ORIGINAL ARTICLE

Associations of Single Nucleotide Polymorphism (MMP-9 -1562 C/T rs 3918242) with the Risk of Severity in Adult Multiple Trauma ICU Admitted Patients: A Pilot Study

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ABSTRACT

Background: *Multiple traumas are injuries involving multiple body parts or organs, with at least one life-threatening injury. Despite advances in trauma care, complications like pneumonia can arise. Genetic variations, such as the MMP9-1562 C/T gene variant, may contribute to these complications.* ***Objectives:*** *This study aimed to investigate the potential association between the MMP-9 -1562 C/T gene variant (rs3918242) and the risk of developing pneumonia in critically injured patients admitted to the Intensive Care Unit (ICU).* ***Methodology:*** *This study included 120 participants: 40 ICU patients with multiple traumas and pneumonia, 40 with non-complicated multiple traumas, and 40 healthy controls. All participants were assessed using the Injury Severity Scale (ISS) scoring system. MMP9 levels were measured using the Human MMP-9 Quantikine ELISA Kit. The study focused on the MMP-9 -1562 C/T gene variant (rs3918242) to investigate its association with pneumonia susceptibility in severe multiple trauma patients in the ICU.* ***Results:*** *MMP9 levels were higher in patients with complicated multiple trauma and pneumonia, the T allele increased complication risk and WBC, CRP, and MMP9 levels helped differentiate between complicated and non-complicated trauma cases.* ***Conclusion:*** *Our study suggests a link between the MMP9-1562C/T gene variation and pneumonia risk in severely injured ICU patients. The T allele may be a genetic indicator for pneumonia risk in these patients.*

INTRODUCTION

Multiple traumas refers to injuries involving multiple anatomical segments or organs (>2), with at least one potentially life-threatening injury [1]. The Injury Severity Score (ISS) assesses trauma patient injuries by rating each injury via the Abbreviated Injury Scale (AIS) across six anatomical regions. The ISS calculation sums the squares of the three highest AIS scores, with scores >15 indicating major trauma [2]. Multiple traumas remain the leading cause of death for those under 40 years old, despite improved treatment options[1].Severe injury treatment remains a formidable challenge in trauma care, despite advancements, optimal treatment protocols are debated. Early assessment is critical to prevent complications, prioritize transport, inform surgical interventions and minimize mortality [3].

Current prehospital systems expedite transportation of critically injured patients to specialized facilities, enabling timely detection and treatment of life-threatening conditions, including hemorrhage, hypoxia, airway compromise and intracranial hypertension. Nevertheless, survivors remain vulnerable to secondary complications, prominently multiple organ dysfunction syndrome (MODS) and sepsis[4]. The development of sepsis and MODS post-trauma is mediated by complex interplay between genetic predisposition, environmental influences, and host responses, requiring further investigation[5].

Genetic variations, including insertions, deletions, duplications, and single nucleotide polymorphisms (SNPs), modulate clinical phenotypes by altering protein function or gene expression, thereby serve as stable predictive indicators of disease susceptibility and trauma outcomes[6]. Advances in genotyping technologies enhance investigation of genetic associations with trauma outcomes. Genetic association studies, utilizing peripheral blood samples, offer a robust approach for identifying susceptibility markers[7]. An epidemiological study revealed a significant correlation between adoptees' mortality from infection and their biological parents, but not adoptive parents, indicating genetic predisposition influences infection risk and outcome[8]. Subsequent research has substantiated the significance of genetic factors in the pathogenesis of post-traumatic complications like pneumonia[7].

Matrix Metalloproteinase 9 (MMP9) is a zinc-dependent enzyme essential for the breakdown and regulation of the extracellular matrix. It is involved in several biological functions such as cell migration, adhesion, apoptosis, and angiogenesis. Matrix Metalloproteinase-9 (MMP-9) also exacerbates sepsis pathophysiology through enhanced vascular permeability, inflammation and contributions to vascular instability, pulmonary injury and hemostatic dysregulation[9]. Increased levels of circulating MMP9 are recognized as a biomarker for serious respiratory illnesses, including Community Acquired Pneumonia and ventilator-associated pneumonia, which can complicate the treatment of multiple trauma patients admitted to intensive care units. Additionally, MMP9 contributes to inflammation by aiding in the infiltration of leukocytes[10]. The present work aims to investigate the link between **MMP-9 -1562 C/T** gene variant rs3918242 and susceptibility of pneumonia in severe multiple trauma patients in ICU.

