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Comparison of Histidine rich glycoprotein, a novel biomarker for sepsis, with traditional biomarkers in adult ICU patients

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Abstract

Sepsis is a major public healthcare problem. It remains a significant cause of morbidity and mortality in intensive care units (ICU) all over the world. A lifesaving early specific diagnosis and treatment is a challenge as no gold standard technique exists that can alone allow a rapid and reliable diagnosis of sepsis. Histidine-rich glycoprotein (HRG) is a promising new biomarker of sepsis that can contribute to enhance current sepsis diagnostic tools. The current study aimed to evaluate HRG as a diagnostic biomarker for sepsis compared to the conventionally used biomarkers, procalcitonin (PCT) and C-reactive protein (CRP). The study included 67 participants classified into 3 groups: Control (n=19), systemic inflammatory response syndrome (SIRS) patients (n=24) and sepsis patients (n=24). Serum HRG, CRP and PCT levels were measured by ELISA techniques. HRG level was significantly reduced in sepsis patients compared with SIRS patients (P<0.001) and controls (P<0.001) with overall statistically significant differences between the three groups (P<0.001). Serum levels of the 3 biomarkers revealed increased PCT level in SIRS and sepsis groups, (P=0.002 and p<0.001 respectively), CRP level significantly increased in sepsis (P<0.001) but not in SIRS patients (P=0.525). The area under the curve (AUC) value was 0.988 for HRG, 0.966 for PCT and 0.859 for CRP respectively. The sensitivity, specificity, PPV and NPV for diagnosis of HRG were 95.8%, 93%, 88.5%, and 97.6%, respectively. In conclusion, HRG could be a good indicator for sepsis, that can discriminate sepsis and SIRS patients in ICU.

Keywords: Sepsis, SIRS, HRG, CRP, PCT, Biomarker, ELISA.

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Introduction

Sepsis (septicemia) is a medical condition of blood infection caused by all types of microbes predominantly bacteria.[1] It is an emergency, life threatening clinical syndrome resulting in exaggerated systemic immune response followed by immune suppression that can lead to multiple organ dysfunction and death. Immediate specific treatment is the key to reduce deaths from severe sepsis, so early accurate diagnosis of sepsis is crucial.[2]

Sepsis diagnosis includes physical examination and vital signs, which are largely nonspecific as well as imaging, laboratory, and microbiological findings.[3]

Blood cultures regarded the gold standard conventional laboratory diagnostic method for sepsis, enable bacterial isolation and identification and subsequently antimicrobial sensitivity testing.[4] However, it depends on the volume of drawn blood, the timing of the blood drawing and any prior antibiotics treatment. Moreover, it is time consuming, insensitive under certain conditions such as slow-growing and non-cultivatable micro-organisms and can give false positive results due to contamination.[5]

Pathogen-focused molecular-based tests have been developed for rapid and more sensitive identification of infectious microbes but still suffer some disadvantages. The need for technical expertise, special equipment and maneuvers for pathogen enrichment to detect the low number of pathogens are among the known drawbacks of such methods.[6]

These limitations disqualify molecular assays from becoming the next “gold standard” approach for sepsis diagnosis. Thus, targeting the host immune response to infection and monitoring abnormal changes in certain serum protein biomarker concentrations have gained increased interest.[7] Various biomarkers have been described to be either associated with sepsis or to have a prognostic value for the outcome of sepsis, but none has a satisfying sensitivity nor specificity to be routinely used in clinical practice.[8]

Procalcitonin (PCT) and C-reactive protein (CRP) are the most studied biomarkers and the most widely used in sepsis. However, they have no or limited ability to differentiate sepsis from systemic inflammatory response syndrome (SIRS) or to predict outcome of disease management.[9] CRP is an acute phase reactant protein synthesized by the liver in response to infection/inflammation, although having low specificity, it is commonly used in diagnosis of sepsis.[10] Procalcitonin is a hormone precursor elevated in response to invasive bacterial infections but can be elevated with noninfectious disorders, especially following trauma. It cannot accurately distinguish sepsis from SIRS in critically ill patient. Therefore, identifying a biomarker that can diagnose and efficiently distinguish sepsis from SIRS is crucial.[11]

Histidine-rich glycoprotein (HRG) is an abundant plasma glycoprotein, produced mainly by liver parenchymal cells but can also be synthesized in immune cells such as monocytes and macrophages.[12] It consists of three main domains (N-terminal domain, central domain and C-terminal domain) its multi-domain structure enables it to regulate various biological processes through interacting by many ligands to different cell receptors.[13]

