Role Of Haptoglobin Binding Proteins In Influencing Immune Response In Sepsis

Ramesh Kande[1], Suma Kantipudi[2], Sandeepta Burgula[1*].

[1] *Department of Microbiology, Osmania University, Hyderabad- 500007, Telangana, India.*
[2]*Prasad Hospitals, Nacharam, Hyderabad – 500 076, Telangana, India.*

Corresponding author:
Dr Sandeepta Burgula*
Mailing address: Department of Microbiology,
Osmania University, Tarnaka, Hyderabad- 500007
E-mail: s_burgula@osmania.ac.in

KEYWORDS

Haptoglobin, sepsis, immunoprecipit-ation, mass spectrometry, biomarkers, inflammation.

ABSTRACT

Sepsis is a life-threatening condition characterized by a dysregulated immune response to infection, leading to widespread inflammation and organ dysfunction. Haptoglobin (Hp), an acute-phase protein, plays a critical role in mitigating oxidative damage by binding free hemoglobin. This study investigated the interaction partners of haptoglobin in serum samples from healthy individuals, sepsis survivors, and non-survivors using immunoprecipitation and mass spectrometry. Distinct protein profiles were identified across the three groups, highlighting the role of haptoglobin-binding proteins in immune regulation, inflammation, and cellular stress responses. In healthy individuals, haptoglobin primarily interacted with proteins involved in maintaining cellular integrity and vascular permeability. Sepsis survivors exhibited interaction of Hp with proteins regulating inflammatory gene expression, oxidative stress, and tissue repair, suggesting a controlled immune response. In contrast, non-survivors showed associations with proteins linked to dysregulated apoptosis, antibiotic resistance, and hyperinflammation, indicative of poor outcomes. Key proteins such as MORF4 family-associated protein 1 (in survivors) and CASP8 and FADD-like apoptosis regulator (in non-survivors) were identified as potential biomarkers and therapeutic targets. These findings underscore the importance of haptoglobin and its binding partners and suggest that targeting these proteins could provide valuable insights into sepsis pathophysiology. Further research is needed to validate these proteins as biomarkers and explore their therapeutic potential in sepsis management.

Introduction

Sepsis is a life-threatening condition characterized by a dysregulated host response to infection, leading to systemic inflammation, multi-organ dysfunction, and high mortality rates (Singer et al., 2016). Despite advances in critical care, sepsis remains a leading cause of death worldwide, with mortality rates exceeding 30% in severe cases (Fleischmann et al., 2016). The pathophysiology of sepsis involves a complex interplay of immune dysregulation, oxidative stress, and endothelial dysfunction, making it a challenging condition to diagnose and treat effectively (van der Poll et al., 2017).

Haptoglobin (Hp), an acute-phase protein, plays a crucial role in the innate immune response by binding free hemoglobin released during hemolysis, thereby preventing oxidative damage caused by hemoglobin's iron content (Delanghe et al., 2020). Beyond its antioxidant properties, haptoglobin has been implicated in modulating immune responses, regulating inflammation, and influencing cellular stress pathways (Wang et al., 2016). In sepsis, haptoglobin levels are significantly elevated as part of the acute-phase response, suggesting its potential role in the host's defense mechanisms (Langlois & Delanghe, 1996). However, the specific interactions of haptoglobin with other proteins

in the context of sepsis, and how these interactions differ between survivors and non-survivors, remain poorly understood.

Recent studies have highlighted the importance of protein-protein interactions in the pathophysiology of sepsis, with specific proteins acting as biomarkers or therapeutic targets (Hotchkiss et al., 2016). Immunoprecipitation followed by mass spectrometry (IP-MS) has emerged as a powerful tool for identifying protein interaction networks, providing insights into the molecular mechanisms underlying disease progression (Rello et al., 2017). By analyzing the haptoglobin interactome in serum samples from healthy individuals, sepsis survivors, and non-survivors, this study aims to uncover key protein interactions that may influence sepsis outcomes.

This study investigates the hypothesis that haptoglobin-binding proteins differ significantly between healthy individuals, sepsis survivors, and non-survivors and that these differences may reflect underlying mechanisms of immune regulation, inflammation, and cellular stress responses. By identifying these proteins, we aim to provide new insights into the pathophysiology of sepsis and Identify potential biomarkers and therapeutic targets to improve patient outcomes.

Materials and methods

Study design and Patient enrolment

The study was approved by the Ethical Committee of Prasad Hospital and Research Foundation, with informed consent obtained from each participant. It included 20 male patients (aged 30-60) from the Nephrology in-patient department (IPD) of Prasad Hospital, Hyderabad, and 10 healthy individuals of the same age group with no history of sepsis or other infections, recruited during the year 2022-2024. Blood samples were collected, serum was separated and stored at -80°C, and the health status of the patients was regularly monitored. Samples were categorized into three groups: Healthy (H), Sepsis Survivors (S), and Non-Survivors (NS). Sepsis diagnosis was based on PCT and CRP values, with renal failure determined through haematological, serological, and biochemical parameters.

Immunoprecipitation of Hp

To purify Hp from serum samples (n=6) in each category (H, S, and NS), Protein A agarose beads were used. Serum samples were pre-cleared to remove abundant immunoglobulins, like IgG. The pre-cleared samples were immunoprecipitated to purify Hp. The beads were then boiled to elute the purified Hp, which was confirmed using 12% SDS-PAGE. The purified protein was further subjected to 2D gel electrophoresis to determine the pI and molecular weight of the proteins present in the samples.

2D-Gel Electrophoresis

10 µL of purified protein sample was diluted with 2DE rehydration buffer. Each sample was loaded onto an IEF strip (pH 4-7 Linear, 7 cm, Biorad) in a rehydration tray for 8-12 hours. IEF was carried out, and the strips were equilibrated in equilibration buffers for 1 hour each. The strips were then placed on a 12% SDS PAGE gel for the second-dimension (2D) run. The gels were run at a constant voltage of 70V until the blue dye front reached the bottom. The gels were removed and placed in fixative overnight. Post washing steps with distilled water, gels were silver stained and stored in 10% acetic acid. The stained gels were scanned using an HP Scanner. The spots identified for Hp-β (45 kDa) were used for profiling the samples by the ESI LC-MS method.