METHODOLOGY

Study Population and Setting:

This study was a case-control study, it recruited participants from Suez Canal University Hospital's ICU Department. Laboratory analyses were conducted in the Clinical Pathology Department. The study comprised three age- and sex-matched groups: Patients with severe multiple traumas complicated by pneumonia (n=40), non-complicated multiple traumas patients (n=40) and healthy controls individuals from the hospital's blood bank (n=40).

Inclusion Criteria:

Adults (18-65 years) diagnosed with severe polytrauma (Revised Trauma Score) complicated by pneumonia for group 1, adults with severe non-complicated polytrauma (Revised Trauma Score) for group 2.

Exclusion criteria for both groups:

Pre-existing comorbidities, pediatric/geriatric populations, autoimmune diseases, and sepsis.

Sample size:

The sample size (n) was calculated using Dawson and Trapp's[11] formula: n = (Zα/2 + Zβ)^2 * (2 * (p1(1-p1) + p2(1-p2) / (p1 - p2)^2, where:

n: sample size, Zα/2: 1.96 (95% confidence), Zβ: 0.84 (80% power), P1: 32.5% (MMP-9 TT/CC genotypes in severe asthma[12]) and P2: 16.9% (MMP-9 TT/CC genotypes in mild asthma[12]) so the results: 120 patients, considering 10% drop-outs.

A probability sampling approach was employed to randomly select 40 patients from each group within the adult ICU and emergency unit populations.

Methods of the study:

The entire study population undergone assessment using the Injury Severity Scale (ISS) scoring system, which evaluates injury severity based on anatomical criteria across n body regions. The Abbreviated Injury Scale (AIS) grades injury severity as follows: (0: No injury, 1: Minor, 2: Moderate, 3: Severe (non-life-threatening), 4: Severe (life-threatening, survival probable), 5: Critical (survival uncertain), 6: Maximal (possibly fatal).

Human MMP-9 Quantikine ELISA Kit : Solid Phase Sandwich ELISA

We used a serum separator tube (SST) and allowed serum samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Serum was removed and assayed immediately in aliquot and stored at ≤ -20 °C, repeated freeze-thaw cycles were avoided.

ELISA Assay Procedure:

MMP9 levels were quantified according to the manufacturer's protocol. Serum samples (50 µL) were added to designated wells, followed by 50 µL of Antibody Cocktail. After sealing and incubating (1 hour, room temperature, 400 rpm), absorbance was measured at 450 nm using a Thermo ELISA reader.

DNA extraction: Peripheral blood DNA extraction (200 µL) from buffy coat was performed using QIAamp DNA Blood Kit (QIAGEN, Germantown, USA), adhering to manufacturer guidelines and stored at -80°C.

SNP selection and genotyping*:* MMP-9 -1562 C/T gene variant rs3918242 was selected: with minor allele frequency >5% in African population (according to data from the 1000 Genome Projects: https://www.internationalgenome.org/) These were validated by the Single Nucleotide Polymorphism database (dbSNP) (https://www.ncbi.nlm.nih.gov/snp/) and the Ensembl genome browser 107 (https://www.ensembl.org/index.html).

Amplification using RT-PCR: TaqMan allelic discrimination analyses were performed according to Applied Biosystems standard protocols (Applied Biosystems, Carlsbad, USA). Primers and probes were designed using Assays-by-Design (Applied Biosystems, Foster City, USA): The probes were labelled at their 5′-ends with FAMTM (the first allele) and VICTM (the second allele), and the 3′-ends contained quenchers. Primers and probes were mixed with PCR amplification of the SNP-containing promoter region utilized forward (5'-GCCTGGTGGCACATAGTGGCCC-3') and reverse (5'-CTTCCTAGCCAGCCGGCATC-3') primers. The probes were labelled at their 5′- ends with FAMTM (the first allele) and VICTM (the second allele), and the 3′-ends contained quenchers. Primers and probes were mixed with TaqMan H Universal PCR Master Mix (Catalog number: 4304437, Applied Biosystems, Thermo Fisher Scientific, Waltham, USA) . PCR was carried out according to Applied Biosystems instructions, and detection of the different genotypes was done using the Bio-Rad CFX96 Real-Time

Detection System C1000 Thermal Cycler (Bio-Rad Laboratories, Hercules, USA). Data was processed using CFX Maestro Software for CFX Real-Time PCR Instruments (Bio-Rad Laboratories, Hercules, USA).

