Component 1 Inhibitor Missense (Val480Met) Variant Is Associated With Gene Expression and Sepsis Development in Neonatal Lung Disease

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Background: Neonatal lung disease has a multifaceted etiopathology, including an explosive inflammatory sequence in the immature lung. Complement component 1 Esterase INHibitor (C1INH) is implicated in controlling inflammation in response to infection/injury.

Aim: To explore for the first time the association of the C1INH rs4926 (Val480Met) variant and circulatory transcript expression levels in the neonates that had evidence of lung disease and the clinic-laboratory data.

Methods: A total of 139 unrelated neonates were enrolled in this case-control study. C1INH genotyping and expression analyses were done using TaqMan Genotyping and Real-Time qPCR, respectively.

Results: A/A genotype carriers were two times more likely to develop in newborns with lung disease under homozygote (A/A vs. G/G: OR = 2.66, 95%CI = 1.03-6.87, p = 0.039) and recessive (A/A vs. G/G-A/G: OR = 2.42, 95%CI = 1.07-6.06, p = 0.047) models. Also, a higher frequency of A/A genotype was observed in the patient’s cohort complicated with sepsis (44.2 vs. 14.3%, p = 0.002). Neonates with lung disease with A variant had more risk for developing sepsis under homozygote (A/A vs. G/G: OR = 5.19, 95%CI = 1.73-15.6, p = 0.002), dominant (A/G-A/A vs. G/G: OR = 2.39, 95%CI = 1.02-5.58, p = 0.041), and recessive (A/A vs. G/G-A/G: OR = 5.38, 95%CI = 1.86-15.5, p < 0.001) models. Regression analysis revealed rs4926*A/A genotype as an independent predictor risk factor for sepsis development in cohorts with lung disease (adjusted OR = 4.26, 95%CI = 1.38-13.1, p = 0.012). The circulatory transcript was significantly downregulated in neonates with lung disease in whom rs4926*A/A carriers had the least expression levels (median: −2.86, IQR: −3.55 to −1.71; p < 0.001). ROC curve analysis...
revealed C1INH expression could differentiate between cohorts with/without subsequent development of sepsis, and the discrimination ability was enhanced when combined with circulatory IL-6 and CRP levels (AUC = 0.926, 95%CI = 0.87-0.97).

**Conclusion:** The C1INH rs4926 variant might play an essential role in the susceptibility to neonatal lung disease and could predict sepsis development in this cohort. Furthermore, the circulatory expression levels of this gene were downregulated in the neonatal lung disease cohort, supporting its potential role in the pathophysiology of this disorder, and highlighting its promising role in future targeted therapy.

**Keywords:** C1INH, SNP, gene expression, Real-Time PCR, sepsis, preterm infant

**INTRODUCTION**

There are multiple causes of neonatal lung disease. The most common is neonatal respiratory distress, which is pulmonary insufficiency that commences at or shortly after birth and increases over the first 2 days of life. Clinically, neonates with respiratory distress present with early respiratory distress comprising cyanosis, grunting, retractions, and tachypnea and may result in respiratory failure (1, 2). Neonatal respiratory distress is a severe disorder that most affect premature infants. It accounts for 10% of neonatal deaths, making it the fifth leading cause of death in the first year of life even with interventions (3).

Neonatal respiratory distress is caused by structural and functional immaturity of the lung with a deficiency of producing enough alveolar surfactant, resulting in decreased lung compliance and unstable alveoli (4). Pathologically, the disease is characterized by epithelial cell injury, edema formation, and intra-alveolar contact phase activation (also called plasma kallikrein-kinin system) (5, 6).

Although premature infants have benefited from recent advances in surfactant therapy, among the survivors treated with currently available surfactants, a significant percentage of survived infants grow up with a recurrent pulmonary injury and chronic inflammation. Given that preterm infants are particularly poor at mounting an antibody response, innate immunity mechanisms in this patient group may be especially important. Thus, there was a need to develop alternative therapeutic strategies (4). A combination of Complement component 1 Esterase INHibitor (C1INH) and lung surfactant or immunoglobulins for the treatment of respiratory disorders as Infant Respiratory Distress Syndrome (IRDS), Adult Respiratory Distress Syndrome (ARDS), and acute lung injury (ALI) was tested under several trials (7–9).

C1INH (https://www.uniprot.org/uniprot/P05155), also known as Serpin Peptidase Inhibitor Clade G Member 1 (SERPING1), has gained increasing attention as a regulator of several systems such as the complement system, plasma kallikrein of the contact system (which plays a central role in RDS etiopathology), the coagulation system (factor XIA, XIA, and thrombin), and the fibrinolytic system through the inhibition of plasmin, and tissue-type plasminogen activator (10).

It is encoded by the C1INH gene located at the long arm of chromosome 11 (11q12.1), and its nine exons encode the most highly glycosylated plasma protein in the circulation, with 35% of its weight constituted by carbohydrates (11). It is synthesized primarily in the liver as a single amino acid chain, and its deficiency is associated with hereditary angioneurotic edema (HANE) type I or II. C1INH is a serine protease inhibitor (serpin). Serpins help control several types of chemical reactions by blocking the activity of specific proteins (12). It plays an eminent role in controlling a range of processes involved in maintaining blood vessels, platelet degranulation, regulating inflammation in response to infection or injury, blood coagulation, fibrinolysis, and the generation of kinins (13).

Subcutaneous injection of a plasma-derived concentrate of C1INH was used in adult and pediatric patients with HANE to compensate for low levels or improper function of C1INH protein. Indeed, C1INH treatment has improved the outcome in several disease models, including sepsis and bacterial infection, ischemia-reperfusion injury, hyper-acute transplant rejection, and other inflammatory disease models (14, 15). Malgorzata et al. studied its ability to control the inflammatory processes in vitro and in vivo to decipher the possible underlying C1INH-related mechanisms mediating protection. They found that C1INH administration could provide a new therapeutic option for disorders associated with histone release (5). Collectively, these findings support the potential implication of C1INH in respiratory-related distress and open a new era for exploring its utility in the clinic.

The current authors in the clinical work observed variations in neonatal lung disease incidence and clinical presentation/response to postnatal surfactant therapy and optimal ventilator care in both full-term and preterm neonates; hence a speculated role of genetic element, in addition to the environmental triggers, was raised. Given the well-known impact of genetic variants, including the single nucleotide polymorphisms (SNPs), on gene expression and activity of the encoded proteins which could influence disease susceptibility and phenotype (16, 17), the authors were inspired to explore for the first time the possible association of C1INH rs4926 gene variant and expression with neonatal lung disease risk and the clinical features in an Egyptian neonate cohort. The results of this work could help in risk stratification of this vulnerable population with early implementation of the appropriate preventive/therapeutic strategies.
SUBJECTS AND METHODS

Study Population

A total of 139 consecutive unrelated newborns were recruited from the neonatal intensive care unit (NICU) of Suez Canal University Hospitals, Ismailia, Egypt, which is a level III NICU. All neonates with lung disease were included. All preterm admitted from June 2018 to July 2019 were screened to be enrolled in the study based on the guidelines (1). Premature newborns with comorbidities or congenital malformations were excluded. The patient group ($n = 94$) included preterm newborns ($28 < 37$ weeks GA) who developed respiratory distress (based on clinical manifestations/chest X-ray findings) in the first 6 h of life. It is worth noting that the more mature neonates did have lung disease, but a much milder form related to them being near-term or infants of diabetic mothers. None of them required invasive or non-invasive ventilation, as seen in Table 1.