HRG is suggested to have an essential role in host defense mechanisms and possibly regulate other physiological processes such as angiogenesis, coagulation, fibrinolysis, soluble immune complex clearance, and phagocytosis of apoptotic/necrotic cells.[14] Plasma HRG limits vascular endothelial damage by reactive oxygen released from damaged circulating neutrophils by conserving their round shape and smooth surface maintaining them in a quiescent state and easing their passage through blood vessels. It also protects vascular endothelium from strong activation and apoptosis induced by lipopolysaccharide or TNF-α and inhibits Zn^{2+} induced aggregation of erythrocytes.[15]

Plasma HRG was reported to be directly related to sepsis pathogenesis. Therefore, based on its levels, it could be a good diagnostic and prognostic indicator superior to other routinely used biomarkers.[16] Consequently, this work aimed to evaluate HRG as a novel biomarker for diagnosis of sepsis in comparison to CRP and PCT the most commonly used diagnostic biomarkers.

Materials and Methods

Study design

The current prospective, case-control study was performed at the Medical Microbiology and Immunology Department, Faculty of Medicine, Benha University during the period from December 2020 to November 2021. The Ethical Committee, Benha Faculty of Medicine, reviewed and approved the study protocol

(date, 28/12/2020). A written informed consent was obtained from all study participants or their legal designates.

Subjects' characteristics

A total of 67 subjects were included in this study. Of these, 48 patients were admitted to intensive care units (ICU), 24 patients with SIRS and 24 patients with sepsis diagnosed according to the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) criteria,[17] and 19 apparently healthy subjects, matched for age and sex with patients, served as controls. Study subjects were aged between 20 and 75 years. Patients were excluded if they were <20 years old, had any immunological disorder, use any antibiotics before hospitalization, pregnant, refused to take part in the study or refused to provide a written and signed consent.

Sample collection and ELISA measurement procedure

Blood samples were collected within 24 hours of ICU admission. The samples were processed and stored at –80°C. HRG levels were measured using a human histidine-rich glycoprotein ELISA kit (BT LAB, China). Procalcitonin levels were measured by a human procalcitonin ELISA kit (DuoSet®, R&D Systems, Inc. USA). CRP was measured using a human CRP ELISA Kit (Quantikine®, R&D Systems, Inc. USA). ELISA procedures were performed according to the manufacturer's instructions. All tests were performed in duplicate and data represented as mean values.

Statistical analysis

Data were expressed as median and interquartile ranges (IQR, 25th to 75th percentiles). The Mann-Whitney U and Kruskal Wallis tests were used to compare groups. All statistical tests were two-sided and a *P* value of < 0.05 was considered statistically significant. The receiver-operating characteristic (ROC) curve analysis was used to determine the diagnostic accuracy of the measured biomarkers.

Results

Levels of the three biomarkers among the study groups

The study included 67 subjects assigned to three groups. The first group included 19 controls, the second group 24 patients with SIRS and the third group 24 patients with sepsis.

The median HRG level in controls was 66.22 µg/mL (IQR 54.2-70.31) which was significantly higher than in SIRS patients 36.85 (IQR 29.15-47.13; *P*<0.001) and also significantly higher than in sepsis patients 8.91 (IQR 7.2-14.58; *P*<0.001). The median HRG level in SIRS patients was significantly higher than in sepsis patients (*P*<0.001). The overall differences in HRG level between the three groups was statistically significant (*P*<0.001).

The differences in HRG, CRP and PCT levels among the study groups are shown in Table 1. The PCT level was significantly higher in SIRS patients than controls (*P*=0.002). However, the HRG level was significantly lower in SIRS patients than controls (*P*<0.001). There was no significant difference in CRP levels between SIRS patients and controls (*P*=0.525). The CRP and PCT levels were significantly higher in patients with sepsis than SIRS patients (*P*<0.001 for both). However, the HRG level was significantly lower in patients with sepsis than in SIRS patients (*P*<0.001).

Table 1. Levels of the three biomarkers among the three study groups.

	Controls	SIRS	Sepsis	**P* value
N (%)	19 (28.40%)	24 (35.80%)	24 (35.80%)	
HRG	66.22 (54.2-70.31)	36.85 (29.15-47.13)	8.91 (7.2-14.58)	<0.001
CRP	20.20 (9.12-32.21)	24.90 (12.67-37.14)	45.21 (40.46-49.9)	<0.001
PCT	0.05 (IQR 0.03-0.97)	0.85 (IQR 0.29-2.76)	19.62 (11.5-28.76)	<0.001

**P* value<0.05 is significant. The *P* values were calculated for comparison of the three groups by Kruskal-Wallis H test. Data are shown as the median and interquartile range.