Data management:

Statistical analysis employed SPSS v26. Qualitative data were presented as frequencies and percentages. Quantitative data, assessed for normality via Kolmogorov-Smirnov test, used median (range) for non-normal distributions and mean ± SD for normal distributions. Significance ($p < 0.05$) was evaluated using Chi-Square, Kruskal-Wallis, Student's t-test, ANOVA with Tukey's post-hoc, ROC curve analysis and Hardy-Weinberg equilibrium.

Ethical consideration:

The study adhered to ethics guidelines established by Suez Canal University Faculty of Medicine's Ethics Committee (5876#). Written informed consent was obtained from participants (or legal guardians) prior to data collection and investigations.

RESULTS

This case-control study investigated the relationships between serum MMP9 levels, SNP MMP-9 -1562 C/T, and susceptibility of pneumonia in severe multiple trauma patients in ICU. The study included 120 participants divided into three age- and sex-matched groups: Group 1: 40 ICU patients with severe multiple traumas complicated by pneumonia, Group 2: 40 patients with non-complicated multiple traumas, Group 3: 40 healthy controls. Mean ages (±SD) were 36.17±12.36, 32.83±10.61, and 31.88±9.48 for groups 1, 2, and 3, respectively, with no significant intergroup differences in age or sex as shown in table (1).

Table 1: demographic characters of studied groups

	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	Test of significance	Within group significance
Age/ years Mean ±SD	36.17±12.36	32.83±10.61	31.88±9.48	F=1.72 P=0.183	P1=0.171 P2=0.08 P3=0.697
Sex n (%) Male Female	 32(80%) 8(20%)	 26(65%) 14(35%)	 25(62.5%) 15(37.5%)	 χ^2=3.36 P=0.186	P1=0.133 P2=0.09 P3=0.816

Group 1: complicated , Group 2:non complicated group , Group3: control group, F:One Way ANOVA test , χ^2= Chi-Square test , p1: difference between group1 & 2, p2: difference between group1 & 3, p3: difference between group 2&3

A statistically significant difference was detected between studied groups as regard body temperature, WBCS, CRP with the highest values detected for group 1 followed by group 2 and the least for group 3 as shown in table (2).

Table 2: Comparison of body temperature and laboratory findings between studied groups

	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	Test of significance	Within group significance
Body temperature	39.14±0.71	36.74±1.59	36.97±0.16	F=68.41 P<0.001*	P1=0.001* P2=0.001* P3=0.302
WBCS*1000	18(13-29)	5(3.8-10)	5(4-8)	KW=81.16 P=0.001*	P1=0.001* P2=0.001* P3=0.409
CRP (mg/dl)	18(10-53)	1.3(1-3)	0.75(0.3-1.0)	KW=54.18 P=0.001*	P1=0.001* P2=0.001* P3=0.001*
CRP -VE +VE	 0 40(100)	 15(37.5) 25(62.5)	 34(85) 6(15)	 χ^2=60.08 P=0.001*	P1=0.001* P2=0.001* P3=0.001*

Group 1: complicated, Group 2:non complicated group , Group3: control group, F:One Way ANOVA test , KW= Kruskal Wallis test , p1: difference between group1 & 2, p2: difference between group1 & 3,p3: difference between group 2&3 , *statistically significant

A statistically significant difference ($p<0.05$) existed between Group 1 (severe multiple trauma with pneumonia) and Group 2 (severe multiple trauma) regarding:

Number of injured systems (higher in Group 1), Frequency of multiple transfusions, coma, tracheal secretions, and pulmonary contusions (higher in Group 1), Chest radiograph findings: diffuse infiltration (90% Group 1 vs. 0% Group 2) and localized infiltration (10% Group 1 vs. 0% Group 2), Abbreviated Injury Scale (AIS) scores: Group 1 (10% serious, 90% severe) vs. Group 2 (45% minor, 55% moderate), as shown in Table 3.