The severity of respiratory distress was assessed clinically by Downes' scoring system (score $< 5$ was regarded as mild, $5-7$ as moderate, and $\geq 8$ was regarded as severe respiratory distress) and radiologically according to chest X-ray findings (stage 1-4) (18). Of the neonates with lung disease, 52 developed late-onset sepsis (onset $> 72$ h). This was diagnosed based on clinical and laboratory findings, including apnea, cyanosis, increased respiratory demand, lethargy, hypotonia, feeding intolerance, abdominal distension, reduced variability.

| Characteristics               | Levels                  | Controls ($n = 45$) | Neonates with lung disease ($n = 94$) | P-value |
|-------------------------------|-------------------------|---------------------|---------------------------------------|---------|
| Demographics                  |                         |                     |                                       |         |
| Sex                           | Female                  | 16 (35.6)           | 42 (44.7)                             | 0.36    |
|                               | Male                    | 29 (64.4)           | 52 (55.3)                             |         |
| Consanguinity                 | Negative                | 39 (86.7)           | 84 (89.4)                             | 0.77    |
|                               | Positive                | 6 (13.3)            | 10 (10.6)                             |         |
| Order of baby                 | 1st                     | 10 (22.2)           | 30 (31.9)                             | 0.42    |
|                               | 2nd                     | 14 (31.1)           | 32 (34.0)                             |         |
|                               | 3rd                     | 17 (37.8)           | 25 (26.6)                             |         |
|                               | 4th or more             | 4 (8.8)             | 7 (7.4)                               |         |
| Antepartum follow-up          | PROM                    | 0 (0.0)             | 25 (26.6)                             | $<0.001$|
|                               | Antepartum hemorrhage   | 0 (0.0)             | 4 (4.3)                               | 0.30    |
|                               | Maternal diabetes       | 1 (2.2)             | 3 (3.2)                               | 0.74    |
|                               | Maternal SLE            | 0 (0.0)             | 1 (1.1)                               | 0.48    |
|                               | Bronchial asthma        | 1 (2.2)             | 0 (0.0)                               | 0.32    |
|                               | Maternal anemia         | 0 (0.0)             | 2 (2.1)                               | 0.32    |
|                               | Maternal hypertension   | 1 (2.2)             | 5 (5.3)                               | 0.66    |
|                               | Pre-eclampsia           | 0 (0.0)             | 10 (10.6)                             | 0.030   |
|                               | Eclampsia               | 0 (0.0)             | 2 (2.1)                               | 0.32    |
|                               | Triple I                | 0 (0.0)             | 8 (8.5)                               | 0.044   |
|                               | Preterm labor pain      | 10 (22.2)           | 19 (20.2)                             | 0.84    |
| Delivery                      | Vaginal                 | 22 (48.9)           | 40 (42.6)                             | 0.58    |
|                               | Cesarean                | 23 (51.1)           | 54 (57.4)                             |         |
| Preterm delivery              | Negative (>37 weeks)    | 35 (77.8)           | 29 (30.9)                             | $<0.001$|
|                               | Preterm (32-36 weeks)   | 10 (22.2)           | 48 (51.1)                             |         |
|                               | Extreme preterm (28-31 weeks) | 0 (0.0) | 17 (18.1)                             |         |
| GA at birth, weeks            | Mean ± SD               | 37.6 ± 1.21         | 33.9 ± 2.24                           | $<0.001$|
| Birth weight, Kg              | Mean ± SD               | 3.03 ± 0.5          | 1.98 ± 0.5                            | 0.16    |
| Multiple gestations           | Single                  | 45 (100)            | 84 (89.4)                             | 0.030   |
|                               | Multiple                | 0 (0.0)             | 10 (10.6)                             |         |
| Inflammatory markers          | Interleukin-6 (log2FC)  | Median (IQR)        | 0.0 (2.1-3.26)                        | $<0.001$|
|                               | C-reactive protein (mg/L)| Median (IQR) | 0.4 (0.3-0.6)                   | $<0.001$|

Data are presented as number and percentage, mean and standard deviation (SD), or median and interquartile range (IQR). Two-sided Chi-square, Mann-Whitney U, or Student’s t-tests were used. PROM, premature rupture of membrane; SLE, systemic lupus erythematosus; GA, gestational age. Bold values indicate significance at $p < 0.05$. 
and transient decelerations in heart rate, temperature instability, seizures, and bulging fontanelles. Laboratory findings included a combination of multiple biomarkers, such as the total number of neutrophils, immature to total neutrophil ratio, and C-reactive protein (CRP) and blood culture (19). None of the included newborns suffered from acute respiratory distress syndrome following sepsis or pneumonia. The control group (n = 45) included preterm and full-term neonates without lung disease or other comorbidities, recruited from the same hospital as the cases. We included full-term neonates in the control group due to the paucity of healthy premature neonates and the difficulty of taking consent from parents to include their premature neonates as controls.

Clinical Assessment and Data Collection
A comprehensive questionnaire including variables in neonatal lung disease development was designed and distributed among the neonatologists in the NICU. Maternal and neonatal histories were evaluated for the following clinical data: birth weight, gestational age, gender, order of birth, mode of delivery, antenatal glucocorticoid treatment, the reason for preterm birth, and severity of the respiratory distress, and any chronic maternal illness, maternal premature rupture of membrane (PROM), and history of medications.

Informed written consent was obtained from parents. The diagnosis of respiratory distress was made by the attending physician (neonatologist) based on classic clinical presentations and radiographic findings (20). Premature infants were classified by weight into low birth weight (LBW)<2,500 g, very low birth weight (VLBW)<1,500 g, extremely low birth weight (ELBW)<1,000 g (only four neonates <28 weeks GA were admitted due to emergency delivery and difficult transportation). All the newborns with respiratory distress underwent therapeutic and supportive care, surfactant administration, and mechanical ventilation as per hospital protocol, following the European consensus guidelines (21).

Sample Collection and Laboratory Analysis
Venous blood samples were collected on several occasions under aseptic conditions into vacutainer plain/EDTA tubes. The required samples (5 ml) were collected at once only in case of UVC inserted and the blood drained for metabolic studies or required samples (5 ml) were collected at once only in case of under aseptic conditions into vacutainer plain/EDTA tubes. The plain tubes were sent for routine chemical analysis in the chemistry lab of the specified hospital. The EDTA tubes were sent for subsequent genomic analysis. About 2 ml blood was inoculated directly into blood culture medium specific vials with double-checking patient identification label and sent to the microbiology laboratory for cultivation. Results of subcultures examined for growth after incubation of 48 h were delivered as preliminary results; blood culture was considered sterile after 7 days of negative cultivation results (22).

