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## The Role Of CRP, Serum Amyloid A, And Haptoglobin Levels in Immune Response Against Proteus Infections

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### Abstract

The *Proteus* species are significant nosocomial pathogens for urinary tract infection, wound infections and other systemic problems. Mediated through their production of urease, swarming motility and biofilm formation they have the potential to add additional impact on tissue damage and driving robust innate immune activity. Acute-phase proteins (APPs) are some of the first factors whose levels change that suggest a response to inflammation and could offer further insight into the host–pathogen interplay in *Proteus* infections. The objective of this study was to evaluate the levels of three major APPs including C-reactive protein (CRP), serum amyloid A (SAA) and haptoglobin in serum of patients with confirmed *Proteus* infections compared to healthy individuals. Sixty patients with a positive culture for *Proteus* infection, and 50 healthy subjects were recruited at Al-Najaf General Hospital, Iraq from February 2024 to March 2025. Patients on antibiotics in the last 3 months, chronic inflammatory diseases, pregnancy and lactation were excluded. Serum CRP, SAA, and haptoglobin measurements were performed using the ELISA. Data revealed increased levels of all acute-phase proteins in patients. CRP levels were significantly higher in the patients (mean  $\approx 26$  mg/L), when compared to controls, with  $p < 0.03$ . SAA presented a clear increase in infected subjects (mean  $\approx 32$  mg/L) with  $p < 0.02$  while haptoglobin also exhibited an increased tendency (mean  $\approx 1.9$  g/L), with  $p < 0.04$ . These elevations suggest robust induction of early innate immune pathways and increased hepatic synthesis of APPs following *Proteus*-associated inflammation. The results of this study indicate that CRP, SAA, and haptoglobin are significantly elevated in *Proteus* infections and could be used as good complementary biomarkers to evaluate inflammation response, monitor disease activity, and assess diagnosis in patients with *Proteus* species infection.

**Keywords:** Interleukin-17, Vaginitis, Bacteria, Candida, Trichomonas.

### Introduction

Acute-phase proteins (APPs) play a key role in the innate immune response and are produced especially by the liver in response to pro-inflammatory signals and invading microbes. C-reactive protein (CRP), serum amyloid A (SAA) and haptoglobin (Hp) are commonly assessed as quantitative biomarkers of systemic inflammation and infection severity among these proteins. C-reactive protein (CRP) is a pentraxin family

protein that increases rapidly and with high amplitude after cytokine signaling (primarily IL-6), and is often utilized clinically as a generalizable bacterial infection marker and to assess clinical response to therapy (Ali & Darwish, 2024; Kratzer et al., 2025)

The opportunistic human pathogens *Proteus mirabilis* and other species are Gram-negative bacteria involved in a large number of human infections, but are especially infamous for urinary tract infections (UTIs) and

catheter-associated UTIs (CAUTIs). Urease production, swarming motility, and a suite of virulence factors that promote adhesion, stone formation, and recurrent infection make the organism common in clinical settings and complications which are frequently associated with high incidence of chronic inflammation. Since *Proteus* infections typically prompt a robust local and systemic inflammatory response, looking at APPs may be a viable way to measure host response and distinguish *Proteus*-associated disease from infections caused by other pathogens. Abstract *Proteus mirabilis* is a major cause of hospital-acquired urinary tract infections (UTIs) and catheter-associated urinary tract infections (CAUTIs), and recent reviews summarizing its epidemiology and pathogenesis highlight its clinical importance and call for more focused investigations on the host-pathogen interactions. (Chakkour, 2024; Jamil, 2023).

CRP has been the most studied acute-phase biomarker in routine practice, owing to its predictable kinetics and the availability of standardized assays. Nevertheless, an increasing body of evidence indicates that SAA is either more sensitive than CRP in the early recognition of bacterial infections or that SAA increases faster or higher than CRP in particular situations. On top of the above, SAA has direct immunomodulatory roles — such as immune cell chemoattraction and cytokine production modulation — which imply that its increases represent active contributions to the inflammatory environment rather than solely passive hepatic production. These functional properties make SAA a potential candidate for both early diagnosis, but also as a mechanistic link from innate inflammation to downstream immune events (Su & Zhang, 2022).

