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Diagnostic and prognostic roles of peripheral blood Toll-like receptor-4 and stanniocalcin-1 genes expression in acute lung injury

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ABSTRACT

Acute lung injury (ALI) is an acute inflammatory disorder. Toll-like receptor-4 (TLR-4) and Stanniocalcin -1 (STC-1) had roles in lung endothelial protection. This study aims to assess TLR-4 and SCT-1 genes expressions in peripheral blood of ALI patients. Total RNA was extracted from peripheral blood of 48 subjects (20 healthy controls, 28 ALI patients) and expressions of genes were assessed by real-Time qRT-PCR. The expression levels of TLR-4 and SCT-1 genes were significantly lower in ALI patients compared to controls ($P < 0.0001$). After 10 days, the expression levels of TLR-4 and SCT-1 were increased compared to their baseline levels ($p = 0.012$ and 0.024, respectively). SCT-1 has 92.9% sensitivity and 100% specificity in ALI detection. SCT-1 gene expression was negatively correlated with severity score ($r = -0.54$, $p = 0.003$). The mortality pattern was higher in ALI patients with lower TLR-4 gene expression ($p = 0.014$). In conclusion, the peripheral blood expressions of TLR-4 and STC-1 genes were decreased in ALI patients. Both genes expressions were increased with patients' recovery. SCT-1 had higher sensitivity and specificity in ALI diagnosis. The peripheral blood expressions of SCT-1 and TLR-4 genes seem to be diagnostic and prognostic markers in ALI.

1. Introduction

Acute lung injury (ALI) is an acute inflammatory disorder caused by damage to the alveolar-capillary wall which leads to increased capillaries permeability and pulmonary edema (Guo et al., 2019). ALI associated with neutrophils recruitment, and the release of pro-inflammatory mediators (Matthay and Zimmerman, 2005). ALI is presented with hypoxemia, hypercapnia, and bilateral pulmonary infiltrates in the absence of left atrial hypertension evidence. ALI is a serious disorder which causes a high mortality rate in critically ill patients (Ragaller and Richter, 2010).

Oxidant-induced endothelial injury plays a major role in the pathogenesis of ALI and the subsequent respiratory failure. Reactive oxygen species and nitric oxide species may cause pulmonary vascular endothelial damage (Perl et al., 2011). Also, the decreased antioxidants levels were stated with the increased levels of the reactive oxygen species (Ciencewicki et al., 2008).

Stanniocalcin (STC) is a calcium-regulating glycoprotein hormone that was first described in the bony fish (Wagner et al., 1986). Two stanniocalcin genes (STC-1 and STC-2) were identified (Sheikh-Hamad, 2010). The mammalian STC-1 has roles in many developmental, physiological and pathological processes. It is expressed in the brain, lung, heart, and other tissues (Itoa et al., 2014). STC-1 is expressed in brain and heart tissues as a response to hypoxia; it induces ischemic tolerance by reducing the inflammatory response and the subsequent apoptosis (Tang et al., 2014). STC-1 has been proved to suppress superoxide production in macrophages, oppose the action of proinflammatory cytokines on lung endothelium, and reduce migration of leukocytes and macromolecules (Sheikh-Hamad, 2010).

Toll-like receptor (TLR) is a family of pattern recognition receptors which are activated upon recognition of a wide variety of microbial and tissue-derived molecules (Medzhitov, 2001). The protective role of TLR4 against oxidant-induced lung injury and hypoxic damage has been reported (Takyar et al., 2016). The antioxidant and antiapoptotic functions of TLR-4 signaling in the endothelium are dependent on endogenous soluble ligands. However, the downstream effectors' mechanisms are under thorough investigations (Kim et al., 2019).

The relationship between TLR-4 and STC-1 in lung injury was

Abbreviations: ALI, acute lung injury; AUC, area under the curve; LISS, lung injury severity score; ROC, receiver operating characteristics; SCT, stanniocalcin; TLR, Toll-like receptor

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studied, both in vitro and in vivo. TLR-4 regulates STC-1 role in lung endothelial protection (Zhang et al., 2019). So, the aim of the current study is to identify specific inflammatory parameters that contribute to ALI and to delineate the relation between TLR-4 and STC-1 genes expression in peripheral blood of ALI patients. Also, the study aims to evaluate their diagnostic values in predicting ALI and their prognostic values in assessing the severity and outcome of ALI.

2. Subjects and methods

2.1. Study design

This study is a case-control study, carried out in the RICU of the Chest Department and Clinical Pathology Department, Faculty of Medicine, Zagazig University during the period from July 2017 to February 2019. The study protocol was approved by the Zagazig University Institutional Review Board. Written informed consent was signed either by the patients or their first degree relatives before inclusion in this study.