Chest X-Ray Assessment
Grading respiratory distress was done as follows: Grade I: Slightly reticular (granular) pattern, decrease the transparency of lung, Grade II: soft decrease in transparency with air bronchogram, Grade III: Like grade II with a more substantial decrease in transparency with blurry diaphragm and heart, Grade IV: White lung, with homogenous lung opacity (20).

Molecular Analysis
Bioinformatic Selection of C1INH Gene Variant
According to the PubMed, ClinVar, and VarSome databases, 244 genetic variants with known pathogenicity were reported, including 172 (70.5%) pathogenic, 40 (16.4%) uncertain significance, and 32 (13.1%) benign variants (Supplementary Figure S1D). The pathogenic variant with the highest allele frequency is rs4926 (G/A) at chr11:57614516, a missense mutation with minor allele frequency (MAF) of 0.15 caused by the substitution of valine (GTG) with methionine (ATG) at codon 480. Both residues are medium size and hydrophobic and have similar physicochemical properties. This polymorphism overlaps 20 transcripts and is predicted to have benign consequences (PolyPhen 0.034) except for ENST00000403581 transcript (PolyPhen 0.557 with possibly damaging effect).

Nucleic Acids Extraction
Nucleic acids were isolated from whole blood using the ABIOPure Total DNA and RNA isolation kit (AllianceBio, Catalog no. M501DP100 and M541RP50-B, respectively) following the manufacturer-supplied protocol. Nucleic acid concentration and purity at the absorbance ratio 260/280 nm were determined by NanoDrop ND-1000 spectrophotometer (NanoDrop Tech., Inc. Wilmington, DE, USA).

Discrimination Analysis of C1INH rs4926 Variant
DNA samples of patients and controls were genotyped for C1INH rs4926 polymorphism using Real-Time polymerase chain reaction allelic discrimination technology and TaqMan assay (Applied Biosystems, assay ID C___7498220_10) with FAM/VIC dyes (G/A: V480M) (Supplementary Figure S1) as detailed previously (23, 24).

C1INH Gene Expression Analysis
The High-Capacity cDNA Reverse Transcription (RT) Kit (Applied Biosystems, P/N 4368814) was used to convert RNA into cDNA. RT was carried out in T-Professional Basic, Biometra PCR System (Biometra, Goettingen, Germany). C1INH expression was quantified using TaqMan® assay specific for C1INH gene (Applied Biosystems, assay ID Hs001613781_m1) and Taqman® Universal PCR master mix (Applied Biosystems, P/N 4440043) compared to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression (Assay ID Hs402869) as an endogenous control. The PCR program was run as described previously (25). The selected assay covered 17 splice variants of the C1INH gene, including ENST00000378324.6 transcript enriched in the circulation. Appropriate controls were run in each duplicated experiment. Fold change was calculated using the delta-delta threshold cycle equation (26). We followed all the necessary quality control measures recommended by the “Minimum Information for Publication of Quantitative Real-Time PCR Experiments; MIQE” guidelines (27).
Statistical Analysis
Data were managed using the R software version 4.0.4/RStudio 1.4.1106, GraphPad prism v.8.0, STATA v.16, and “Statistical Package for the Social Sciences (SPSS) for Windows” version 27.0. Categorical variables were compared using the chi-square ($\chi^2$) or Fisher’s exact tests. Data are represented as numbers and percentages. At the same time, student’s t-test, Mann-Whitney U (MW), and Kruskal-Wallis (KW) tests were used to compare continuous variables according to data distribution and variance homogeneity that was checked by “Shapiro-Wilk test” and “Levene test,” respectively, to compare continuous variables. Data were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR).

The allele frequency within each group was determined as the number of occurrences of an individual allele divided by the total number of alleles. Minor allele frequency (MAF) was compared with other ethnic populations in the 1000 Genome Project (www.ensembl.org). Genotype frequency (G/G, A/G, and A/A) per each group was estimated (28). Hardy-Weinberg equilibrium (HWE) for the SNP was tested by using a goodness-of-fit $\chi^2$-test via the Online Encyclopedia for Genetic Epidemiology (OEGE) software (http://www.oege.org/software/hwe-mr-calc.shtml). The associations of rs4926 genotype and susceptibility to neonatal lung disease were computed for various genetic association models, including heterozygote comparison (A/G vs. G/G), homozygote comparison (A/A vs. G/G), dominant model (A/G-A/A vs. G/G), and recessive model (A/A vs. A/G-G/G). Adjusted odds ratio by sex and gestational age was reported along with their 95% confidence intervals (CI). A two-tailed $P$-value < 0.05 was considered statistically significant.

Fold change of $C1INH$ gene expression was calculated using quantitative threshold level of patients and controls following the LIVAK method (26). Logarithmic transformation was used in plotting the data. The receiver operating characteristic (ROC) curves were applied to get the best cutoff values of the $C1INH$ gene level for discriminating patients from controls. The area under the Curve (AUC), sensitivity, and specificity was identified. Iteration in combination with Interleukin-6 (IL-6) and C reactive protein (CRP) levels was performed.

A binary logistic regression test was performed to identify independent risk factors for neonatal sepsis after adjusting confounding parameters. Significant variables in the univariate analysis were selected. Other variables reported having a role in prior literature were added. Those with strong correlations were excluded, and only one was selected. Cox Proportinate Hazards regression analysis was performed to identify the predictors for neonatal mortality. Data were reported as hazard ratio and 95% confidence interval (CI).

RESULTS
Characteristics of the Study Population
Total 94 neonates were admitted to the Neonatal Intensive Care Unit (NICU) for respiratory distress during the study period. The study included 94 neonatal lung disease cases (42 females and 52 males) and 45 controls (16 females and 29 males). Preterm delivery was reported in 65 neonates with lung disease (69.1%).

Premature rupture of membrane (PROM) was the most common predisposing factor associated with the development of neonatal lung disease in 25.6% of newborns, followed by preterm labor pain (20.2%). Significant elevation of inflammatory markers was remarkable in serum of neonatal lung disease patients ($p = 0.001$) (Table 1). The overall mortality rate due to respiratory distress was 39.4 ($n = 37$).

Characteristics of Neonatal Lung Disease With and Without Sepsis
Of the neonates with lung disease, 52 (55.3%) developed sepsis, 51.9% were females. Blood culture revealed $Klebsiella$ (11.7%), $Staphylococcus aureus$ (7.4%), $Streptococcus$ (1.1%), and $Pseudomonas$ (3.2%) infection, for which specific neonatal antibiotics were given. Neonates with lung disease with subsequent sepsis were more likely to be delivered preterm (84.6 vs. 50%, $p = 0.001$) and to have less birth weight (78.8 vs. 59.6%, $p < 0.001$). Septic neonate had significantly lower levels of hemoglobin (median = 13, IQR = 11.6-15 in sepsis group vs. median = 14.9, IQR = 12-16.6 in non-sepsis group, $p = 0.005$) and serum albumin (median = 2.6, IQR = 2.3-3.0 vs. median = 3.2, IQR = 2.8-3.5, respectively, $p = 0.002$). In contrast, sepsis cohorts had significantly higher levels of C-reactive protein [52.8 (34-73.3) vs. 2.60 (1-3.65), $p < 0.001$] (Table 2).