ESI LC-MS method is used for profiling of the samples

100 µg of the sample was reduced with 100 mM DTT at 95 °C for 1 hour at 400 rpm and alkylated with 250 mM IDA with incubation in the dark at room temperature for 45 minutes. The sample was then incubated with trypsin at 37°C overnight. The resulting samples were vacuum dried and dissolved in 50 µl of 0.1% formic acid in water. Desalting of the peptides was carried out, followed by centrifugation at 13,000 g. Subsequently, 10 µl of the supernatant was injected on a UPLC BEH

C18 column for peptide separation, with LC parameters set as follows: Buffer A: Water with 0.1% FA; Buffer B: ACN with 0.1% FA; Flow rate: 0.3 ml/min; Column oven temperature: 40°C. The analysis was performed on a Q-TOF instrument for MS and MS/MS. The raw data was processed using MassLynx 4.1 WATERS, and the individual peptides in the MS/MS spectra were matched to the theoretical tryptic peptides. The raw data was processed using MassLynx software version 4.1 from WATERS. The individual peptides' MSMS spectra were matched to the database sequence for protein identification on PLGS software, WATERS. Bioinformatic analysis was carried out with peptide tolerance of 10ppm, fragment tolerance of 20 ppm, carbamidomethyl C as fixed modification, oxidation M and N-Glycosylation as variable modifications, and missed cleavages were allowed up to 1. The glycan pattern on specific Aminoacids were checked on the glycopeptides.

Results

Immunoprecipitation

Haptoglobin and Hp binding proteins were analysed by Immunoprecipitation of Serum samples using Agarose A beads and separated on 2D Gel Electrophoresis to confirm the purification based on the PI values represented in fig1, indicating the purified Hp proteins and its interacting proteins, Hpβ was observed at 55KDa, and other proteins at 20KDa and 10 KDa in all H, S and NS category purified serum samples (**Fig 1)**.

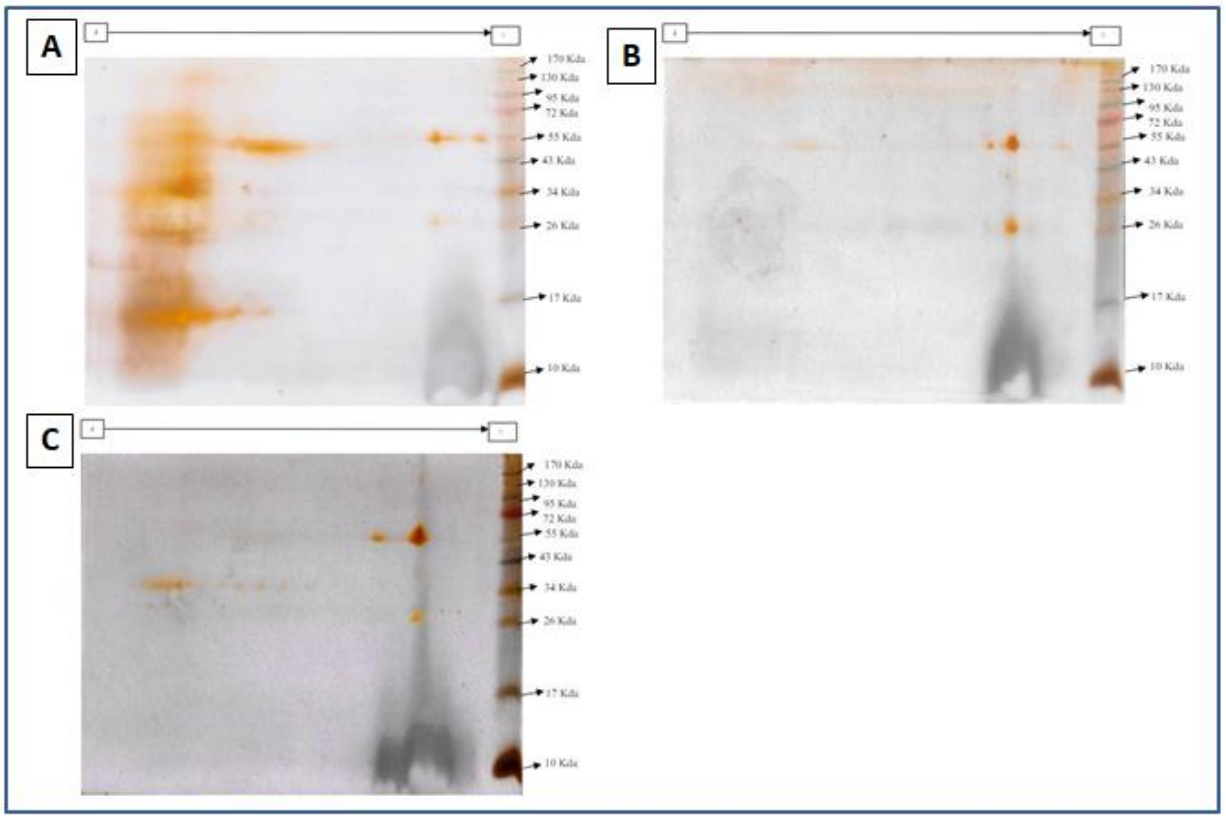
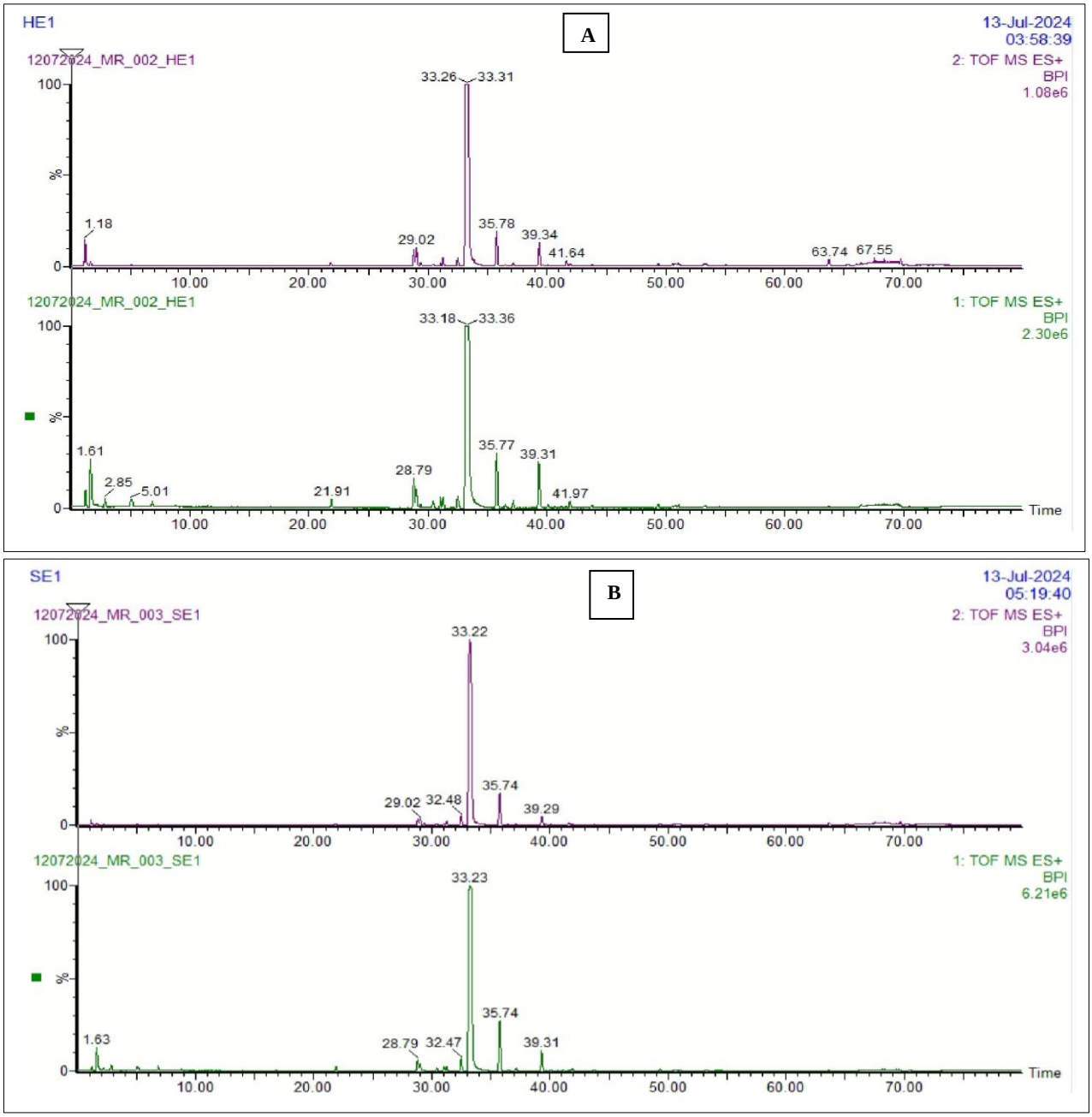