Table 3: comparison of injury characters between studied groups

	Group 1 (n=40)	Group 2 (n=40)	Test of significance
Type of injury Penetrating Blast	 18(45%) 22(55%)	 12(30%) 28(70%)	 χ^2=1.92 P=0.166
Number of injured systems TBI only TBI+1 TBI+2 TBI+3	 17(42.5%) 13(32.5%) 6(15%) 4(10%)	 36(90%) 4(10%) 0% 0%	 χ^2=21.58 P=0.001*
Multiple transfusion	29(72.5%)	4(10%)	χ^2=32.24 P=0.001*
Coma	28(70%)	3(7.5%)	χ^2=32.92 P=0.001*
Tracheal secretions	38(95%)	2(5%)	χ^2=64.8 P=0.001*
Pulmonary contusions	21(52.5%)	6(15%)	χ^2=12.58 P=0.001*
Chest radiograph No infiltration Diffuse infiltration Localized infiltration	 0% 36(90%) 4(10%)	 40(100%) 0% 0%	 χ^2=80 P=0.001*
AIS SCORE Minor Moderate Serious Sever	 0% 0% 4(10%) 36(90%)	 18(45%) 22(55%) 0% 0%	 χ^2=80 P=0.001*

Group 1: complicated, Group 2:non complicated group, χ^2= Chi-Square test, *statistically significant

Serum MMP9 levels differed significantly ($p < 0.001$) across groups: Group 1 (100.5 ng/ml, range 56-165), Group 2 (43 ng/ml, range 21-76), and Group 3 (7.4 ng/ml, range 1-24). Genotype distributions varied: Group 1 (37.5% CC, 40% CT, 22.5% TT), Group 2 (70% CC, 25% CT, 5% TT), and Group 3 (75% CC, 22.5% CT, 2.5% TT). Hardy-Weinberg equilibrium analysis revealed no significant deviations in genotype frequencies between studied groups and the population (p-values: 0.250, 0.396, and 0.745; Table 4), as shown in table (4).

Table 4: Frequency distribution of serum MMP9 serum level and genotype among studied groups

	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	Test of significance	Within group significance
MMP9serum level (ng/ml)	100.5(56-165)	43(21-76)	7.4(1-24)	KW=103.89 P=0.0001*	P1=0.001* P2=0.001* P3=0.001*
MMP9 rs 3918242 CC CT TT	 15(37.5%) 16(40%) 9(22.5%)	 28(70%) 10(25%) 2(5%)	 30(75%) 9(22.5%) 1(2.5%)	 χ^2=17.41 P=0.002*	P1=0.001* P2=0.001* P3=0.001*
HWE	P=0.250	P=0.396	P=0.745		

Group 1: complicated , Group 2:non complicated group , Group3: control group, HWE: Hardy Weinberg equilibrium, χ^2= Chi-Square test, KW= Kruskal Wallis test , p1: difference between group1 & 2, p2: difference between group1 & 3,p3: difference between group 2&3 , *statistically significant

Comparative analysis revealed statistically significant differences in CT and TT genotype distributions between Group 1 (trauma patients) and Group 2 (non-complicated cases), with increased complication risks (OR = 2.98 and 8.4, respectively). Similar differences existed between Group 1 and Group 3 (controls), with OR = 3.56 and 18. No significant differences were observed between Group 2 and Group 3. Presence of the T allele significantly increased complication risk by 3.48-fold (Group 1 vs. 2) and 4.64-fold (Group 1 vs. 3) compared to the C allele, as illustrated in Table 5.

Table 5: Odds ratio of MMP9 rs 3918242 in differentiating between groups

	Group 1 (n=40)	**Group 2 (n=40)**	**Group 3 (n=40)**	**Group 1 versus 2**		**Group 1 versus 3**		**Group 2 versus 3**	
MMP9 rs 3918242				P	Odds ratio (95%CI)	P	Odds ratio (95%CI)	P	Odds ratio (95%CI)
CC (R)	15(37.5%)	28(70%)	30(75%)		R				R
CT	16(40%)	10(25%)	9(22.5%)	0.03*	2.98 (1.09-8.19)	0.01*	3.56 (1.28-9.91)	0.74	1.19 (0.42-3.36)
TT	9(22.5%)	2(5%)	1(2.5%)	0.005*	8.4 (1.60-43.98)	0.001*	18 (2.08-155.61)	0.53	2.14 (0.18-24.95)
Allele C (R) T	 46(57.5%) 34(42.5%)	 66(82.5%) 14(17.5%)	 69(86.3%) 11(13.7%)	0.0005*	3.48 (1.68-7.21)	0.0005*	4.64 (2.14-10.07)	0.51 3	0.752 (0.318-1.77)