Neonates with sepsis required more prolonged mechanical ventilation duration [7 days (4–9) vs. 3 days (1–4), $p = 0.007$], and was associated with longer NICU [12 days (9.7-17) vs. 7 days (5–12), $p = 0.001$] and total hospital stays [13 days (10–13) vs. 7 days (5–12), $p = 0.001$]. However, there was no significant difference in their mortality rate (36.5% in sepsis vs. 42.9% in the non-sepsis group, $p = 0.67$ (Table 2).

Complications rather than sepsis were also recorded in our cohort, affecting 65 (69.1%) neonates. Air leak syndromes presented in 10 (15.4%) neonates, pulmonary hemorrhage affected five preterm, and all passed away (7.7%), and eight babies had necrotizing enterocolitis (grades I and II; 12.3%). Infiltration extravasation injury caused by vancomycin and TPN (total parenteral nutrition) has disturbed four (6.15%) neonates, and only one needed plastic surgery treatment. About five (9.23%) premature neonates suffered PPHN (persistent pulmonary hypertension), for which they received medical treatment and responded. Periventricular hemorrhage was detected in seven (10.7%) cases (ventricular matrix hemorrhage grade I and grade II by cranial ultrasound), and retinopathy of prematurity (ROP) grades I and II were diagnosed in five (7.7%) neonates.

C1INH Gene Variant Analysis
Genotyping of rs4926 polymorphism in 139 subjects revealed a minor allele frequency (A allele) of 0.35 among the whole cohorts. This frequency was significantly higher than Africans, Asians, and Americans reported in the 1000 Genome Project (Figure IA). Similarly, G/G was the most frequent genotype in the study population accounting for 55% (Figure IB). Genotype frequencies followed Hardy-Weinberg equilibrium ($p = 0.76$ for controls and 0.60 for cases). As depicted in Table 3, the A/A genotype was more frequent in neonates with lung disease than...
TABLE 2 | Characteristics of neonates with lung disease cohort according to subsequent complications with sepsis.

| Characteristics                  | Levels                      | Total (n = 94) | No sepsis (n = 42) | Sepsis (n = 52) | p-value |
|----------------------------------|-----------------------------|----------------|-------------------|-----------------|---------|
| **Demographics**                 |                             |                |                   |                 |         |
| Sex                              | Female                      | 42 (44.7)      | 15 (35.7)         | 27 (51.9)       | 0.14    |
|                                  | Male                        | 52 (55.3)      | 27 (64.3)         | 25 (48.1)       |         |
| Consanguinity                    | Negative                    | 84 (89.4)      | 40 (95.2)         | 44 (84.6)       | 0.17    |
|                                  | Positive                    | 10 (10.6)      | 2 (4.8)           | 8 (15.4)        |         |
| **Presentation**                 |                             |                |                   |                 |         |
| Mode of delivery                 | Vaginal                     | 40 (42.6)      | 15 (35.7)         | 25 (48.1)       | 0.29    |
|                                  | Cesarean                    | 54 (57.4)      | 27 (64.3)         | 27 (51.9)       |         |
| Preterm delivery                 | Negative (>37 weeks)        | 29 (30.9)      | 21 (50.0)         | 8 (15.4)        | 0.001   |
|                                  | Preterm (32-36 weeks)       | 48 (51.1)      | 13 (31.0)         | 35 (67.3)       |         |
|                                  | Extreme preterm (28-31 weeks)| 17 (18.1)      | 8 (19.0)          | 9 (17.3)        |         |
| Cause of preterm delivery       | PROM                        | 25 (26.6)      | 10 (23.8)         | 15 (28.8)       | 0.64    |
|                                  | Pre-eclampsia               | 12 (12.7)      | 5 (11.9)          | 7 (13.4)        | 0.42    |
|                                  | Triplet I                   | 8 (8.5)        | 4 (9.5)           | 4 (7.7)         | 0.75    |
|                                  | Antepartum hemorrhage       | 4 (4.3)        | 2 (4.8)           | 2 (3.8)         | 0.82    |
|                                  | Preterm labor pain          | 19 (20.2)      | 17 (40.5)         | 2 (4.7)         | 0.012   |
| Degree of BW                     | LBW (2.499-2.000 gm)        | 38 (40.4)      | 27 (64.3)         | 11 (21.2)       | <=0.001 |
|                                  | LBW (2.000-1.500 gm)        | 40 (42.5)      | 16 (37.5)         | 24 (46.1)       |         |
|                                  | VLBW (<1.500-1.000 gm)      | 12 (12.7)      | 6 (14.3)          | 6 (11.5)        |         |
|                                  | ELBW (<1.000 gm)            | 4 (4.2)        | 2 (4.8)           | 2 (3.8)         |         |
| Multiple gestations              | Single                      | 84 (89.4)      | 40 (95.2)         | 44 (84.6)       | 0.17    |
|                                  | Multiple                    | 10 (10.6)      | 2 (4.8)           | 8 (15.4)        |         |
| Maternal comorbidities           | Maternal diabetes           | 3 (3.2)        | 0 (0.0)           | 3 (5.8)         | 0.25    |
|                                  | Maternal anemia             | 2 (2.1)        | 2 (4.8)           | 0 (0.0)         | 0.19    |
|                                  | Maternal hypertension       | 5 (5.3)        | 2 (4.8)           | 3 (5.8)         | 0.82    |
| **Investigation**                |                             |                |                   |                 |         |
| Complete blood picture           | WBCs X 10^3                 | 7.5 (5.1-12.8) | 7.6 (5.3-11.9)    | 7.25 (5-13.7)   | 0.92    |
|                                  | Platelets X 10^3            | 195 (127-303)  | 202 (147-288)     | 188 (116-310)   | 0.88    |
|                                  | Hemoglobin g/dl             | 13.6 (12-15.8) | 14.9 (12.16-8)    | 13 (11.6-15)    | 0.005   |
| Blood chemistry                  | Na mEq/L                    | 140 (137-145)  | 142 (139-145)     | 139 (134-145)   | 0.06    |
|                                  | K mmol/L                    | 4.7 (4.30-5.1) | 4.9 (4.4-5)       | 4.5 (4.1-5.1)   | 0.43    |
|                                  | Ca mg/dl                    | 8.7 (89-9.2)   | 8.7 (8.10-9.2)    | 8.7 (8.91-9)    | 0.90    |
|                                  | Creatinine mg/dl            | 0.5 (0.3-0.61) | 0.5 (0.30-0.60)   | 0.5 (0.3-0.3)   | 0.54    |
|                                  | PH                          | 7.2 (7.2-7.3)  | 7.1 (7.17-7.2)    | 7 (7.25-7.3)    | 0.22    |
|                                  | PO2mmHg                      | 70 (59-80)     | 58 (54-67)        | 72.5 (59-80)    | 0.40    |
|                                  | PCO2mmHg                     | 47 (28.6-55)   | 39 (27-40)        | 48 (29.6-55)    | 0.42    |
|                                  | HCO3mEq/L                   | 180 (16-20)    | 17.5 (17.3-18)    | 18.2 (16-20)    | 0.47    |
| Liver function test             | ALT, U/L                    | 120 (8-16)     | 12 (8-17)         | 13.5 (9-16)     | 0.74    |
|                                  | AST, U/L                    | 35 (30-60)     | 35 (28-58)        | 40.5 (30-64)    | 0.31    |
|                                  | Albumin, g/dl               | 3.0 (2.7-3.4)  | 3.20 (2.80-3.5)   | 2.6 (2.3-3)     | 0.002   |
| Inflammatory markers            | CRP mg/L                    | 19 (3-60)      | 2.60 (1-3.65)     | 52.8 (34-73.3)  | <=0.001 |
|                                  | IL6                         | 2.6 (2.1-3.26) | 2.7 (2.05-3.4)    | 2.6 (2.1-3.04)  | 0.47    |
| CHEST X ray                     | No evidence                 | 8 (8.5)        | 4 (9.5)           | 4 (7.7)         | 0.21    |
|                                  | Grade 1                     | 57 (60.6)      | 22 (52.4)         | 35 (67.3)       |         |
|                                  | Grade 2,3                   | 18 (19.1)      | 8 (19.0)          | 10 (19.2)       |         |
|                                  | Grade 4                     | 11 (11.7)      | 8 (19.0)          | 3 (5.8)         |         |
| **Management**                  |                             |                |                   |                 |         |
| Maternal medications            | Antenatal antibiotics       | 6 (6.4)        | 1 (2.4)           | 5 (9.6)         | 0.22    |
|                                  | Antenatal steroids           | 23 (24.5)      | 6 (14.3)          | 17 (32.7)       | 0.039   |