Fig 1: Haptoglobin from serum samples of patients was immunoprecipitated with Hp antibody coupled to protein A agarose beads and eluted samples were separated by two dimensional gel electrophoresis (12 % SDS-PAGE) and gels were stained by silver staining. Figure shows images of silver stained gels in A) Healthy B) Survivors C) Non-survivors. Arrows indicate bands from protein molecular weight marker.

Separation of compounds from the purified serum

UPLC chromatograms of elutes from immunoprecipitation show distinct peaks and retention times, which include Hp and Hp interacting proteins. The peaks are observed at approximately

29.03 minutes and between 35.78-39.24 minutes. The peak at 29.03 minutes has a relatively high intensity, suggesting a significant presence of Hp or its fragments. Additional peaks in the range of 60.74-67.05 minutes indicate the presence of Hp-interacting proteins, which may include known binding partners such as hemoglobin or other serum proteins in Healthy samples (**Fig 2A)**. The peaks were observed at around 30.73 minutes and between 35.77-39.21 minutes. The peak at 30.73 minutes shows a moderate intensity, indicating a good yield of Hp in survivors. Peaks in the range of 25.00-23.91 minutes and 41.97 minutes suggest the presence of Hp-interacting proteins, though the intensity is lower compared to Sample H **(Fig 2B)**. The peak at 29.07 minutes has a lower intensity compared to the other samples, indicating a lower concentration of the Hp in non-survivors. Peaks in the range of 30.67-39.29 minutes and 30.64 minutes indicate the presence of Hp-interacting proteins, though the overall yield is lower compared to H and S group **(Fig 2C)**.



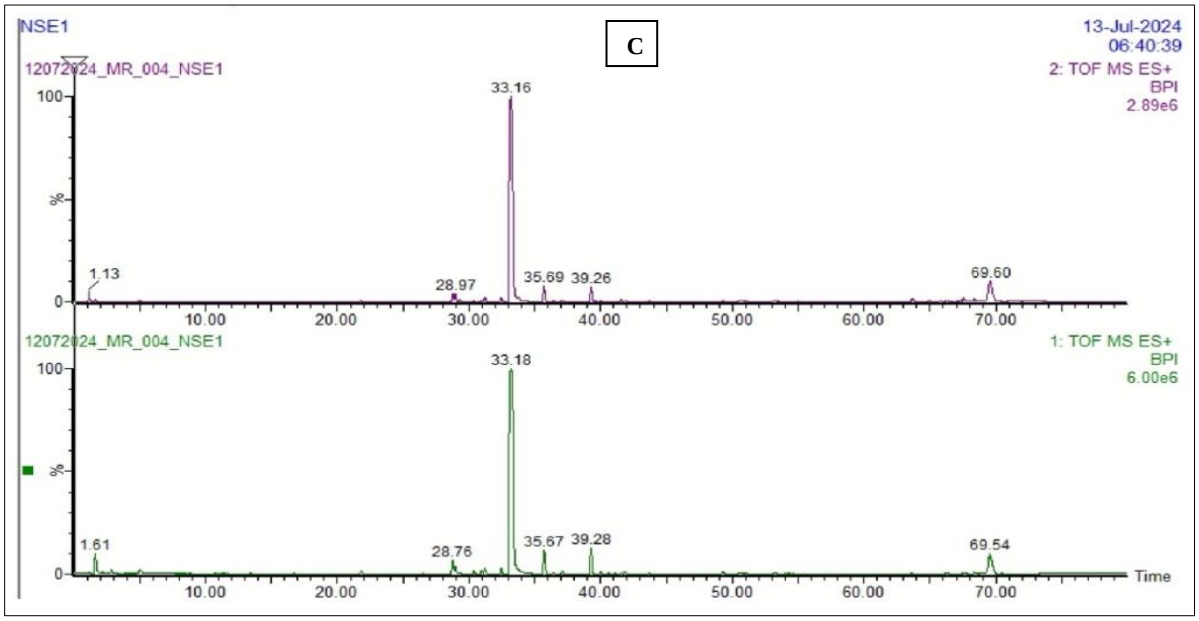
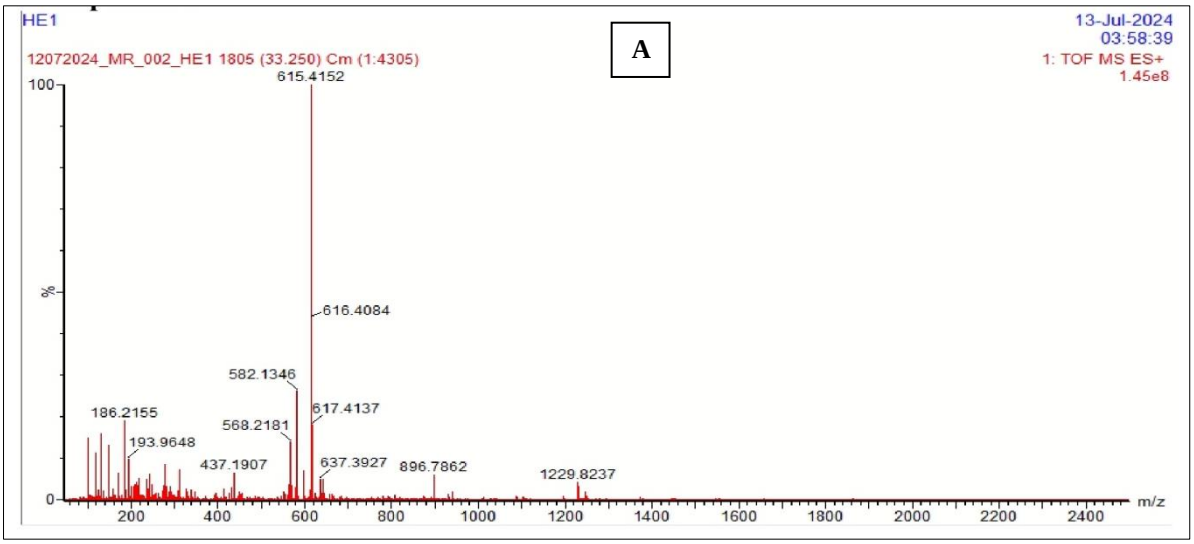