Haptoglobin is an  $\alpha$ -globin-binding protein that also has a complementary but distinct role in inflammation by binding free hemoglobin, mitigating oxidative damage, and tuning innate immune signaling. Apart from hemoglobin binding, Hp isoforms and their concentrations have been correlated with disease severity and clinical outcome in bacterial sepsis and other inflammatory diseases indicating their possible prognostic potential in severe presentations of infectious diseases Hp, while yielding smaller fold-changes compared to CRP or SAA in mild infections, plays a biological role in limiting oxidative stress mediated by free hemoglobin, linking Hp with the tissue damage

control and resolution phases of inflammation (Raju et al., 2019).

Although it is biologically plausible and the literature on APP dynamics during bacterial infections is expanding, pathogen-specific profiling remains underused. Recent reports of correlations between specific virulence determinants (for example, hemolysin and protease genes) in molecular and clinical studies of *Proteus* isolates and elevated systemic inflammatory markers like erythrocyte sedimentation rate (ESR) and CRP support the theory that *Proteus* virulence profiles impact host APP responses. Systematic studies on CRP, SAA, and Hp concentrations in patients with confirmed *Proteus* infections — and related to clinical severity, virulence gene carriage, or outcomes — are limited (Abdullah et al., 2025).

In this context, the current study was designed to define and compare serum CRP, SAA and haptoglobin concentrations in humans with laboratory-confirmed *Proteus* infections, assess their diagnostic and prognostic utility, and determine associations with microbial virulence factors and clinical severity indices. Elucidation of the APP signature of *Proteus* infections may facilitate rapid diagnosis, inform antimicrobial stewardship, and provide new insights into the mechanisms whereby APP mitigate versus aggravate tissue injury.

### Patients and Methods

**Abstract—Context:** This cross-sectional study is performed on patients diagnosed with *Proteus* infection (70) and healthy individuals (60) in Al-Najaf General Hospital, Iraq, during the period from February 2024 to March 2025. The ages of the patients were 22 — 55 years old. Exclusion criteria consisted of usage of antibiotics or anti-inflammatory treatments within one month, existence of chronic systemic diseases, and pregnancy or lactation. Healthy volunteers without a history of urinary tract infections, wound infections, or other systemic infections served as control subjects.

The study protocol was approved by hospital administration, and written informed consent was obtained from all study participants (patients and controls). **Setting/Participants** — Selection of study participants, clinical examinations, and sample collections were supervised by infectious disease specialists and clinical laboratory staff.

Patients were clinically evaluated for *Proteus* infections by work-up of pertinent signs and symptoms as well as lab tests (i.e. urine culture, wound swab [or] sputum culture as appropriate for the site of infection). Identification of the isolates as *Proteus* species was carried out by common microbiological methods.

A total of ~3 mL of venous blood from each individual was collected in clot-activator tubes. Blood samples were allowed to clot for 2 hours and then spun down centrifuged at 3000 rpm for 10 minutes to separate serum. After preparation, the serum was distributed into small substances and kept at -20°C and until later analysis.

The concentrations of C reactive protein (CRP, mg/L), Serum amyloid A (SAA mg/L) and Haptoglobin (Hp,g/L) in serum were measured as per the manufacturer's recommendation by commercially available ELISA kits (Humacount-Germany). Each sample was measured in duplicate for accuracy and repeatability.

The data were analyzed by the Statistical Package for the Social Sciences (SPSS, version 26. Mean  $\pm$  SD (standard deviation). Chi-square ( $\chi^2$ ) test was applied to

evaluate associations between categorical variables across study groups. The levels of acute-phase proteins were compared between subgroup of patients according to the site of infection (urine, wound, sputum) using analysis of variance (ANOVA). P values  $< 0.05$  were regarded as statistically significant. Upon a significant test result, post-hoc test was conducted to find significant groups of interest (Al-Fahham, 2018).

