Surprisingly, the levels of HO-1 and bilirubin were in upper limits in the recovered individuals (HO-1:2.333 and bilirubin 4.295) compared to the control group but lower in the glomerulonephritis patients. Further, it was found from the regression analysis that there was no association among the level of HO-1 and bilirubin of the subjects in all study groups.

Conclusion: From the current study, it was concluded that there was no effect of HO-1 on the level of bilirubin in blood plasma. Moreover, it was still unknown why HO-1 and bilirubin levels were in upper limits in the recovered patients. Future research should focus on the levels of HO-1 and bilirubin in recovered patients to see whether they indicate any underlying medical issues.

Keywords: Heme-oxygenase-1; Bilirubin; Glomerulonephritis; Recovered patients

INTRODUCTION

The release of excessive amounts of heme from intracellular proteins is inherently dangerous[1]. There are numerous conditions associated with the release of heme, or free heme, which can lead to oxidative and inflammatory harm [2-3]. As a result, removing excess free heme at the site of injury is of utmost importance [4]. Free heme is oxidatively degraded in the presence of microsomal enzyme heme oxygenase (HO) to produce biliverdin (BV), carbon monoxide (CO), and ferrous iron (Fe2+) [5]. BV is converted by BV reductase into bilirubin (BR) during this reaction, and the ferritin rapidly sequesters ferrous iron and recycles it for heme synthesis [6]. The HO-1 enzyme can metabolize large amounts of free heme to produce enzymatic by-products in high concentrations under a variety of pathological conditions and consequently can influence many biological events, and in recent years, it has been the subject of considerable medical attention [7-9]. HO-1 is expressed by a wide variety of proinflammatory stimuli as well as its substrate and free heme [10]. The evidence suggests that HO-1 plays an important role in the resolution of inflammation in addition to its fundamental role in heme degradation [11]. Several kidney inflammatory diseases are associated with an increased risk of oxidative stress [12-13]. Among these, Glomerulonephritis (GN) is one of the inflammatory disorders that affect the glomeruli as a result of immune-mediated damage [14]. Most studies have used a model of nephrotoxic nephritis (NTN) to examine HO-1 expression in acute GN [15-16]. HO-1 expression also appeared to be primarily present in tubular cells rather than in glomeruli in this model [17]. Several studies have shown that inflammation in the glomeruli induces HO-1 production in the renal tubules [18-19]. High blood pressure and generalized swelling are symptoms of Glomerulonephritis. It affects the blood's ability to filter out waste products and clean them from within the body [20]. Several studies have been conducted on the relationship between HO-1 and bilirubin, as well as their relationship to kidney inflammation [21-22]. It is important to note, however, that no study examines the levels of HO-1 and bilirubin in patients who have recovered from inflammatory kidney disease. The current study aimed to assess the relationship of these factors with each other and further present study also evaluate the concentration of HO-1 and bilirubin levels in the recovered patients.

METHODOLOGY

Sampling: Subjects were recruited from the Lady Reading Hospital (LRH) in Peshawar, Pakistan. Males were selected for the final analysis because of the difference between the male and female blood parameters level, which can also lead to bias in the results. Recovered patients' data that were discharged 6 months ago were obtained from the hospital record and were communicated telephonically. In the case of the glomerulonephritis patient, subjects that were visiting the hospital were asked and after obtaining the informed consent blood and urine sample were collected. Further, the sample was preferably collected from those individuals, in which infection was confirmed by the kidney CT scan or ultrasound as directed by the in-charge medical doctor. Healthy individuals were randomly selected who were not suffered from any kind of medical complication such as diabetes, Cardiovascular or any other inflammatory diseases that can alter protein or antibodies level in the body. After obtaining informed consent from all subjects' 7cc blood was collected in the blood vials containing EDTA as an anticoagulant. Blood was stored in the laboratory at -80 degree centigrade until further analysis. Urine was collected in the urine jar and was taken to the diagnostic Khyber Medical University (KMU) laboratory for analysis.

Evaluating heme-oxygenase-1 and bilirubin level: Plasma HO-1 levels were measured using an "enzyme-linked immunosorbent assay (ELISA)" with a commercially available kit (Human HO-1

ELISA Kit; Enzo Life Sciences Inc., Farmingdale, NY, USA) at KMU, and serum total bilirubin levels, urea and antibodies were measured using an enzymatic method using an auto-analyzer (MSLAB01). Further urine analysis was carried out according to the Canny Edge Detection and Circular Hough Transform as described by Cruz et al [23], and protein was evaluated by bimolecular interaction analysis mass spectrometry as described by Nedelkov and Nelson [24].