Fig 2; UPLC chromatograms showing MS/MS spectra of peptides obtained after tryptic digestion of Hp obtained after Immunoprecipitation of serum samples from sepsis patients. The figure shows peak values obtained indicating peptides from Hp interacting proteins from A) Healthy B) Survivors C) Non-survivors.

MS and MSMS Analysis

Mass spectrometry analysis reveals key peptide fragments and variations across samples H, S, and NS. A highly intense peak at m/z 615.4152 in Sample H indicates a significant presence of the Hp protein or its fragments, with isotopic variants at m/z 616.4084 and 617.4137. Additional peaks at m/z 582.1345 and 186.2155 suggest smaller fragments or contaminants (Fig. 3A). Similar patterns appear in Sample S but with lower intensity, while Sample NS shows the weakest signal, indicating reduced Hp levels. Unique peaks, such as m/z 118.0230 in NS, may represent distinct fragments (**Fig. 3B, 3C**).



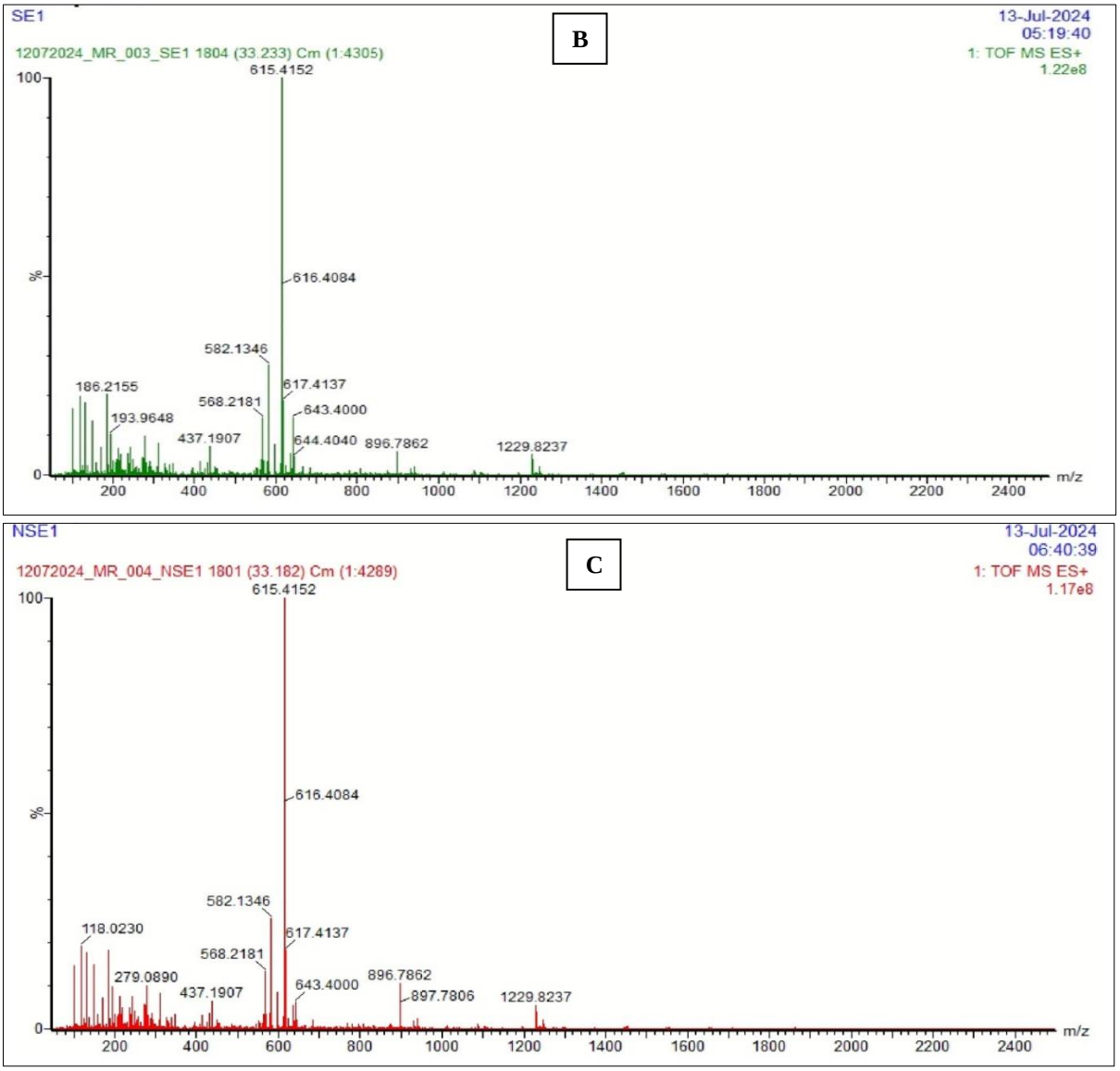


Fig 3; MS spectra showing mass to charge ratio [m/z] of Hp binding proteins in serum samples after separation by UPLC. The figure shows peak values obtained indicating peptides from Hp interacting proteins from A) Healthy B) Survivors C) Non-survivors.

MS/MS spectra confirm distinct fragmentation patterns, with common peaks at m/z 384.3090, 250.3336, and 168.9881 across all samples, suggesting shared peptide fragments (**Fig. 4A, 4B, 4C**). The peak at m/z 900.2990, strongest in Sample H, indicates significant Hp presence but decreases in intensity from H to NS. A unique peak at m/z 412.3180 in Sample S may suggest a modified peptide or interacting protein (**Fig. 4B**). Overall, Hp and its interacting proteins show a decreasing trend from H to NS, as reflected in MS/MS spectra (**Fig. 4C**).