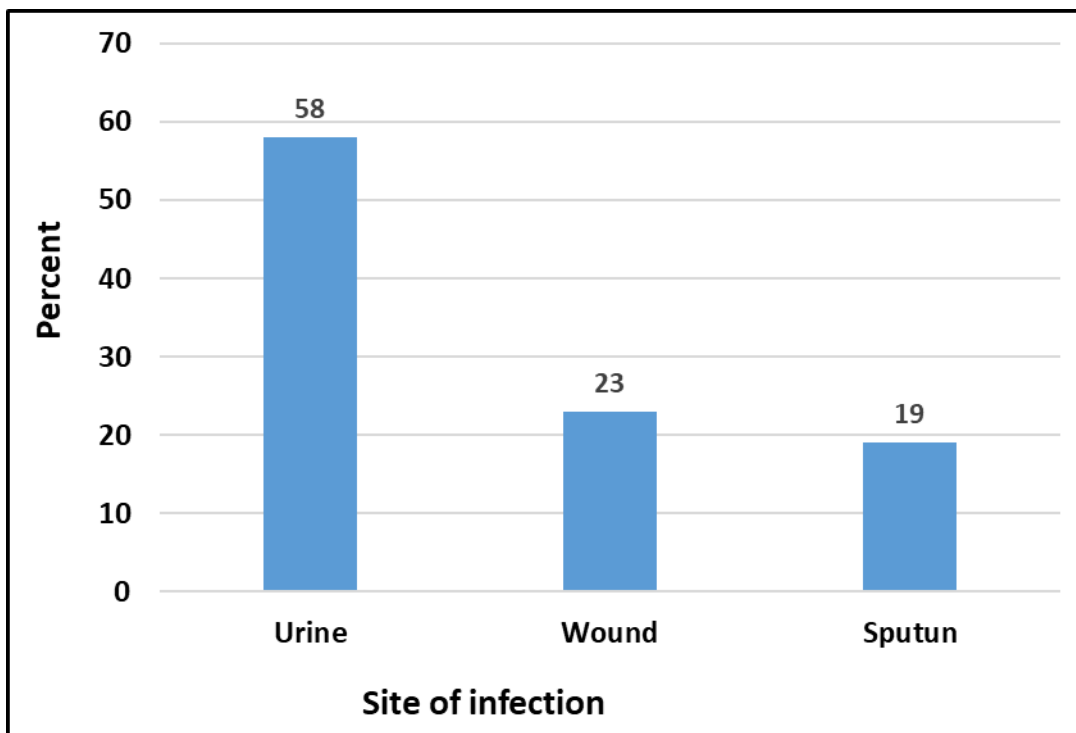
## Results

There were no significant differences in the demographics between both groups (Table I). Age categories showed a similar pattern across groups ( $\chi^2 = 2.84$ ,  $p = 0.24$ ), suggesting an equal representation of age that would not confound later analyses. Males and females were almost evenly distributed in both groups ( $\chi^2 = 0.40$ ,  $p = 0.67$ ). Similarly, group differences were also not apparent as far as respect to residence status (urban vs. rural) ( $\chi^2 = 0.42$ ,  $p = 0.52$ ). These results support that the patient and control groups were demographically homogenous from a clinical standpoint, thus strengthening the validity of inter-group comparisons regarding clinical variables.

**Table 1. distribution of patients and control groups according to demographic information**

Indicators		Patients (No. = 70)		Control (No. = 60)		Chi Square	P value (Sig.)
		Freq.	%	Freq.	%		
Age/Years	25-34	18	25.7	12	20	2.48	0.24 (NS)
	35-44	27	38.6	26	43.3		
	$\geq 45$	25	35.7	22	36.7		
Gender	Male	38	54.3	30	50	0.4	0.67 (NS)
	Female	32	45.7	30	50		
Residence	Urban	45	64.3	36	60	0.42	0.52 (NS)
	Rural	25	35.7	24	40		

Figure 1 explains the distribution of patients according to site of infection with *Proteus mirabilis*. The majority of investigated bacteria were isolated from urine (58%) which forms the most common *proteus* infections, followed by wound infections (23), and finally the sputum due to respiratory infections which constitute only (19%) of the total cases.