R: reference group

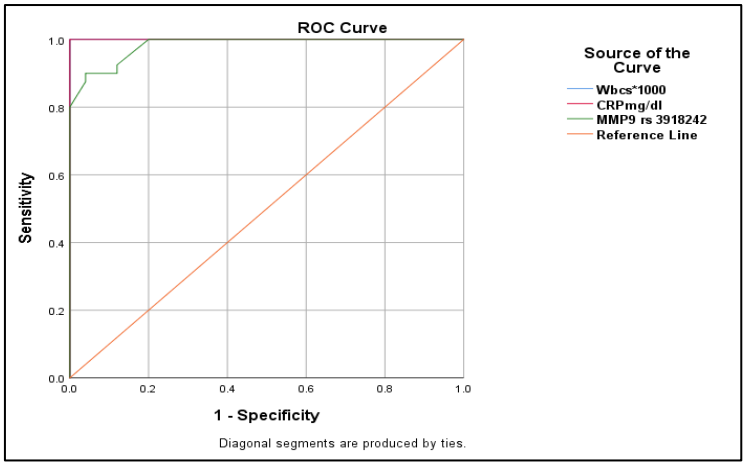

Fig. 1: ROC Curve for MMP9 rs 3918242, CRP, WBCS count in differentiating between complicated versus non complicated cases

Receiver operating characteristics curve showed that that area under curve were excellent which indicated that highest Validity was detected for WBCS, CRP then followed by MMP9 serum in differentiating between complicated versus non complicated multiple traumas patients figure (1), also table 6.

Table 6: validity of MMP9serum level, WBC and CRP in differentiating between complicated versus non complicated

	AUC (95%CI)	**P value**	**Cut off point**	**Sensitivity %**	**Specifiicty %**	**PPV%**	**NPV%**	**Accuracy%**
MMP9serum level	0.988 (0.973-1.0)	0.001*	≥58	92.5	92.5	92.5	92.5	92.5
Wbcs	1.0 (1.0-1.0)	0.001*	≥11.4	100.0	100.0	100.0	100.0	100.0
CRP	1.0(1.0-1.0)	0.001*	≥6.5	100.0	100.0	100.0	100.0	100.0

AUC: Area under curve , PPV: Positive predictive value , NPV: Negative predictive value

No statistically significant relation was detected between genotype distribution and all studied demographic and clinical findings among group 1 as shown in table (7).

Table 7: Relation between genotype distribution and clinical, demographic characters among group 1

Group 1	CC N=15	CT N=16	TT N=9	Test of significance
Age/ YEARS	35.27±11.59	35.81±13.57	38.33±12.55	F=0.177 P=0.839
Sex Male Female	 11(73.3) 4(26.7)	 15(93.8) 1(6.3)	 6(66.7) 3(33.3)	 χ^2=3.31 P=0.191
Body temperature	39.02±0.89	39.12±0.64	39.39±0.42	F=0.766 P=0.472
WBCS*1000	19(13-29)	18(13-26)	18(16-26)	KW=0.215 P=0.898
CRP(mg/dl)	19(12-40)	15(10-53)	16(12-23)	KW=1.62 P=0.445
Type of injury Penetrating Blast	 6(40) 9(60)	 6(37.5) 10(62.5)	 6(66.7) 3(33.3)	 χ^2=2.22 P=0.329
Number of injured systems TBI only TBI+1 TBI+2 TBI+3	 6(40) 5(33.3) 2(13.3) 2(13.3)	 7(43.8) 5(31.3) 2(12.5) 2(12.5)	 4(44.4) 3(33.3) 2(22.2) 0	 χ^2=1.62 P=0.951
Multiple transfusion	11(73.3)	11(68.8)	7(77.8)	χ^2=0.244 P=0.885
Coma	10(66.7)	11(68.8)	7(77.8)	χ^2=0.351 P=0.839
Tracheal secretions	14(93.3)	15(93.8)	9(100)	χ^2=0.614 P=0.736
Pulmonary contusions	7(46.7)	8(50)	6(66.7)	χ^2=0.969 P=0.616
Chest radiograph Diffuse infiltration Localized infiltration	 14(93.3) 1(6.7)	 15(93.8) 1(6.3)	 7(77.8) 2(22.2)	 χ^2=1.92 P=0.381
AIS SCORE Serious Sever	 1(6.7) 14(93.3)	 2(12.5) 14(87.5)	 1(11.1) 8(88.9)	 χ^2=0.309 P=0.857

KW: Kruskal Wallis test , F:One Way ANOVA test , χ^2=Chi-Square test , -*statistically significant

No statistically significant relation was detected between genotype distribution and all studied demographic and clinical findings among group 2 as shown in table (8).