2.2. Subjects

This study included 20 healthy controls and 28 ALI patients. Controls group is age and sex-matched to the patients. They were 17 males and 3 females. Their age ranged between 38 and 60 years with a median of 47.5 years. Patients were included within the first 24 h of the onset of ALI. Patients presented with a partial pressure of arterial oxygen/fraction of inspired oxygen ratio (PaO2/FIO2) between 200 and 300 mmHg. Pregnancy, leucopenia, corticosteroid use within 2 weeks before inclusion and immunosuppressive therapy within the last month, were excluded from this study.

2.3. Study protocol

The first point of the study was on the 1 st day of patient RICU admission, ALI patients were ventilated. The severity of lung injury was evaluated by the estimation of the Lung Injury Severity Score (LISS) (Murray et al., 1988). Samples from patients and controls for TLR-4 and SCT-1 expression levels assessment were collected. The second point of the study was on the 10th day. Respiratory parameters of the survived patients were collected. Another sample was collected from survived patients and controls for genes expression evaluation. The primary outcome was the mortality. The secondary clinical outcome was the assessment of respiratory and ventilator parameters (Fig. 1).

2.4. Sample collection

Samples were collected at 2 time points; the 1st-day of patient RICU admission which represents the time of ALI diagnosis and the 10th-day, that is, the time after acute inflammatory phase subsides (Huang et al., 2017; Cheung, et al., 2018). Samples were collected from the peripheral blood of patients and controls (2 ml) in BD Vacutainer® EDTA tubes. Samples were processed within 2 h.

2.5. RNA isolation, cDNA preparation

The Total RNA Purification kit **(Jena Bioscience, Germany)** was used to extract total RNA from peripheral blood cells according to the manufacturer's protocol. The production of RNA assessed spectrophotometrically at 260 and 280 nm. The ratio of optical density (OD 260/ 280) is measured to ensure the quality of RNA using Nanodrop 2000 spectrophotometer **(ThermoScientific)**.

The SCRIPT Reverse Transcriptase kit **(Jena Bioscience, Germany)** which uses Moloney murine leukemia virus reverse transcription enzyme. Oligo $(dT)_{15}$ primer **(Jena Bioscience, Germany)** was selected for RNA reverse transcription. RNA was reverse-transcribed on ice in 20 µl mixture containing: 1.5 µl of RNase-free water, 10 µl of total RNA and 1 µl of primer were mixed together. Then, 4 µl of SCRIPT RT buffer, 1 µl of dNTP Mix, 1 µl of RNase inhibitor, 1 µl of Dithiothreitol stock solution and 0.5 µl SCRIPT reverse transcriptase were mixed. The reverse transcription mixture was incubated at 30 °C for 10 min then at 50 °C for 60 min. The cDNA stored at −80 °C until analysis.

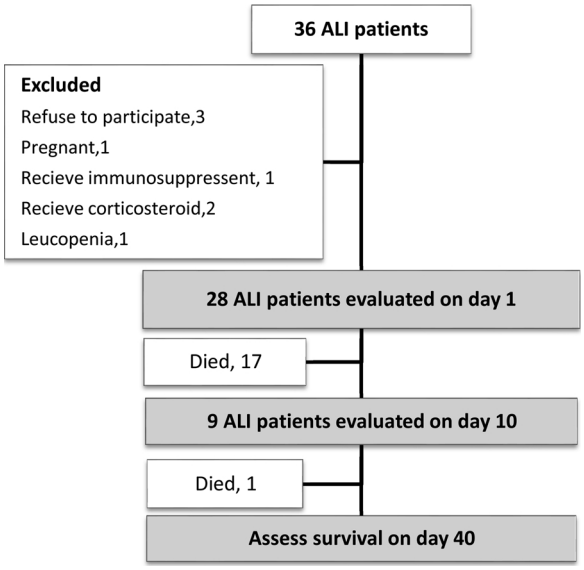

Fig. 1. Flow chart of the study. Day 1 is the day of inclusion.

Table 1
Baseline patient's demographics, clinical characteristicsand outcome.