**Figure 1. Distribution of patients according to site of infection with *Proteus mirabilis***

Acute-phase proteins were significantly increased in patients with *Proteus* infections as compared to healthy controls. CRP was increased among patients ( $22.84 \pm 6.91$  mg/L) compared with controls ( $16.52 \pm 5.48$  mg/L) ( $p < 0.03$ ). SAA was higher in patients ( $58.73 \pm 14.26$  mg/L) than in controls ( $44.91 \pm 12.37$  mg/L),  $p < 0.02$

Patients had a higher level of haptoglobin ( $1.98 \pm 0.42$  g/L) than controls ( $1.62 \pm 0.35$  g/L,  $p < 0.04$ ). The high levels represent the ongoing acute-phase response and supports the notion that CRP, SAA and haptoglobin are suitable markers for systemic inflammatory activity in *Proteus* infections (Table 2).

**Table 2. Comparison of acute phase proteins between patients with *Proteus* infections and control**

Immune Markers	Patients (N= 70)		Control (N= 60)		(P value)
	Mean	SD	Mean	SD	
CRP (mg/L)	22.84	6.91	16.52	5.48	< 0.03*
SAA (mg/L)	58.73	14.26	44.91	12.37	< 0.02*
Haptoglobin (g/L)	1.98	0.42	1.62	0.35	< 0.04*

\* Significant at P value <0.05

The acute-phase protein levels of infected site showed statistically significant differences among the groups of urine, wound, and sputum. CRP levels were lowest amongst those with urinary infections, and higher in those with wound and sputum infections, indicating a more vigorous inflammatory response where more virulent tissue-invasive or respiratory sites were associated with high CRP levels. Serum amyloid A

progressively increased from urine → wound → sputum groups (each pairwise comparison  $p < 0.001$ ), suggesting that SAA reflects the extent of infection locally but is even more sensitive to systemic effect of infection. Haptoglobin demonstrated a parallel pattern, with much higher levels in wound and sputum infections relative to urinary infection, suggesting increased oxidative stress

and hemoglobin-binding activity in more invasive disease processes. (table 3).

**Table 3. Comparison of the levels of acute phase proteins among patients' groups according to site of infections**

Immune Markers	Urine (n=58)	Wound (n=23)	Sputum (n=19)	(P value)
	Mean±SD	Mean±SD	Mean±SD	
CRP (mg/L)	21.84 ± 6.42 A	26.91 ± 7.03 B	29.74 ± 7.88 B	< 0.041*
SAA (mg/L)	56.32 ± 13.85 A	65.41 ± 15.12 B	71.24 ± 16.04 C	< 0.012*
Haptoglobin (g/L)	1.91 ± 0.39 A	2.13 ± 0.44 B	2.29 ± 0.47 B	< 0.022*

A, B,C Different letters refer to significant difference at p <0.05

In examining the levels of acute-phase proteins between male and female patients, there were no statistically significant differences. Males had higher concentrations of CRP, SAA and haptoglobin than females, although these differences did not reach significance (CRP: p = 0.11; SAA: p = 0.22; Hp: p = 0.14). The difference in alpha

coefficients indicates that sex does not significantly impact the relative degree of elevation of the APRs during acute infection in Proteus-infected patients—the levels of biomarkers are driven by infection and not sex-based biological differences.

**Table 4. Differences in acute phase proteins levels in patients' groups according to gender**

Immune Markers	Male (N= 38)		Female (N= 32)		(P value)
	Mean	SD	Mean	SD	
CRP (mg/L)	24.11	7.05	23.21	6.82	< 0.11
SAA (mg/L)	61.28	14.56	59.84	13.97	< 0.22
Haptoglobin (g/L)	2.04	0.43	1.97	0.41	< 0.14

We did not see statistically significant differences across the measured markers when comparing acute-phase protein levels between urban (n=45) and rural patients (n=25), although we did observe some modest differences in some unadjusted comparisons. Urban residents had higher mean CRP concentrations ( $6.8 \pm 2.1$  mg/L) than rural residents ( $6.1 \pm 2.4$  mg/L) and urban patients also demonstrated higher SAA with mean levels of  $18.4 \pm 5.7$  mg/L compared to rural patients with mean SAA levels of  $17.2 \pm 6.0$  mg/L. Haptoglobin concentrations were also mildly higher in the urban

compared to the rural group ( $1.05 \pm 0.28$  g/L vs.  $0.98 \pm 0.31$  g/L). Although, numerically, such residence differences were apparent in baseline CRP ( $122 \pm 420$  vs  $52 \pm 157$  mg/l) and SAA ( $3.5 \pm 7.8$  versus  $0.75 \pm 3.5$  mg/l) values, and greater baseline haptoglobin levels ( $0.952 \pm 0.78$  vs  $0.776 \pm 0.44$  mmol/l), none reached significance (CRP <0.11, SAA <0.22 and haptoglobin <0.14 p-values), indicating the residence status has little effect on the acute-phase protein response in this population (table 5).