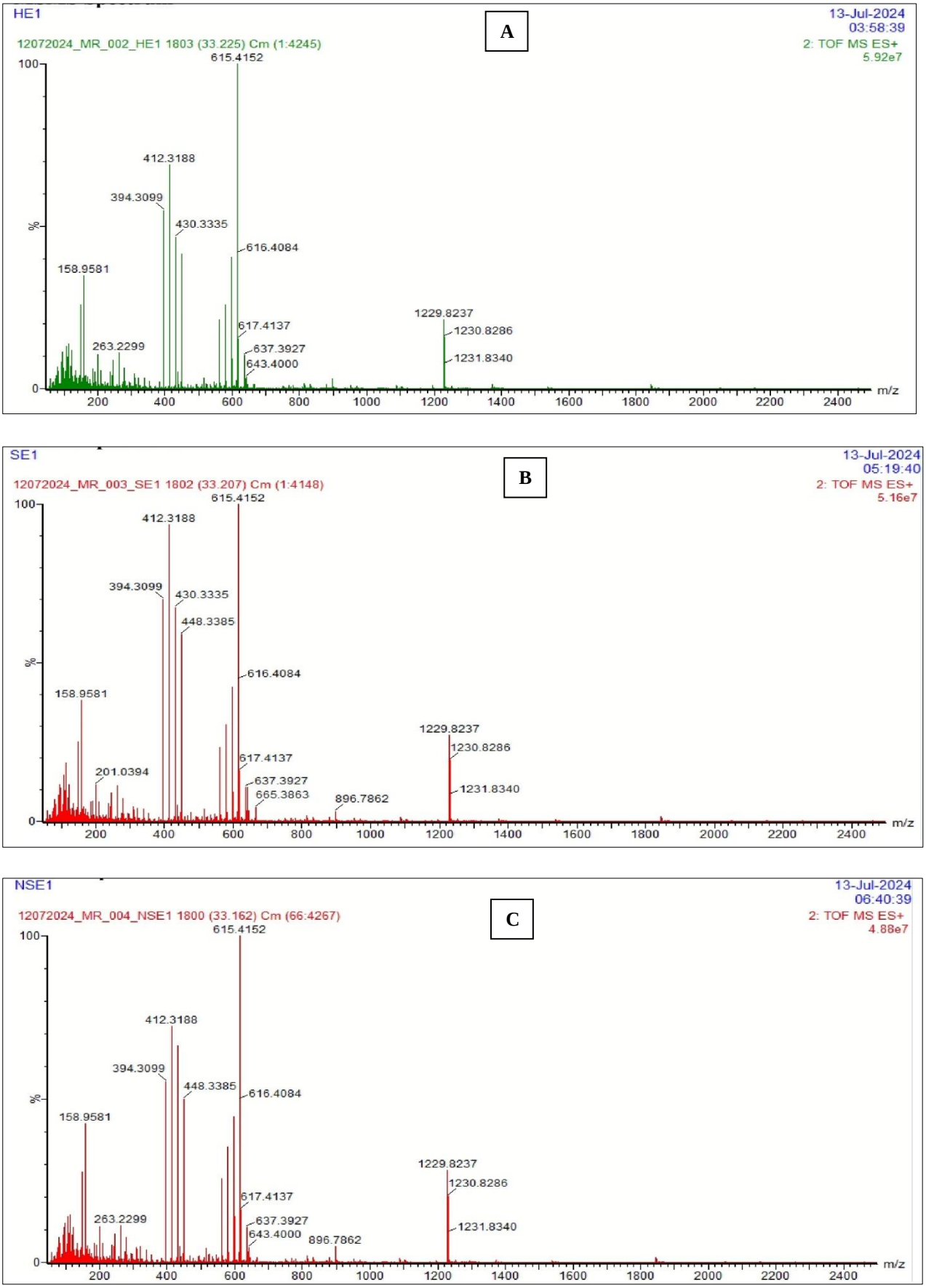


Fig 4; MS/MS spectra showing relative intensity of peptides obtained after tryptic digestion of Hp along with Hp binding proteins at different mass to charge obtained from A) Healthy B) Survivors C) Non-survivors serum samples.

Hp binding protein pattern changes in Sepsis

The mass spectrometry analysis revealed distinct protein profiles in healthy individuals, sepsis survivors, and non-survivors (**Table; 1,2,3**). These differences suggest that the interaction partners of haptoglobin may influence the outcome of sepsis. In healthy individuals, haptoglobin primarily interacts with including Homeobox Protein TGIF1 Involved in transcriptional regulation and developmental processes. Phosphatase and Actin Regulator 1 is essential for cytoskeletal organization and cell movement. Kinesin-like Protein KIF3A contributes to intracellular transport and cell division. UDP-glucuronosyltransferase 2B11 plays a key role in detoxification and drug metabolism. The distinct proteins identified in the H group include Transmembrane Protein 42, whose function is not well-defined but may be involved in cell signaling, and Putative Homeobox Protein Meis3-like 1, which is potentially associated with developmental processes and gene regulation. In S group Splicing Regulatory Glutamine/Lysine-rich Protein 1 Involved in RNA processing and splicing regulation, Ras and Rab Interactor 3 Plays a role in vesicle trafficking and signal transduction. The unique proteins found including MORF4 Family-associated Protein 1 Role not well-defined, potentially involved in chromatin remodeling. Peroxisome Assembly Protein 12 Crucial for peroxisome biogenesis and metabolic functions.

S. No	Protein Entry	Protein Accession	Protein Description
01	TMM42_HUMAN	Q69YG0	Transmembrane protein 42 OS=Homo sapiens OX=9606 GN=TMEM42 PE=1 SV=2
02	TGIF1_HUMAN	Q15583	Homeobox protein TGIF1 OS=Homo sapiens OX=9606 GN=TGIF1 PE=1 SV=3
03	PHAR1_HUMAN	Q9C0D0	Phosphatase and actin regulator 1 OS=Homo sapiens OX=9606 GN=PHACTR1 PE=1 SV=3
04	ME3L1_HUMAN	A6NDR6	Putative homeobox protein Meis3-like 1 OS=Homo sapiens OX=9606 GN=MEIS3P1 PE=5 SV=2
05	SREK1_HUMAN	Q8WXA9	Splicing regulatory glutamine/lysine-rich protein 1 OS=Homo sapiens OX=9606 GN=SREK1 PE=1 SV=1
06	ZC3HD_HUMAN	Q5T200	Zinc finger CCCH domain-containing protein 13 OS=Homo sapiens OX=9606 GN=ZC3H13 PE=1 SV=1
07	TMEM9_HUMAN	Q9P0T7	Proton-transporting V-type ATPase complex assembly regulator TMEM9 OS=Homo sapiens OX=9606 GN=TMEM9 PE=1 SV=1
08	KIF3A_HUMAN	Q9Y496	Kinesin-like protein KIF3A OS=Homo sapiens OX=9606 GN=KIF3A PE=1 SV=4
09	UDB11_HUMAN	O75310	UDP-glucuronosyltransferase 2B11 OS=Homo sapiens OX=9606 GN=UGT2B11 PE=1 SV=1
10	KI3S1_HUMAN	Q14943	Killer cell immunoglobulin-like receptor 3DS1 OS=Homo sapiens OX=9606 GN=KIR3DS1 PE=1 SV=2