RESULTS

The laboratory analysis of all the three groups showed a significant difference in urine results and blood results. Detail is given in table 1. RBCs, WBCs and Proteins level was found higher in the glomerulonephritis patient. The same parameters were found lower in the control group. Interestingly in the case of the recovered patients' the same parameters were found to be higher in the control group but lower than patients. However, values were in the normal range.

Table 1: Laboratory analysis (urine and blood analysis).

Parameters		Control	Glomerulonephritis	Recovered	References values
Urine	RBCs	2	4	3	≤2 RBCs/hpf
	WBCs	3	4	3	≤2-5 WBCs/hpf
	Protein	140	160	150	≤150 mg/d
	Color	pale yellow to deep amber	Cola-pink	pale yellow to deep amber	pale yellow to deep amber
blood	Urea	12	35	20	6 to 24 mg/dL
	Anti-bodies	750	1255	1200	688 to 1251 mg/100ml

Table 2: Heme-oxygenase and bilirubin concentration in three different groups.

	Groups	Average	Variance	P value
Heme oxygenase-1	Control	1.402	0.284	P<0.00
	Glomerulonephritis	3.220	0.391	
	Recovered	2.333	0.128	
Bilirubin	Control	2.637	1.279	P<0.00
	Glomerulonephritis	5.536	1.010	
	Recovered	4.295	0.238	

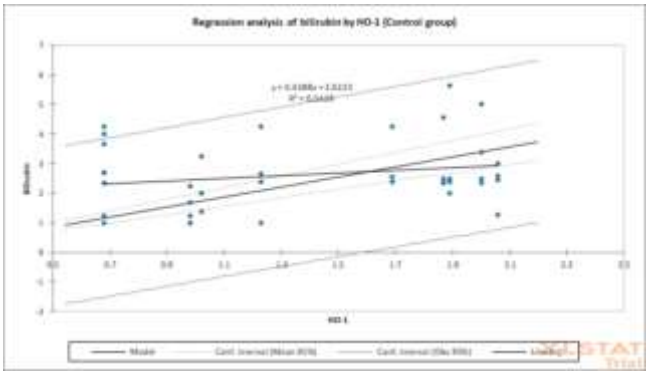

Figure 1: Regression analysis of HO and bilirubin from the control group.

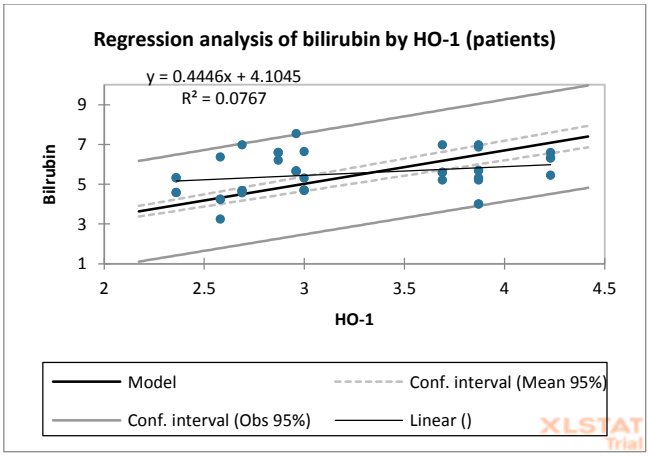

Figure 2: Regression analysis of HO and bilirubin from the patients with glomerulonephritis.

After evaluating the concentration of heme-oxygenase and bilirubin level it was found that heme-oxygenase was significantly higher in the glomerulonephritis patients (3.220), and lowered in the control group (1.402). In the case of recovered patients, HO was found higher than the control group and lower glomerulonephritis. Detail can be seen in table 2.

Bilirubin level was higher in the patients with glomerulonephritis (5.536), followed by the recovered subjects (4.295). Lowest values were recorded for the control group (2.637). Detail is depicted in table 2.

It was found from the regression analysis, that there was no association between the HO-1 and bilirubin levels of the subjects in all three groups. There was no linear arrangement of the data as shown by the blue dots in figure 1(control), figure 2 (patients with glomerulonephritis) and figure 3 (recovered).