Diagnostic accuracy of the 3 biomarkers for sepsis

The diagnostic accuracy of the three biomarkers was determined by receiver operating characteristic (ROC) curve analysis and compared for differentiating patients with sepsis from patients with SIRS and the control group.

A ROC curve analysis (Figure 1 and Table 2) was done to find out the diagnostic accuracy of these 3 biomarkers for diagnosis of sepsis. The results showed that HRG achieved the highest area under curve (AUC) value among the investigated biomarkers (AUC=0.988) followed by the value of PCT (AUC=0.966) and CRP (AUC=0.859).

For diagnosis of sepsis, HRG had achieved sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 95.8%, 93%, 88.5%, and 97.6%, respectively when using a cut-off value of 19 µg/mL. Thus, HGR was superior biomarker compared with the other markers in diagnosing sepsis (Figure 2 and Table 2).

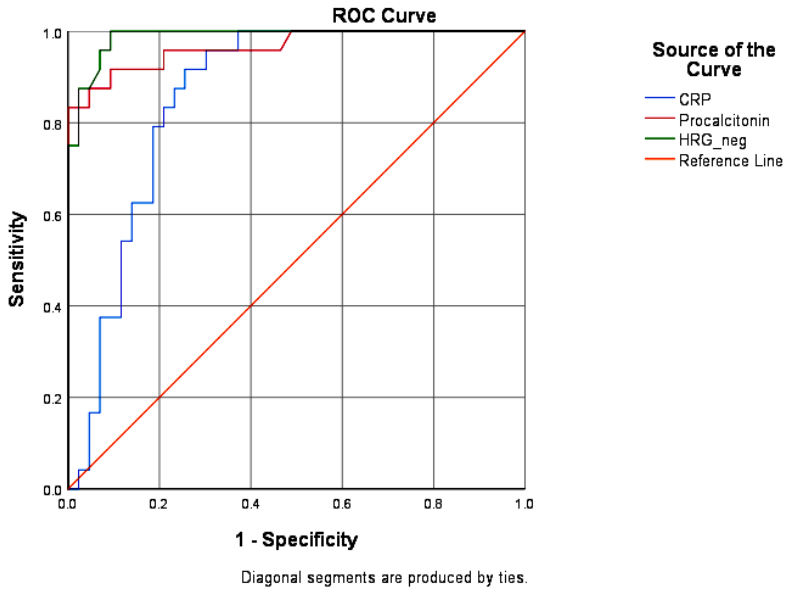

Figure 1. Receiver-operating characteristic (ROC) curve analysis for diagnosis of sepsis. ROC curves of HRG, PCT, and CRP. The area under the curve (AUC) in ROC curve analysis for HRG was 0.988. AUC for HRG was higher than that of PCT (0.966) and CRP (0.859).

Table 2. Area under curve (AUC) from the ROC curve analysis and the optimal cut-off value for diagnosis of sepsis using the 3 biomarkers, and their diagnostic accuracy at the optimal cut-off value.

Test Result Variable(s)	AUC	Cut-off	Sensitivity	Specificity	PPV	NPV
HGR	0.988	19 µg/mL	95.83%	93.02%	88.46%	97.56%
CRP	0.859	38 mg/L	83.33%	79.07%	68.97%	89.47%
PCT	0.966	3.9 ng/mL	91.67%	90.70%	84.62%	95.12%

The optimal cut-off was calculated by the point closest to the corner method.