Table 1 A list of Hp binding proteins in serum samples of healthy donors.
Peptides obtained upon tryptic digestion and Mass Spectrometric analysis of Hp extracted from serum samples of sepsis patients by Immunoprecipitation with Hp antibody were identified by MS/MS analysis. The table shows a list of Hp interacting proteins in serum samples from healthy donors.

S.no	Protein Entry	Protein Accession	Protein Description
01	MOFA1_HUMAN	Q9Y605	MORF4 family-associated protein 1 OS=Homo sapiens OX=9606 GN=MRFAP1 PE=1 SV=1
02	PEX12_HUMAN	O00623	Peroxisome assembly protein 12 OS=Homo sapiens OX=9606 GN=PEX12 PE=1 SV=1
03	SREK1_HUMAN	Q8WXA9	Splicing regulatory glutamine/lysine-rich protein 1 OS=Homo sapiens OX=9606 GN=SREK1 PE=1 SV=1
04	RIN3_HUMAN	Q8TB24	Ras and Rab interactor 3 OS=Homo sapiens OX=9606 GN=RIN3 PE=1 SV=4

Table 2 A list of Hp binding proteins in serum samples of sepsis survivors.
Peptides obtained upon tryptic digestion and Mass Spectrometric analysis of Hp extracted from serum samples of sepsis patients by Immunoprecipitation with Hp antibody were identified by MS/MS analysis. The table shows a list of Hp interacting proteins in serum samples from patients who were able to recover from sepsis (survivors)

S.no	Protein Entry	Protein Accession	Protein Description
01	MBLC1_HUMAN	A4D2B0	Metallo-beta-lactamase domain-containing protein 1 OS=Homo sapiens OX=9606 GN=MBLAC1 PE=1 SV=1
02	OR1FC_HUMAN	Q8NHA8	Putative olfactory receptor 1F12P OS=Homo sapiens OX=9606 GN=OR1F12P PE=5 SV=1
03	CTBP1_HUMAN	Q13363	C-terminal-binding protein 1 OS=Homo sapiens OX=9606 GN=CTBP1 PE=1 SV=2
04	NHRF3_HUMAN	Q5T2W1	Na(+)/H(+) exchange regulatory cofactor NHE-RF3 OS=Homo sapiens OX=9606 GN=PDZK1 PE=1 SV=2
05	CFLAR_HUMAN	O15519	CASP8 and FADD-like apoptosis regulator OS=Homo sapiens OX=9606 GN=CFLAR PE=1 SV=1
06	HMGN5_HUMAN	P82970	High mobility group nucleosome-binding domain-containing protein 5 OS=Homo sapiens OX=9606 GN=HMGN5 PE=1 SV=1
07	ZC3HD_HUMAN	Q5T200	Zinc finger CCCH domain-containing protein 13 OS=Homo sapiens OX=9606 GN=ZC3H13 PE=1 SV=1
08	GSH0_HUMAN	P48507	Glutamate--cysteine ligase regulatory subunit OS=Homo sapiens OX=9606 GN=GCLM PE=1 SV=1

Table 3 A list of Hp binding proteins in serum samples of sepsis non-survivors.
Peptides obtained upon tryptic digestion and Mass Spectrometric analysis of Hp extracted from serum samples of sepsis patients by Immunoprecipitation with Hp antibody were identified by MS/MS analysis. The table shows a list of Hp interacting proteins in serum samples from patients who were unable to recover from sepsis (non-survivors).

C-terminal-binding Protein 1 Involved in transcriptional repression and cell cycle regulation. CASP8 and FADD-like Apoptosis Regulator Plays a role in apoptosis and cell death pathways. Zinc Finger CCCH Domain-containing Protein 13 Involved in RNA binding and processing. The unique proteins includes Metallo-beta-lactamase Domain-containing Protein 1: Function not well-defined, potentially involved in metal ion binding. Putative Olfactory Receptor 1F12 Possibly involved in sensory perception and signal transduction.

S.no	Healthy	Survivors	Non Survivors
1	**Transmembrane protein 42** Maintenance of cellular integrity, potential role in immune signaling, ion and molecular transport, regulation of vascular permeability and interaction with inflammatory pathways.	**MORF4 family-associated protein 1** Regulation of inflammatory gene expression, control of cellular stress responses, immune cell proliferation and differentiation, epigenetic modulation of key genes, regulation of cell death pathways and potential role in tissue repair and recovery	**Metallo-beta-lactamase domain-containing protein 1** Antibiotic resistance, role in inflammation, cellular stress response, regulation of cell survival and apoptosis, interaction with pathogens, role in cellular signaling pathways, influence on endothelial function and potential biomarker for sepsis
2	**Transmembrane protein 42** Maintenance of cellular integrity, Potential role in immune signaling, ion and molecular transport, regulation of vascular permeability and interaction with inflammatory pathways.	**Peroxisome assembly protein 12** Regulation of oxidative stress, lipid metabolism and inflammatory modulation, immune cell function and inflammation, cell survival and poptosis prevention, mitochondrial interaction and metabolic stability and Potential role in immune modulation.	**Putative olfactory receptor 1F12P** Immune system modulation, detection of pathogen-associated molecular patterns (PAMPs), influence on inflammatory pathways, regulation of cell signaling, potential impact on neuroinflammation, regulation of vascular function, role in metabolic regulation and potential for therapeutic targeting.
3	**Phosphatase and actin regulator 1** Regulation of endothelial integrity and vascular permeability, immune cell migration and activation, modulation of inflammatory response via phosphatase interaction, influence on coagulation pathways and Potential for cardiovascular protection	**Splicing regulatory glutamine/lysine-rich protein 1** Regulation of immune response genes, control of cytokine and inflammatory pathways, modulation of apoptosis and cell survival, maintenance of endothelial integrity, regulation of metabolic genes, influence on RNA stability and translation efficiency and potential role in epigenetic modulation of immune genes.	**C-terminal-binding protein 1** Regulation of inflammatory gene expression, support for cellular metabolism and redox balance, prevention of immune cell exhaustion, modulation of apoptosis and cell survival pathways, influence on vascular integrity and endothelial function, protection against oxidative stress, epigenetic regulation of immune response genes and role in mitochondrial function and energy production.