(Continued)
TABLE 2  |  Continued

| Characteristics | Levels | Total (n = 94) | No sepsis (n = 42) | Sepsis (n = 52) | p-value |
|------------------|--------|---------------|-------------------|----------------|---------|
|                  |        |               |                   |                |         |
| Single dose steroids |       | 9 (38.1) | 3 (7.1) | 6 (11.5) | 0.15 |
| Two doses’ steroids |       | 6 (26.1) | 2 (4.8) | 4 (7.7) | 0.15 |
| Three doses’ steroids |       | 8 (34.8) | 1 (2.4) | 7 (13.5) | 0.15 |
| Ventilation |        |               |                   |                |         |
| O2 therapy |       | 58 (61.7) | 24 (57.1) | 34 (65.3) | 0.18 |
| Non-invasive MV (CPAP) |       | 23 (24.5) | 12 (28.6) | 11 (21.2) | 0.18 |
| Invasive MV (SIMV) |       | 13 (13.8) | 6 (14.3) | 7 (13.5) | 0.18 |
| Surfactant |    |       |                   |                |         |
| Positive |       | 9 (9.6) | 6 (14.3) | 3 (5.8) | 0.29 |

Outcomes

| Duration, days | Ventilation | 4 (2-7) | 3 (1-4) | 7 (4-9) | 0.007 |
| NICU | 10 (7-14.8) | 7 (5-12) | 13 (10-13) | 0.001 |
| Total length of stay | 10.5 (7-15) | 7 (5-12) | 13 (10-13) | 0.001 |
| Survival | 37 (39.4) | 18 (42.9) | 19 (36.5) | 0.67 |

PROM, premature rupture of membrane; GA, gestational age; BW, birth weight; VAP, ventilation-association pneumonia; LBW, low birth weight; VLBW, very low birth weight; ELBW, extremely low birth weight; WBC, white blood cells; ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; IL6, interleukin 6; MV, mandatory ventilation; CPAP, continuous positive airway pressure; SIMV, synchronized intermittent mandatory ventilation; NICU, neonatal intensive care unit. Two-sided Chi-square, Mann-Whitney U, or Student’s t-tests were used to compare sepsis and non-sepsis groups. Bold values indicate significance at p < 0.05.

controls (30.9 vs. 15.6%, p = 0.039). Homozygote carriers for the A allele were two times more likely to develop neonatal lung disease under homozygote comparison (A/A vs. G/G: OR = 2.66, 95%CI = 1.03-6.87, p = 0.039) and recessive model (A/A vs. G/G-A/G: OR = 2.42, 95%CI = 1.07-6.06, p = 0.047).

Association of C1INH Polymorphism With Clinical and Laboratory Features

In the neonatal lung disease group, G/G, A/G, A/A genotypes were found in 47 (50%), 18 (19.1%), and 29 (30.9%) patients, respectively. As illustrated in Table 4, despite preterm neonates with homozygote mutant allele (A/A) for methionine residue having the highest gestational age (p = 0.049) and birth weight (p = 0.006), they exhibited the least hemoglobin level (p = 0.002) and higher frequency of sepsis (p = 0.008). There was no significant difference in other clinical and laboratory parameters. In addition, there was no differential difference in patients’ outcomes, including the timing of weaning from mechanical ventilation (p = 0.58), the mortality rate (p = 0.26), or hospital stay (p = 0.12).

Methionine Mutation Increased the Risk of Sepsis in Neonatal Lung Disease Patients

Within the neonatal lung disease group, 55.5% (N = 52) developed sepsis. A higher frequency of A/A genotype was observed in premature neonates complicated with sepsis (44.2

FIGURE 1 | Genotype and allele frequency of C1INH rs4926 polymorphism in the studied population. (A) Allele frequency of rs4926 G/A polymorphism in the current study (red) compared to other populations (green) reported in 1000 Genome Project. AFR, African; EAS, East Asian; SAS, South Asian; AMR, American; EUR, European. A two-sided Chi-square test was used. [Data source: ensembl.org]. (B) Genotype frequency of rs4926 (c.1438G > A) across the whole cohorts. *Indicates the p-value < 0.05.
vs. 14.3%, \( p = 0.002 \)). Neonates with lung disease with A variant had more risk for developing sepsis than their counterparts under homozygote comparison (A/A vs. G/G: OR = 5.19, 95%CI = 1.73-15.6, \( p = 0.002 \)), dominant model (A/G-A/A vs. G/G: OR = 2.39, 95%CI = 1.02-5.58, \( p = 0.041 \)), and recessive model (A/A vs. G/G-A/G: OR = 5.38, 95%CI = 1.86-15.5, \( p < 0.001 \)) (Table 5).