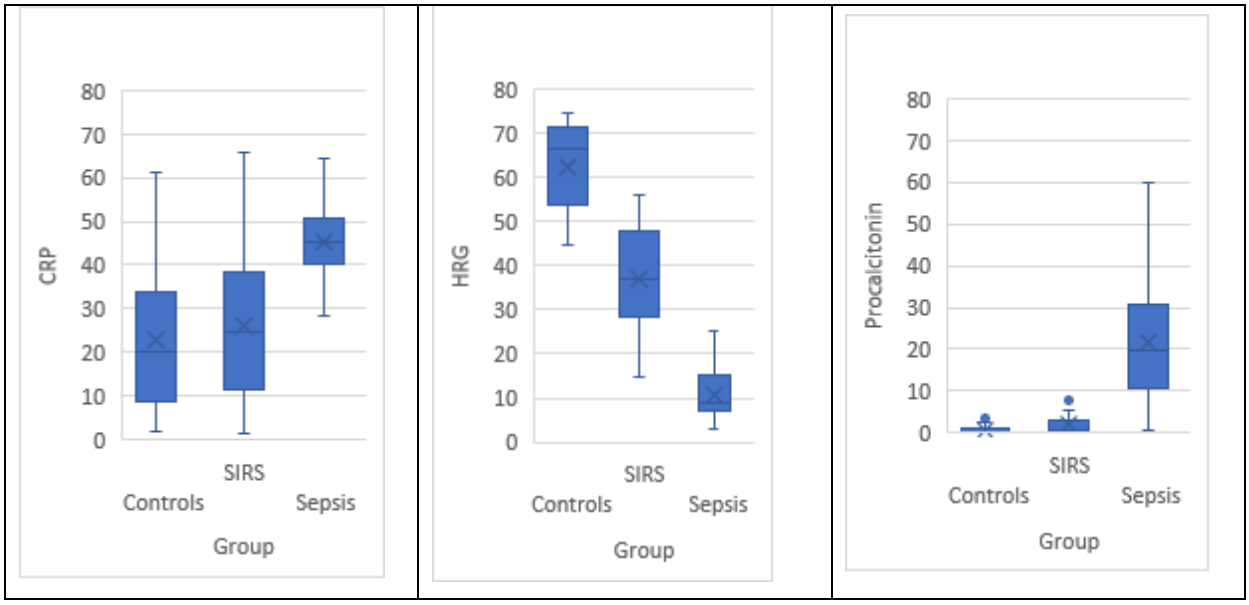

Figure 2. Comparison of the CRP, PCT and HRG levels as measured by ELISA between the three groups: Controls, SIRS and Sepsis. A box-and-whisker plot showing median, 25th, and 75th percentiles. The bars represent the 5th and 95th percentiles.

Discussion

HRG is a novel biomarker for diagnosis of sepsis, it was first discovered in mouse models of sepsis. The levels of HRG were found to rapidly decrease in subjects with sepsis conditions.[18] In the present study, the median HRG level in the control group was 66.22 μg/mL (IQR: 54.2-70.31), which is close to the normal level in control subjects in other published data.[19] In this study, HRG levels were significantly lower in sepsis patients than in SIRS patients (P<0.001) which is similar to results observed in a previous study.[20]

When comparing controls with SIRS patients, the differences in CRP were not statistically significant. However, PCT was significantly elevated (P=0.002) and HRG was significantly reduced in SIRS patients (P<0.001). Kuroda & co-workers (2018) also found significant difference in HRG level between control, and SIRS patient's groups.[20]

In the present study, at the optimum cutoff level for diagnosis of sepsis (19 μg/mL), HRG showed higher sensitivity (95.8%), specificity (93%), positive predictive value (88.5%) and negative predictive value (97.6%) than both CRP and PCT.

The calculated optimum cut off for CRP in this study was 38 mg/L, which is close to the value calculated in another published research (50.7 mg/L),[21] however the calculated PCT cut off value in this study was 3.9 ng/mL which is higher than the calculated level (1.1 ng/ml) by Li et al (2014).[21] However, using the cut-off value calculated in this paper (3.9 ng/mL) archived higher specificity (90.7%) than specificity associated with the 1.1 ng/ml cut of level (68%) as determined by Li et al (2014).[21] The method of calculation of cut-off value in this paper is through the nearest point to the corner in ROC curve, which is used to find the best combination of sensitivity and specificity. Furthermore, the difference in the study population may also had contributed to the difference in the calculated cut-off point. In this study SIRS patients were included in the study population which is not the case in the other study conducted by Li and colleagues (2014) [21] which only compared between patients with sepsis and a control group.

HRG showed more diagnostic accuracy in diagnosis of sepsis than the other traditional

biomarkers. In this study it was superior to PCT, and CRP as shown by the area under curve in ROC curve (0.988, 0.966 and 0.859, respectively). Kuroda and co-workers (2018) also found that HRG had superior diagnostic accuracy for diagnosis of sepsis when compared to procalcitonin and presepsin.[20]

In conclusion, our findings indicated that HGR provides a better diagnostic ability for detecting SIRS and sepsis in ICU patients than traditional biomarkers. This superior performance may allow ICU specialists to start specific treatment earlier which may be lifesaving.

Author Contributions

Authors contributed equally to sample collection and processing, performing ELISA, writing, data collection and interpretation and revision.

Declaration of Conflicting Interests

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Ethical approval

The Ethical Committee, Benha Faculty of Medicine, reviewed and approved the study protocol (date, 28/12/2020).

Informed consent

A written informed consent was obtained from all study participants or their legal designates.

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