4	**Putative homeobox protein Meis3-like 1** Regulation of inflammatory response, impact on immune cell differentiation, role in endothelial integrity,apoptosis regulation and epigenetic regulation of gene regulation.	**Ras and Rab interactor 3** Regulation of immune cell activation and signaling, control of phagocytosis and pathogen clearance, endocytosis and cytokine receptor recycling, regulation of cell migration and adhesion, influence on oxidative stress responses, role in apoptosis and cell survival pathways and endothelial function and vascular integrity.	**Na(+)/H(+) exchange regulatory cofactor NHE-RF3** Regulation of cellular pH and ion homeostasis, protection against cellular damage, maintenance of endothelial barrier function, modulation of inflammatory signaling pathways, support for immune cell function, influence on renal function and fluid balance, protection of organ function through metabolic regulation and potential role in modulating apoptosis.
5	**Splicing regulatory glutamine/lysine-rich protein 1** Regulation of inflammatory gene expression, control of immune cell function, response to cellular stress maintenance of endothelial and barrier function, impact on coagulation pathways and adaptive immune modulation.		**CASP8 and FADD-like apoptosis regulator** Inhibition of apoptosis, regulation of necroptosis, modulation of cytokine production, protection against immune cell depletion, support for endothelial integrity, prevention of organ failure and balance between inflammatory and anti-inflammatory responses.
6	**Zinc finger CCCH domain-containing protein 13** Regulation of inflammatory response, post-transcriptional regulation, cellular stress response, immune cell differentiation and function, viral response and antiviral activity and interplay with other signaling pathways.		**High mobility group nucleosome-binding domain-containing protein 5** Regulation of inflammatory gene expression, modulation of immune cell activation, influence on cellular stress response mechanisms, control of cell death and survival pathways, maintenance of vascular integrity, epigenetic modulation of sepsis-responsive genes and potential role in dampening hyperinflammatory responses.
7	**Proton-transporting V-type ATPase complex assembly regulator TMEM9** Regulation of acid-base homeostasis, influence on immune cell function, control of cytokine secretion, endosomal and lysosomal function, impact on cellular metabolism and potential role in vascular function.		**Zinc finger CCCH domain-containing protein 13** Regulation of inflammatory response, post-transcriptional regulation, cellular stress response, immune cell differentiation and function, viral response and antiviral activity and interplay with other signaling pathways.
8	**Kinesin-like protein KIF3A** Intracellular transport of immune components, cytokine secretion, vesicle trafficking and endocytosis, regulation of cell motility, microtubule dynamics and cell function, potential role		**Glutamate--cysteine ligase regulatory subunit** Regulation of oxidative stress via glutathione production, protection against organ dysfunction, modulation of immune cell function, influence on inflammatory cytokine production, role in endothelial

	in cellular stress responses and influence on endothelial function.		function and vascular integrity, prevention of cell death pathways and regulation of mitochondrial function.
9	**UDP-glucuronosyltransferase 2B11** Detoxification of metabolic products, metabolism of drugs, regulation of inflammatory mediators, role in hormone regulation, protective role against oxidative stress and influence on gut microbiome interactions.		
10	**Killer cell immunoglobulin-like receptor 3DS1** Regulation of NK cell activity, modulation of cytokine release, interaction with HLA class I Molecules, influence on T cell responses, potential role in immune regulation, genetic variability and sepsis outcomes and interactions with other immune cells.		

Table 4: A list of Haptoglobin binding proteins observed in sepsis patients and their functions in immune response.

Discussion

The findings from the immunoprecipitation of haptoglobin (Hp) from serum samples of sepsis patients, followed by mass spectrometry analysis, reveal significant differences in the protein profiles among healthy individuals, sepsis survivors, and non-survivors. These differences highlight the complex role of haptoglobin and its binding partners in the pathophysiology of sepsis, particularly in immune regulation, inflammation, and cellular stress responses. Haptoglobin is an acute-phase protein that binds free hemoglobin, preventing oxidative damage. In sepsis, haptoglobin levels are elevated as part of the systemic inflammatory response (Wang et al., 2016). The immunoprecipitation of haptoglobin from serum samples allowed for the identification of proteins that interact with haptoglobin, which may play roles in the immune response, inflammation, and cellular stress during sepsis (Delanghe et al., 2020). The mass spectrometry analysis revealed distinct protein profiles in healthy individuals, sepsis survivors, and non-survivors. These differences suggest that the interaction partners of haptoglobin may influence the outcome of sepsis. In healthy individuals, haptoglobin primarily interacts with proteins involved in maintaining cellular integrity, immune signaling, and vascular permeability. These interactions are part of normal physiological processes, such as ion and molecular transport, and regulation of inflammatory pathways (Langlois & Delanghe, 1996). The presence of proteins like transmembrane protein 42 and Phosphatase and actin regulator 1 suggests a role in maintaining endothelial integrity and regulating immune responses under normal conditions (Gutteridge, 1987).

In sepsis survivors, haptoglobin interacts with proteins that are involved in regulating inflammatory gene expression, controlling cellular stress responses, and promoting tissue repair. These

interactions may contribute to a more controlled immune response, which is crucial for survival in sepsis (Levy et al., 2010). Proteins such as MORF4 family-associated protein 1 and Peroxisome assembly protein 12 are upregulated in survivors. These proteins are involved in epigenetic modulation, oxidative stress regulation, and immune cell proliferation, which may help in mitigating the excessive inflammatory response seen in sepsis (Hotchkiss et al., 2016). The presence of Splicing regulatory glutamine/lysine-rich protein 1 suggests a role in maintaining endothelial integrity and regulating immune cell function, which could be protective against organ failure (Singer et al., 2016).

In non-survivors, haptoglobin interacts with proteins that are associated with dysregulated immune responses, antibiotic resistance, and cellular stress (E Homer et al., 2023). These interactions may contribute to the hyperinflammatory state and organ dysfunction observed in fatal sepsis cases (van der Poll et al., 2017). Proteins like Metallo-beta-lactamase domain-containing protein 1 and C-terminal-binding protein 1 are upregulated in non-survivors. These proteins are involved in antibiotic resistance, inflammation, and cellular stress responses, which may exacerbate the severity of sepsis (Rello et al., 2017). The presence of CASP8 and FADD-like apoptosis regulator suggests a dysregulation of apoptosis and necroptosis pathways, which could lead to immune cell depletion and organ failure (Hotchkiss et al., 2013). Transmembrane protein 42 is involved in maintaining cellular integrity and regulating vascular permeability (mehatha D et al., 2006). Its interaction with haptoglobin may play a role in modulating the inflammatory response in sepsis (Gutteridge, 1987). MORF4 family-associated protein 1 found in survivors is protein regulates inflammatory gene expression and may help control the immune response, promoting tissue repair and recovery (Levy et al., 2010). Metallo-beta-lactamase domain-containing protein 1 observed to be upregulated in non-survivors, is associated with antibiotic resistance and inflammation, which may contribute to the poor outcomes in these patients (Rello et al., 2017). CASP8 and FADD-like apoptosis regulator is involved in regulating apoptosis and necroptosis. Its dysregulation in non-survivors may lead to immune cell depletion and organ failure (Hotchkiss et al., 2013).