### C1INH Gene Expression Analysis

The C1INH gene was significantly downregulated in neonatal lung disease patients compared to controls (\( p < 0.001 \)) (Figure 2A). Median fold change was \(-1.16\) (interquartile range: \(-2.29\) to \(0.007\)). The ROC analysis revealed an area under the curve (AUC) of 0.73 (95%CI:0.64-0.82, \( p < 0.001 \)) at the cutoff value of \(-0.054\) (72.3% sensitivity and 92.1% specificity). Discrimination ability was enhanced when combined with circulating IL-6 and CRP levels (AUC = 0.926, 95%CI = 0.87-0.97).

In neonatal lung disease patients, patients with A/A genotype exhibited the least expression levels (median: \(-2.86\), IQR: \(-3.55\) to \(-1.71\)) compared to their counterparts (A/G: median: \(-1.01\), IQR: \(-1.49\) to \(-0.28\); G/G: median: \(-0.17\), IQR: \(-1.36\) to \(0.55\); \( p < 0.001 \)) (Figure 2B).

### Association of C1INH Expression With Clinical Features and Outcomes

Neonatal lung disease patients who developed sepsis in the NICU had significantly lower expression of C1INH than those who did not. The median fold change of septic newborns was \(-1.58\) (IQR: \(-3.0\) to \(-0.025\)), while those who did not have sepsis had values of median: \(-0.84\) (IQR: \(-1.59\) to \(0.26\); \( p = 0.011 \)) (Figure 2C). At the threshold of \(-1.28\), C1INH gene expression can differentiate between cohorts with and without subsequent sepsis with 57.7% sensitivity and 64.3% specificity (AUC = 0.652, 95%CI = 0.54-0.76).

Next, patients were categorized according to the optimum log fold change of circulatory level of the C1INH gene into low expression (\( N = 67 \)) and high expression (\( N = 25 \)) subgroups. There was no observed difference between neonates with different gestational age (median = 35 weeks, IQR = 33-36 vs. median = 34 weeks, IQR = 31.5-36, \( p = 0.26 \)) and birth weight (median = 2.01 Kg, IQR = 1.58-2.53 vs. median = 1.8 Kg, IQR = 1.5-2.03, \( p = 0.17 \)). Additionally, there was no effect on duration of mechanical ventilation (median = 3 days, IQR = 2-6.5 vs. median = 6 days, IQR = 3-8, \( p = 0.33 \)), NICU stay (median = 10 days, IQR = 5-16 vs. median = 11 days, IQR = 7-14, \( p = 0.82 \)), and total hospital days (median = 10 days, IQR = 5-16.5 vs. median = 11 days, IQR = 7-14, \( p = 0.80 \)) (Figure 3).

**C1INH**

**Gene-Host Interaction**

The C1INH gene is a key player in the complement system, responsible for the regulation of inflammation and immune response. Its expression levels in neonatal lung disease patients are significantly altered, with a decreased expression in neonates with sepsis compared to controls (\( p < 0.001 \)). This finding supports the role of C1INH in immune regulation and disease susceptibility.

**Clinical Relevance**

The decrease in C1INH expression in neonatal lung disease patients suggests a potential role in the pathogenesis of these disorders. Understanding the underlying mechanisms could lead to targeted interventions and improved outcomes for neonates at risk.

**Future Perspectives**

Further research is needed to elucidate the specific mechanisms linking C1INH expression to neonatal lung disease. This could include investigations into the genetic and environmental factors that influence C1INH expression and their role in disease development.

**Conclusion**

The study highlights the importance of C1INH in neonatal lung disease, emphasizing the need for targeted therapies that can modulate its expression. Improved understanding of the genetic and environmental factors influencing C1INH expression could lead to more effective prevention and treatment strategies for neonatal lung disease.

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**TABLE 3 | Genetic association models for neonatal respiratory distress risk assessment.**

| Model        | Genotype | Controls (n = 45) | Neonates with respiratory distress (n = 94) | Adjusted OR (95%CI) | P-value |
|--------------|----------|------------------|--------------------------------------------|---------------------|---------|
| Codominant   | G/G      | 30 (66.7%)       | 47 (50%)                                   | Reference           |         |
|              | A/G      | 8 (17.8%)        | 18 (19.1%)                                 | 1.52 (0.58-3.97)    | 0.45    |
|              | A/A      | 7 (15.6%)        | 29 (30.9%)                                 | 2.66 (1.03-6.87)    | 0.039   |
| Dominant     | G/G      | 30 (66.7%)       | 47 (50%)                                   | Reference           |         |
|              | A/G-A/G  | 15 (33.3%)       | 47 (50%)                                   | 2.06 (0.98-4.34)    | 0.06    |
| Recessive    | G/G-A/G  | 38 (84.4%)       | 65 (69.2%)                                 | Reference           |         |
|              | A/A      | 7 (15.6%)        | 29 (30.9%)                                 | 2.42 (1.07-6.06)    | 0.047   |
| Over-dominant| G/G-A/A  | 37 (82.2%)       | 76 (80.8%)                                 | Reference           |         |
|              | A/G      | 8 (17.8%)        | 18 (19.1%)                                 | 1.15 (0.46-2.93)    | 0.85    |

Data are presented as numbers and percentages. A two-sided Chi-square test was employed. Adjusted odds ratio (OR) and 95% confidence interval (CI) by sex and gestational age were estimated for different inheritance models using logistic regression analysis. Bold values indicate significance at \( p < 0.05 \).
### TABLE 4 | Association of C1INH polymorphism with clinical and laboratory characteristics of neonatal lung disease cohort.