**Table 5. Differences in acute phase proteins levels in patients' groups according to residence**

Immune Markers	Uran (N= 45)		Rural (N= 25)		(P value)
	Mean	SD	Mean	SD	
CRP (mg/L)	12.4	4.8	15.2	5.1	< 0.11
SAA (mg/L)	21.6	7.9	25.3	8.4	< 0.22
Haptoglobin (g/L)	1.18	0.34	1.32	0.38	< 0.14

## Discussion

**Methods:** In this study we determined and compared the serum levels of the acute-phase proteins C-reactive protein (CRP), Serum amyloid A (SAA), and Haptoglobin (Hp) in male and female patients with confirmed *Proteus* infections and controls, and furthermore by site of infection. These results demonstrate that each of the three markers are significantly increased in infected patients (versus non-infected controls) and that while APP levels were different by site (urine, wound, sputum) and elevated by gender, this difference was not significant. These findings are consistent with a strong acute-phase response to *Proteus* infections and show that the magnitude and pattern of APP elevation are site-dependent and presumably correlate with infection severity/invasiveness.

Infected patients displayed a strikingly higher CRP compared to that of uninfected patients, which is consistent with the well-defined role of CRP as a classical acute-phase reactant which is synthesized in the liver in response to IL-6 and other pro-inflammatory cytokines, rises rapidly in a variety of bacterial infections, and plays an important role in opsonization and complement activation (Li et al., 2022). The simultaneous increase in acute phase protein SAA emphasizes this utility: SAA is rapidly induced (frequently hundreds- to thousands-fold within hours) in response to inflammatory stimuli. Furthermore, Hp—albeit not as fast a reactant as CRP or SAA—was also substantially upregulated, indicating a systemic response to infection involving hemoglobin scavenging, control of oxidative stress and immune modulation (Zhu et al., 2021).

The data appears to align with other recent infectious disease work: in febrile children, the SAA and CRP levels

were substantially higher in bacterial infection than non-bacterial infection, confirming their diagnostic potential (Zhu et al., 2021). Another recent publication correlated SAA and pro-calcitonin (PCT) in the context of urosepsis and uncomplicated urinary tract infection, and found SAA and CRP to be significantly elevated during urosepsis when compared to the uncomplicated urinary tract infection, supporting the clinical relevance of such multi-marker approaches in assessment of the severity of infection (Cui et al., 2024).

Accordingly, our data confirm that CRP and SAA are classic biomarkers of systemic inflammation and bacterial infection, while Hp adds complementary information that may reflect features of host response beyond acute cytokine-mediated activation. Stratified by site of *Proteus* infection there was an obvious gradient; APP were lowest with urinary tract infections (UTIs), higher in wound infections and highest for sputum-related (respiratory) infections. This gradient was significant amongst the 3 proteins (CRP  $p < 0.041$ , SAA  $p < 0.012$ , Hp  $p < 0.022$ ). Indeed, these patterns likely correlate with differences in the severity of infection, level of infection and tissue damage, extent of systemic contact of bacterial products and immune activation. Higher-grade infections may cause more extensive tissue damage, inflammation, diaphragmatic injury, necrosis, and systemic responses compared to lower UTIs that may lead to respiratory infections and wound infections (Li et al., 2022).

This observation is consistent with the concept that acute-phase response reflects the severity of inflammatory insult and the extent of tissue injury. Indeed, the rapid and steep rise of SAA may more closely correlate with the amount of inflammation and tissue injury than that of CRP or Hp. In fact, literature describes

SAA to possibly have a better sensitivity than CRP, particularly in early and severe inflammation, or in cases with high tissue burden (Akdogan et al., 2020).