Table 8: Relation between genotype distribution and clinical , demographic characters among group 2

Group 2	CC N=28	CT N=10	TT N=2	Test of significance
Age/ YEARS	35.04±10.81	28.3±9.11	24.5±2.12	F=2.27 P=0.117
Sex Male Female	 20(71.4) 8(28.6)	 6(60) 4(40)	 0 2(100)	 χ^2=4.33 P=0.155
Body temperature	36.59±1.89	37.06±0.26	37.10±0.14	F=0.352 P=0.705
WBCS*1000	5(3.8-10)	5.85(4-8)	5.5(5-6)	KW=1.02 P=0.598
CRP (mg/dl)	1.35(1-3)	1.2(1-3)	1.75(1.5-2)	KW=1.45 P=0.483
CRP -VE +VE	 12(42.9) 16(57.1)	 3(30) 7(70)	 0 2(100)	 χ^2=1.78 P=0.41
Type of injury Penetrating Blast	 7(25) 21(75)	 4(40) 6(60)	 1(50) 1(50)	 χ^2=1.19 P=0.551
Number of injured systems TBI only TBI+1	 26(92.9) 2(7.1)	 9(90) 1(10)	 1(50) 1(50)	 χ^2=3.81 P=0.149
Multiple transfusion	4(14.3)	0	0	χ^2=1.91 P=0.386
Coma	2(7.1)	1(10)	0	χ^2=0.257 P=0.879
Tracheal secretions	1(3.6)	1(10)	0	χ^2=0.752 P=0.687
Pulmonary contusions	4(14.3)	2(20)	0	χ^2=0.560 P=0.756
AIS SCORE Minor Moderate	 12(42.9) 16(57.1)	 4(40) 6(60)	 2(100) 0	 χ^2=2.59 P=0.273

KW: Kruskal Wallis test , F:One Way ANOVA test , χ^2=Chi-Square test , -*statistically significant

DISCUSSION

Despite improved trauma care, post traumatic complications persist. Research implicates immune cells and inflammatory mediators. Targeted therapies have failed to improve outcomes, suggesting genetic variability undermines treatment efficacy. Advances in nucleic acid/protein analysis now facilitate identification of genetic variations impacting inflammatory responses, offering new avenues for investigation.[13]. Multiple trauma's complex pathophysiology involves various immune mediators, leading to systemic inflammation and increased pneumonia risk. Trauma-induced immune dysregulation, exacerbated by necrotic tissues and ischemia-reperfusion injury, predispose patients to pneumonia, also critically ill trauma patients are predisposed to pneumonia due to mechanical ventilation. Pneumonia development significantly worsens outcomes, reducing discharge to home and increasing rehabilitation/skilled nursing and facility admissions[14,15]. Matrix Metalloproteinase 9 (MMP9) is an enzyme involved in cell functions and tissue repair, High MMP9 levels indicate severe respiratory illnesses like pneumonia[10]. This case-control study investigated relationships between serum MMP9 levels, SNP MMP-9 -1562 C/T, and susceptibility of pneumonia in severe multiple trauma patients in ICU.

This study enrolled 120 participants, divided into three age- and sex-matched groups: Group 1 (40 ICU patients with severe multiple traumas complicated by pneumonia), Group 2 (40 patients with non-complicated multiple traumas) and group 3 (40 healthy controls from the hospital's blood bank), with no significant intergroup differences in age or sex. A small number of studies have specifically investigated the role of gender in the development of post-injury pneumonia. Gannon et al.[16]

found that male gender is an independent risk factor. However, in agreement with the current study, another report failed to demonstrate this association, and a potential influence remains to be fully understood[17].