| Characteristics                  | Levels | G/G (n = 47) | A/G (n = 18) | A/A (n = 29) | P-value |
|----------------------------------|--------|--------------|--------------|--------------|---------|
| **Demographics**                 |        |              |              |              |         |
| Sex                              |        |              |              |              |         |
| Female                           | 22 (46.8) | 7 (38.9)    | 13 (44.8)    | 0.84        |
| Male                             | 25 (53.2) | 11 (61.1)   | 16 (55.2)    |             |
| Consanguinity                    |        |              |              |              |         |
| Positive                         | 5 (10.6) | 0 (0.0)      | 5 (17.2)     | 0.17        |
| **Presentation**                 |        |              |              |              |         |
| Mode of delivery                 |        |              |              |              |         |
| Vaginal                          | 19 (40.4) | 8 (44.4)    | 13 (44.8)    | 0.91        |
| Cesarean                         | 28 (59.6) | 10 (55.6)   | 16 (55.2)    |             |
| Gestational age, weeks           |        |              |              |              |         |
| Median (IQR)                     | 34 (32-36) | 35 (33-36)  | 35 (34-36)   | 0.049       |
| **Birth weight, Kg**             |        |              |              |              |         |
| Median (IQR)                     | 1.68 (1.5-2.06) | 1.96 (1.68-2.5) | 2.2 (1.76-2.6) | 0.006       |
| **Preterm labor pain**           |        |              |              |              |         |
| Positive                         | 9 (19)  | 1 (5.6)      | 9 (31.0)     | 0.14        |
| PROM                             | 11 (24.4) | 6 (33.3)    | 8 (28.0)     | 0.71        |
| Preeclampsia/fits                |        |              |              |              |         |
| Positive                         | 4 (8.5) | 5 (27.7)      | 3 (10.3)     | 0.10        |
| Triple I                         |        |              |              |              |         |
| Positive                         | 5 (10.6) | 1 (5.6)       | 2 (6.9)      | 0.75        |
| **Investigation**                |        |              |              |              |         |
| Laboratory findings              |        |              |              |              |         |
| Positive blood culture           | 13 (27.7) | 5 (27.8)    | 4 (13.8)     | 0.54        |
| WBCs, x10³                       | 7.5 (5.3-12.1) | 7.1 (4.6-13.5) | 8.3 (5.0-13.3) | 0.83        |
| Platelets, x10³                  | 184 (104-264) | 211 (161-321) | 271 (134-328) | 0.09        |
| Hemoglobin, g/dL                 | 14.9 (12.4-16.3) | 12.9 (12.3-14.9) | 12.4 (9.2-14.7) | 0.002       |
| CRP, mg/L                        | 4.6 (2.0-60.1) | 7.0 (2.7-60.0) | 39.6 (16-61.1) | 0.21        |
| IL6-fold change                  | 2.54 (1.7-3.2) | 3.08 (2.1-3.5) | 2.69 (2.3-3.1) | 0.42        |
| **Chest X ray**                  |        |              |              |              |         |
| Grade 2                          | 6 (12.8) | 6 (33.3)      | 6 (20.7)     | 0.33        |
| Grade 3                          | 4 (8.5) | 2 (11.1)      | 5 (17.2)     | 0.35        |
| **Management**                   |        |              |              |              |         |
| Maternal medications             |        |              |              |              |         |
| Antepartum steroids              | 11 (23.4) | 6 (33.3)    | 6 (20.7)     | 0.60        |
| Ventilation                      | 11 (23.4) | 4 (22.2)    | 8 (27.6)     | 0.84        |
| Invasive MV (SIMV)               | 6 (12.8) | 3 (18.7)     | 4 (13.8)     | 0.79        |
| Surfactant                       | 6 (12.8) | 1 (5.6)       | 2 (6.9)      | 0.56        |
| **Outcomes**                     |        |              |              |              |         |
| Duration, days                   |        |              |              |              |         |
| Ventilation                      | 4.5 (2.0-7.0) | 3.0 (1.0-6.0) | 4.0 (2.0-7.0) | 0.58        |
| NICU                             | 9.0 (5.0-12.0) | 10 (7.0-17.5) | 13 (9.0-17.3) | 0.12        |
| Total length of stay             | 9.0 (5.0-13.0) | 10 (7.0-17.0) | 13 (9.0-17.0) | 0.04        |
| Time to death                    | 9.0 (4.5-14.5) | 9.5 (4.5-17.5) | 12.5 (7.0-17.3) | 0.04        |
| Sepsis                           | 26 (55.3) | 10 (55.6)    | 6 (20.7)     | 0.008       |
| Positive                         | 21 (44.7) | 8 (44.4)     | 23 (79.3)    |             |
| Survival                         | 31 (66.0) | 12 (66.7)    | 14 (48.3)    | 0.26        |
| Died                             | 16 (34.0) | 6 (33.3)     | 15 (51.7)    |             |

Data are presented as the median and interquartile range (IQR). PROM, premature rupture of membrane; WBC, white blood cells; CRP, C-reactive protein; IL6, interleukin 6; MV, mandatory ventilation; CPAP, continuous positive airway pressure; SIMV, synchronized intermittent mandatory ventilation; NICU, neonatal intensive care unit. Two-sided Chi-Square and Kruskal-Wallis tests were used. Bold values indicate significance at p < 0.05.

### TABLE 5 | Genetic association models for risk of developing sepsis in neonatal lung disease cohort.

| Model          | Genotype | No sepsis (n = 42) | Sepsis (n = 52) | Adjusted OR (95%CI) | P-value |
|----------------|----------|--------------------|----------------|---------------------|---------|
| **Codominant** | G/G      | 26 (61.9%)         | 21 (40.4%)     | Reference           |         |
|                | A/G      | 10 (23.8%)         | 8 (15.4%)      | 0.87 (0.28-2.69)    | 0.98    |
|                | A/A      | 6 (14.3%)          | 23 (44.2%)     | 5.19 (1.73-15.61)   | 0.002   |
| **Dominant**   | G/G      | 26 (61.9%)         | 21 (40.4%)     | Reference           |         |
|                | A/G-A/A  | 16 (38.1%)         | 31 (59.6%)     | 2.39 (1.02-5.58)    | 0.041   |
|                | G/G-A/G  | 36 (85.7%)         | 29 (55.8%)     | Reference           |         |
| **Recessive**  | G/G-A/A  | 36 (85.7%)         | 29 (55.8%)     | Reference           |         |
|                | A/A      | 6 (14.3%)          | 23 (44.2%)     | 5.38 (1.86-15.55)   | <0.001  |
| **Over-dominant** | G/G-A/A | 32 (76.2%)         | 44 (84.6%)     | Reference           |         |
|                | A/G      | 10 (23.8%)         | 8 (15.4%)      | 0.51 (0.17-1.47)    | 0.21    |

Data are presented as numbers and percentages. A two-sided Chi-square test was employed. Adjusted odds ratio (OR) and 95% confidence interval (CI) by sex and gestational age were estimated for different inheritance models using logistic regression analysis. Bold values indicate significance at p < 0.05.
interactions are implicated in the morbidity/mortality associated with this disorder (17, 32–34). In this study, we identified for the first time, up to the authors’ knowledge, the C1INH rs4926G>A (Val480Met) variant implication in neonatal lung disease risk and outcome in the population under the study. The A/A genotype carriers were two times more likely to develop neonatal lung disease under homozygote and recessive genetic models. Also, a higher frequency of the rs4926* A/A genotype was associated with sepsis development in the neonatal lung disease cohort and considered an independent predictor risk factor for neonatal sepsis by regression analysis.

Although this variant is classified as one of the C1INH benign variants (https://www.ncbi.nlm.nih.gov/clinvar/variation/254786/) and yielded conflicting results with age-related macular degeneration (35), interestingly, it showed a trend of association with susceptibility to staphylococcal aureus colonization of the nose (36). The latter researchers found an increased prevalence of the G/G genotype in noncarriers than persistent carriers.

Despite previously no differences between the carriers of wild (Val 480) and the mutant (Met 480) C1INH protein were observed concerning “structure, protein stability, serum concentration or inhibition of C1INH protein” (37), however, in the current study, we found a significant association of the studied variant with circulating mRNA transcript levels. In the neonatal lung disease cohort, neonates with the A/A genotype exhibited the least expression levels of C1INH mRNA compared to their counterparts (G/G and G/A). This interesting finding is congruent with that the rs4926 variant is identified as one of the cis-expression quantitative trait locus (cis-eQTL) for the C1INH gene (https://www.eqtlgen.org/cis-eqtls.html) (38). This indicates that the specified variant could partly explain the RNA expression variation across conditions (39).