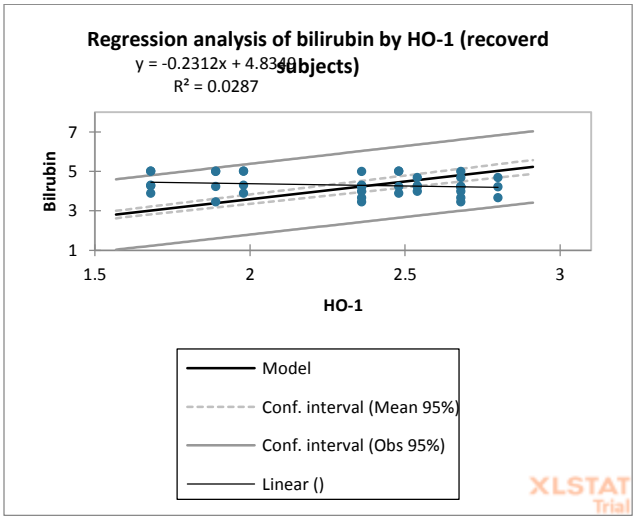

Figure 3: Regression analysis of HO and bilirubin from the recovered patients with glomerulonephritis.

DISCUSSION

The results reveled that bilirubin and HO-1 concentration were higher in the patients suffering from kidney inflammatory diseases as compared to the control group but HO-1 and bilirubin were also found high in the recovered patients as compared to the control group. Further after regression analysis, it was found that bilirubin level was not associated with the heme-oxygenase in all three groups. Some previous studies [25-26], assess the relationship between heme-oxygenase and bilirubin concentration and their findings were consistent with the current study. Further, [27] reported that the patients with cardiovascular disease have lower level of bilirubin, while the level of HO-1 was higher. These findings suggest that the concentration of bilirubin behaves differently among different diseases. A meta-analysis conducted [28] suggests that the elevated serum bilirubin levels within a physiological range, regardless of study variables, are associated with a

decreased risk of chronic kidney disease and correspond with a linear dose-response relationship. However, it is unclear if high serum bilirubin levels constitute a protective factor against mortality. Further assessing the serum bilirubin level can act as a marker for the early diagnosis of chronic kidney infection. In the current study, the high level of bilirubin in the recovered patients is still unclear and there were no such studies that evaluate the level of bilirubin in the recovered patients.

The concentration of bilirubin is significantly influenced by heme-oxygenase. A protein called heme oxygenase (HO) catalyses the transformation of heme into biliverdin, iron, and carbon monoxide, which are eventually reduced to bilirubin (BR) [26]. As a result, the concentration of bilirubin rises due to the catabolism of heme. However, bilirubin is also formed by the breakdown of various heme-containing proteins found in other organs, most notably the liver and muscles [29]. The majority of blood bilirubin is produced by the breakdown of haemoglobin from senescent red blood cells. Heme oxygenase is the factor that restricts the rate of bilirubin production. Unconjugated bilirubin is expelled from cells and transported by albumin in plasma for conjugation in the liver and subsequent excretion by bile ducts into the intestines. It has been demonstrated that an increase in bilirubin concentration has significant anti-inflammatory and antioxidant benefits, as well as therapeutic implications in neurodegenerative diseases like Parkinson's disease. Recent studies have also revealed that bilirubin stimulates fatty acid metabolism by activating the PPAR, or peroxisome proliferator-activated receptor alpha [30].

Several studies demonstrate the role of bilirubin in anti-oxidant activity in different inflammatory diseases such as chronic kidney infections [31-35]. In current study, the elevated level of bilirubin level in the patients suggests the protective role of this compound in the body.

CONCLUSIONS

HO-1 and bilirubin level was found higher in the subjects that suffered from a kidney infection, as compared to the control group. Interestingly the level of HO-1 and bilirubin was found higher in the recovered subjects as compared to the control group but lower than in subjects who suffered from glomerulonephritis. Further, there was no association between the HO-1 and bilirubin levels in all three groups. This was still unclear why HO-1 and bilirubin level was found higher among the recovered patients. Future studies should focus on the level of HO-1 and bilirubin in the recovered patients if it indicates any underlying medical conditions of the individuals.

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