Trauma patients face higher pneumonia risks due to severe injuries, low blood pressure, transfusions, chest/brain injuries, aspiration risks and intubation[18]. Our study revealed that trauma patients with pneumonia had more injured body systems, more transfusions, coma, and lung damage, worse chest X-ray results, higher injury severity scores this indicates that Pneumonia worsened trauma patient outcomes. Dasdar et al.[19] also showed that ISS was significantly associated with in-hospital complications in multiple trauma patients, as the rate of complications increased by 17% for every 1-point increase in ISS. Geiger et al.[20] study revealed that the presence of relevant lung injury, male gender, PMCs, transfusion of more than 10 PRBCs as well as ISS and age were identified as predisposing factors that were independently associated with the development of pulmonary failure. A study of blunt trauma patients found that several factors increase the risk of pneumonia, including gender, injury severity, type of injury, and certain medical conditions like heart disease and cancer. Additionally, a patient's condition at admission, such as respiratory rate and trauma score, also play a role[21].

In response to traumatic injury and the subsequent release of damage-associated molecular patterns (DAMPs), the body's immunological and inflammatory processes at the cellular and humoral levels are activated to neutralize non-infectious tissue insults, bacterial infections, and initiate repair mechanisms. This post-injury phase is known as the systemic inflammatory response syndrome (SIRS)[18]. The pathophysiology of infectious post-injury consequences is thought to be mostly influenced by neutrophils, which are important White Blood inflammatory cells that guard against bacterial infections. Neutrophils are the first line of defense against rapidly dividing bacteria, fungus, and yeast because of their several microbicidal defensive mechanisms, which include the generation of reactive oxygen species (ROS) [22]. C-reactive protein (CRP), synthesized primarily in the liver, serves also as a biomarker for systemic inflammation. CRP production, stimulated by cytokines like interleukin-6, reflects inflammatory activity in diverse disease states [23].

Matrix Metalloproteinase 9 (MMP9) regulates tissue breakdown and inflammation. High levels indicate severe respiratory illnesses like pneumonia[10]. In our study serum MMP9 levels varied significantly ($p < 0.001$) among groups being higher in group 1 (100.5 ng/ml) than group 2 (43 ng/ml), and Group 3 (7.4 ng/ml). MMP9. Receiver operating characteristics curve in our study showed that the highest validity was detected for WBCS, CRP then followed by MMP9 in differentiating between complicated versus non complicated multiple trauma cases. Stupnytskyi & Biletskyi[24] found that risk factors for adverse outcome from multiple trauma patients associated with high neutrophils and white blood cells count. The matrix metalloproteinase (MMP) was also related to a number of lung tissue damage and disorders such as asthma[25], idiopathic pulmonary fibrosis (IPF) [26], emphysema[27], ARDS[28] and Chronic Obstructive Pulmonary Disease (COPD)[29].

The MMP9 gene, located on chromosome 20, harbors functionally significant polymorphisms. Notably, the MMP9-1562 C/T variant enhances transcriptional activity in macrophages by disrupting nuclear protein binding. Polymorphisms within MMP9's regulatory regions, such as MMP9-1562 C/T, influence gene expression. The T allele (CT/TT genotypes) exhibits increased transcriptional activity compared to the CC genotype[30]. Comparative analysis of our study revealed statistically significant differences in CT and TT genotype distributions between Group 1 and Group 2 with increased complication risks (OR = 2.98 and 8.4, respectively). Presence of the T allele significantly increased complication risk by 3.48-fold (Group 1 vs. 2) and 4.64-fold (Group 1 vs. 3) compared to the C allele.

The SNP MMP-9 -1562 C/T polymorphism has been associated with the development of diabetic microvascular complication and coronary artery disease, sepsis[31-33], Cai et al.'s[10] research suggests that other MMP9 single nucleotide polymorphisms (SNPs) influence susceptibility to severe pneumonia.

Study Limitations:

Our study has some limitations. First, the number of participants was relatively small, so larger studies are needed to confirm our findings. Second, our study only included Egyptian participants, so more research is needed in other ethnic groups. Finally, further studies on other genetic variations in the MMP9 gene could help clarify its role in pneumonia among multiple trauma patients.

Conclusion: Our study suggests a link between a specific genetic variation (MMP9-1566C/T) and the risk of pneumonia in severely injured ICU patients. The results support the hypothesis that the MMP9-1562 T allele variant may be a genetic marker for pneumonia risk in these patients.

Disclosure Statements

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