The differences in haptoglobin-binding proteins between survivors and non-survivors suggest that these proteins could serve as potential biomarkers for predicting sepsis outcomes NK Sharma et al., 2019. For example, the presence of proteins associated with tissue repair and controlled inflammation in survivors may indicate a better prognosis, while proteins associated with dysregulated apoptosis and antibiotic resistance in non-survivors may indicate a worse prognosis (Singer et al., 2016). Targeting these proteins or their pathways could offer new therapeutic strategies for sepsis. For instance, enhancing the activity of proteins involved in tissue repair and inflammation control (e.g., MORF4 family-associated protein 1) or inhibiting proteins associated with dysregulated apoptosis (e.g., CASP8 and FADD-like apoptosis regulator) could improve outcomes in sepsis patients (van der Poll et al., 2017).

In conclusion, the immunoprecipitation of haptoglobin and subsequent mass spectrometry analysis revealed distinct protein interaction profiles in healthy individuals, sepsis survivors, and non-survivors. These findings underscore the importance of haptoglobin and its binding partners in regulating immune responses, inflammation, and cellular stress during sepsis. Further research into these proteins and their pathways could lead to the development of novel biomarkers and therapeutic targets for sepsis.

Acknowledgements: Authors thank the Director and Technical Staff at PTNR-CFRD, Osmania University, for the technical and logistic support provided to carry out the work presented in the study.

Funding: This work was supported by:

1. DBT-Builder, Osmania University. File No. 25/DBT-BUILDER/2023.
2. DBT-BME Grant F. No. BT/PR48662/MED/32/943/2023.

References

1. Delanghe, J. R., Langlois, M. R., & De Buyzere, M. L. (2020). Haptoglobin polymorphism and sepsis. *Clinical Chemistry and Laboratory Medicine (CCLM)*, 58(6), 887-894.
2. Gutteridge, J. M. (1987). The antioxidant activity of haptoglobin towards haemoglobin- stimulated lipid peroxidation. *Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism*, 917(2), 219-223.
3. Hotchkiss, R. S., Monneret, G., & Payen, D. (2013). Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nature Reviews Immunology*, 13(12), 862- 874.
4. Hotchkiss, R. S., Moldawer, L. L., Opal, S. M., Reinhart, K., Turnbull, I. R., & Vincent, J.L. (2016). Sepsis and septic shock. *Nature Reviews Disease Primers*, 2, 16045.
5. Langlois, M. R., & Delanghe, J. R. (1996). Biological and clinical significance of haptoglobin polymorphism in humans. *Clinical Chemistry*, 42(10), 1589-1600.
6. Levy, M. M., Fink, M. P., Marshall, J. C., Abraham, E., Angus, D., Cook, D., ... & Vincent,J. L. (2010). 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Critical Care Medicine*, 31(4), 1250-1256.
7. Rello, J., Valenzuela-Sánchez, F., Ruiz-Rodriguez, M., & Moyano, S. (2017). Sepsis: A review of advances in management. *Therapeutics and Clinical Risk Management*, 13, 427- 437.
8. Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., & Angus, D. C. (2016). The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*, 315(8), 801-810.
9. van der Poll, T., van de Veerdonk, F. L., Scicluna, B. P., & Netea, M. G. (2017). The immunopathology of sepsis and potential therapeutic targets. *Nature Reviews Immunology*, 17(7), 407-420.
10. Delanghe, J. R., Langlois, M. R., & De Buyzere, M. L. (2020). Haptoglobin polymorphism and sepsis. *Clinical Chemistry and Laboratory Medicine (CCLM)*, 58(6), 887-894.
11. Fleischmann, C., Scherag, A., Adhikari, N. K., Hartog, C. S., Tsaganos, T., Schlattmann, P., Reinhart, K. (2016). Assessment of global incidence and mortality of hospital-treated sepsis: current estimates and limitations. *American Journal of Respiratory and Critical Care Medicine*, 193(3), 259-272.
12. Hotchkiss, R. S., Monneret, G., & Payen, D. (2013). Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nature Reviews Immunology*, 13(12), 862- 874.
13. Hotchkiss, R. S., Moldawer, L. L., Opal, S. M., Reinhart, K., Turnbull, I. R., & Vincent,J. L. (2016). Sepsis and septic shock. *Nature Reviews Disease Primers*, 2, 16045.
14. Horner, E., Lord, J. M., & Hazeldine, J. (2023). The immune suppressive properties of damage associated molecular patterns in the setting of sterile traumatic injury. *Frontiers in immunology*, *14*, 1239683.
15. Langlois, M. R., & Delanghe, J. R. (1996). Biological and clinical significance of haptoglobin polymorphism in humans. *Clinical Chemistry*, 42(10), 1589-1600.
16. Mehta, D., & Malik, A. B. (2006). Signaling mechanisms regulating endothelial permeability. *Physiological reviews*, *86*(1), 279-367.
17. Sharma, N. K., Ferreira, B. L., Tashima, A. K., Brunialti, M. K. C., Torquato, R. J. S., Bafi, A., ... & Salomao, R. (2019). Lipid metabolism impairment in patients with sepsis secondary to hospital acquired pneumonia, a proteomic analysis. *Clinical proteomics*, *16*, 1-13.

18. Rello, J., Valenzuela-Sánchez, F., Ruiz-Rodriguez, M., & Moyano, S. (2017). Sepsis: A review of advances in management. *Therapeutics and Clinical Risk Management*, 13, 427- 437.
19. Singer, M., Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., & Angus, D. C. (2016). The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*, 315(8), 801-810.
20. van der Poll, T., van de Veerdonk, F. L., Scicluna, B. P., & Netea, M. G. (2017). The immunopathology of sepsis and potential therapeutic targets. *Nature Reviews Immunology*, 17(7), 407-420.
21. Wang, Y., Lam, K. S., Kraegen, E. W., Sweeney, G., Zhang, J., Tso, A. W, Xu, A. (2016). Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. *Clinical Chemistry*, 53(1), 34-41.