C1INH is a highly glycosylated plasma serine-protease inhibitor (105 KDa) and is considered an acute-phase protein that targets several physiological pathways, including the complement activation, blood coagulation (factor XIIa), platelet degranulation, fibrinolysis, and the contact system protease kallikrein (https://www.uniprot.org/uniprot/P05155). Recently, C1INH has been implicated in neutralizing the extracellular histones (40), which are considered as significant components of
“damage-associated molecular patterns,” released after cell death, in particular, from the inflammatory cells such as neutrophils during the “neutrophil extracellular trap; NET” process (41). Previous reports confirmed the inflammatory role that the released extracellular histones play in several disorders, including sepsis, acute lung injury, and acute respiratory distress syndrome (40, 42, 43).

Dependent on the C1INH carbohydrate structure (associated with an excessive negative charge), the protease inhibition capability, endotoxin scavenging property, and leukocyte rolling inhibition, C1INH could be a promising protective biomolecule that prevents the deleterious effects of exaggerated inflammation in acute respiratory distress (40). As illustrated in Figure 6, the administration of C1INH therapeutic agents is predicted to ameliorate the respiratory distress via several molecular pathways, such as inhibition of the classical complement enzymes (C1s), the activated factors XIIa/XIIa, plasma kallikrein, plasmin, tissue-type plasminogen activator (tPA) and thrombin (44). C1INH inhibits the lectin pathway of complement activation via inactivation of “mannan-binding lectin-associated serine protease-1 and−2 (MASP1 and MASP2)” and inhibits the alternative pathway of activation by binding to C3b. In this sense, C1-INH is a significant regulator of all three pathways of complement activation (45). Given the heavily glycosylated structure of C1INH and bearing a “sialyl Lewis-related moiety” through which it binds to endothelial cell-surface selectins E and P, in competition with white blood cells binding, it can exert its anti-inflammatory function (46). These data could support the current study observations of decreased C1INH circulatory transcript levels in neonatal lung disease cases (with a subsequent decline in its protective roles) compared to healthy neonates and could explain, in part, the association of the risky allele genotype (AA) carriers with less circulatory gene transcript expression levels and increased susceptibility to be complicated by sepsis. Due to limited blood sampling of the enrolled neonates, the C1INH protein levels assessment was not feasible to correlate with the circulating transcript expression. Hence, further replication studies that quantify both C1INH protein and transcript levels are required to confirm the study findings.

The ROC analysis revealed a good area under the curve for circulatory C1INH to discriminate neonatal lung disease group from healthy neonates, and this discriminative ability was enhanced when combined with the traditional circulatory marker levels of IL-6 and CRP. This finding confirms that no single biomarker can predict outcome in acute respiratory distress patients, as several different pathways are involved in disease development (47). The acute phase reactant CRP was extensively studied in several contexts, including neonatal lung disease and neonatal sepsis, and it remains the preferred biomarker in many NICUs (48, 49). Also, the pleiotropic pro-inflammatory cytokine IL-6 exhibited a prolonged response to the pathogenic challenge, on several occasions, compared to other cytokines (50, 51). It was implicated in multiple biological processes, including T cell maturation/differentiation, cell survival, apoptosis, and inflammation (52). Taken together, we can conclude that C1INH exp could be an ancillary test with the currently used biomarkers like IL6 and CRP based on our results.

It is worth noting that the authors referred to some studies related to acute respiratory distress cases in the discussion section (as there was a limitation of available literature that explore the
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FIGURE 5 | Predictors for mortality in neonatal lung disease patients. (A) Kaplan-Meier curve analysis shows C1INH gene polymorphism rs4926 (G/A) with neonatal mortality. Log Rank (Mantel-Cox) test was used for comparison. (B) Kaplan-Meier curve analysis showing the association of circulatory C1INH gene expression with neonatal mortality. Patients were categorized according to the optimum log fold change of circulatory level of the C1INH gene of $-0.054$ into low expression ($n = 67$) and high levels ($n = 25$). (C) Cox proportional hazards regression analysis revealed independent risk factors for neonatal mortality in neonatal lung disease patients. Hazard ratio and 95% confidence interval (CI) are reported. Correlation analysis was performed to exclude highly correlated variables from the model (absolute correlation coefficient of $>0.5$).

| Predictor risk factors               | Hazard ratio [95%CI] | p-value |
|--------------------------------------|----------------------|---------|
| Male vs female                       | 1.34 [ 0.55, 3.29]   | 0.52    |
| Cesarean vs vaginal delivery         | 1.47 [ 0.70, 3.08]   | 0.30    |
| Gestational age                      | 0.64 [ 0.53, 0.78]   | <0.001  |
| PROM vs none                         | 3.23 [ 1.32, 7.90]   | 0.010   |
| Triple I vs none                     | 1.22 [ 0.33, 4.58]   | 0.76    |
| CRP level                            | 1.01 [ 1.00, 1.02]   | 0.23    |
| IL6 expression                       | 1.20 [ 0.89, 1.61]   | 0.22    |
| C1INH expression                     | 1.05 [ 0.78, 1.42]   | 0.73    |
| A/G vs G/G genotype                  | 0.54 [ 0.16, 1.78]   | 0.31    |
| A/A vs G/G genotype                  | 1.48 [ 0.67, 3.26]   | 0.33    |

Despite the preventive and therapeutic measurements followed in our NICU, there was a high rate of late-onset sepsis in our cohort. It is a challenge that the pediatricians face in our local NICU and can be attributed to several factors, including low resources affecting the availability of personal protective equipment, more strains of antibiotic-resistance bacteria, and lack of Group B Strep (GBS) screening of mothers. This concern has been raised by Amer et al. as "one of the unfinished agenda in developing countries" (53). It is also of importance to emphasize the Molloy et al. notion in that "the lack of the majority of clinical signs/symptoms complicate the identification of sepsis on the..."
neonate. The difference in the NICU is that it may be difficult to differentiate contamination from true infection, and the long-term impact of these infections is greater on the developing brain. Therefore, a consensus definition is required that can be universally generalizable and validated in international datasets and correlated with neurodevelopmental outcomes (54).

The main limitations of this study are the relatively small sample sizes, the lack of simultaneous investigation of other possible SNPs that could influence the neonatal lung disease risk and outcome, and the characteristics of the control group. Furthermore, due to the small volume of blood was available in this work, the authors could only investigate the SERPING1 transcript expression. It would be helpful to demonstrate also the SERPING1 protein level in future studies as “the mRNA level may not always reflect the corresponding protein expression due to the posttranscriptional processing” (45). However, our findings highlight the potential role of the C1INH rs4926 variant in increased neonatal lung disease risk and prediction of sepsis development in this type of preterm infants cohort. Also, this variant could impact gene expression, which supports its role in neonatal lung disease etiopathology. Nevertheless, additional more extensive genomic and functional studies are required to confirm the study conclusions.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Suez Canal University, Faculty of Medicine, Ismailia, Egypt (Approval No. 3435). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2022.779511/full#supplementary-material

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