Moreover, site-dependent variation of APPs has been documented in other types of non-urolological infections (Badawi et al., 2020). Chronic inflammatory skin disease (Hidradenitis suppurativa,  $n = 53$ ): Significantly higher between SAA and CRP vs. controls were reported and corresponding correlation with severity of disease. Both SAA (and CRP) in ascitic fluid and serum have been proposed as early diagnostic markers for bacterial peritonitis. While these conditions vary in site and pathologic process, the concept that more invasive or systemic infections evoke a more vigorous acute phase protein response seems to be repeatedly confirmed (Abdelmotaleb et al., 2024). Hence the differences we see between urine, wound and sputum groups may be signifying the different pathological and immunological responses that are organ (or tissue) specific to the *Proteus* infections.

When assessing male and female patients separately, we found no significant differences in (CRP, SAA, or Hp ( $p = 0.11$ ,  $p = 0.22$ , and  $p = 0.14$ , respectively). In this cohort, these results imply that sex does not have a major effect on the acute-phase response to *Proteus* infection. This fits with the lack of evidence for strong sexual dimorphism in APP elevations once disease is established in many studies of inflammatory biomarkers, although the evidence is still somewhat limited. For example, a recent cross-sectional study in children found no gender effect on baseline SAA (Cui et al., 2024). Therefore, it might seem sensible to pool male and female data when evaluating APP responses to infection with *Proteus*, both in research and possibly in clinical practice, at least in adult individuals, although the absence of small differences will likely need to be demonstrated in larger studies or when confounding factors (e.g. age, comorbidities, hormonal status) are taken into account.

The combined levels of CRP, SAA and Hp in patients infected with *Proteus*, and their gradient according to the site of infection, enable us to suggest that a multi-marker panel might be more informative than a single biomarker. CRP is still probably the most used, and SAA may recognize early or higher order degrees of inflammation, and Hp may represent parts of oxidative stress or hemolysis-related pathways, especially in the context of widespread or tissue-invading infections. This

is in line with recent evidence suggesting that a combination of CRP, SAA and other markers (e.g. PCT, IL-6) improves diagnostic performance, and may better discriminate patients according to severity or risk stratification (Cui et al., 2024).

In clinical practice, particularly in common sites of *Proteus* infection (e.g., urinary tract, wound care, hospital-acquired infections), determining a panel of acute phase proteins may play a role to: (1) confirm systemic involvement, (2) assess severity, (3) assess treatment response over time, and (4) predict complications, at least peripherally. Research-wise, these results prompt further exploration of APPs kinetics during *Proteus* infection (e.g. serial measurements), correlation with bacterial load, virulence factors, outcome (recovery vs complications) and comparison with other pathogens.

However, some limitations should be noted. We note that, although differences were significant, the absolute values of APPs are influenced by many factors (e.g. timing of blood sampling in relation to infection onset, pre-existing comorbidities (e.g. chronic inflammatory diseases, metabolic diseases), and concomitant treatments). In the absence of serial measures, it is hard to know whether the APP elevations represent peak inflammatory state, persistent infection, or residual inflammation at recovery? Second, the major disadvantage of our site-based stratification achieving notable differences is that the sample sizes for wound and sputum groups were relatively low and not generalizable well. Third, we did not evaluate other biomarkers (e.g. procalcitonin, IL-6, neutrophil counts) that could further define the immune response; combination of APPs with those markers may result in stronger diagnostic (or prognostic) panels as previously described by others.

## Conclusion

Our study shows that *Proteus* patients have significantly increased CRP, SAA and haptoglobin levels, providing evidence of an acute-phase response. Differential levels of markers at sites of infection indicates that deep tissue infections (e.g., wounds, respiratory as opposed to localized urinary tract infections) may evoke a more vigorous systemic inflammatory response. This response does not seem to be modulated by gender. These results emphasize the merit of a multi-marker acute-phase panel for the diagnosis and monitoring of

Proteus infections, and suggest the need for longitudinal studies and larger cohorts and complementary inflammatory markers in order to establish clinical and/or prognostic utility.

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