Parameters	ALI (No. : 28)
Age	45.5 [19 – 72]
Sex (Male/Female)	23/5 (82.1/17.9)
Cause of ALI	
Pneumonia	17 (60.7)
Extra-pulmonary infection	1 (3.6)
Miscellaneous	10 (35.7)
Co-morbidities	
Diabetes	6 (21.4)
Hypertension	5 (17.9)
Cardiac problems	2 (7.14)
Liver cirrhosis	1 (3.57)
Chronic renal impairment	1 (3.57)
Respiratory parameters and ventilator settings	
Tidal volume (mL/ kg PBW)	6.3 [6.19 – 6.5]
Respiratory rate (c/min)	25 [22 – 29.25]
Total PEEP (cmH2O)	9.5 [8.75 – 11]
Plateau pressure (cmH2O)	12.5 [10 – 15]
Tidal compliance (mL/ cmH2O)	33.5 [28.75 – 39]
FiO2	0.4 [0.4 – 0.42]
PaO2/FiO2	220 [207.32 – 240]
PaCO2 (mmHg)	44 [40 – 45]
PH	7.28 [7.26– 7.32]
LISS	2 [2 – 2.4]
ICU stay	5 [3 – 10.25]
Mortality within 40 days	20 (71.4)

Data are expressed as median [Interquartile range] or number (%).
ALI = acute lung injury; PBW: predicted body weight; PEEP: Positive end expiratory pressure; PaO2/FiO2: Partial pressure of arterial oxygen to fraction of inspired oxygen; LISS: Lung Injury Severity Score.
Plateau pressure was measured during a 1 s end-inspiratory pause.
Total PEEP: total positive end-expiratory pressure was measured during a 5 s end-expiratory pause.

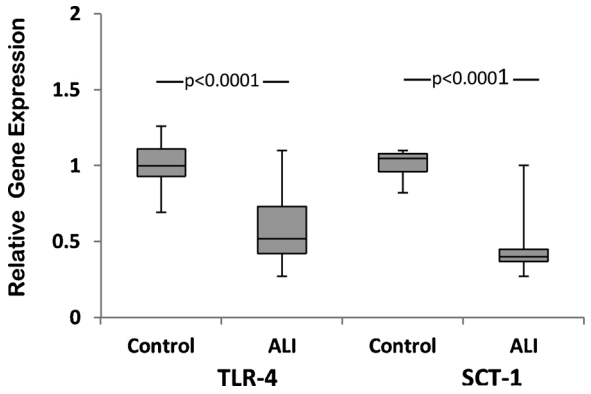

Fig. 2. A: Box and whisker plots of baseline TLR-4 & STC-1 relative gene expression Levels in ALI group compared to controls group.

2.6. Real-Time qRT-PCR (Quantitative Reverse Transcription-PCR)

Detection of TLR-4 and SCT-1 mRNA was performed by real-Time qRT-PCR.on Stratagene Mx3005 P qPCR System **(Agilent Technologies, Germany)**. The transcription levels of TLR-4 and SCT-1 genes were normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) which used as reference gene. The primers are specific for TLR-4, SCT-1, and GAPDH that obtained from the BLAST program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Primers were synthesized by **(Metabion international AG)** as follows:

TLR-4 (Forward primer: 5′-ATATTGACAGGAAACCCCATCCA-3′ and Reverse primer: 5′-AGAGAGATTGAGTAGGGGCATTT-3′)

SCT-1 (Forward primer: 5′- ACCAAGGCTGCTCTGTTTCT-3′ and Reverse primer: 5′- GGTAAGGAGTGGCATTTCTA-3′)

GAPDH (Forward primer: 5′- GAGCCACATCGCTCAGACAC-3′ and Reverse primer:5′-CATGTAGTTGAGGTCAATGAAGG -3′)

PCR reaction of 20 µl volume was prepared by mixing 10 µl of qPCR Green Master **(Jena Bioscience, Germany)**, 0.5 µl of the forward primer (10 µM), 0.5 µl of the reverse primer (10 µM), 5 µl of the template cDNA and 4 µl of PCR grade water into Real-Time qRT-PCR wells. The temperature conditions were initial 95 °C for 10 min then 40 cycles of (95 °C for 30 s, 58 °C for 1 min). The target gene fold change which represents the relative expression of the target gene was calculated as $2^{-\Delta\Delta CT}$ (Livak and Schmittgen, 2001).

2.7. Statistical analysis

The Epi Info program 6 (Atlanta, Georgia, USA) was used to calculate the required sample size. The sample size for unmatched case-control study option was utilized. This study included 20 healthy controls and 28 ALI patients to detect a 0.75–fold difference in genes expression with a ratio of cases to controls of 1:0.7. The Mortality rate reduction was of 76% between low and high genes expression group (Máca et al., 2017). The sample size calculation criteria used were 95% confidence limit and 95% power. This study data were non-normally distributed (Shapiro-Wilk approach). Non-normally distributed variables were presented as median values and interquartile range while as numbers and percentage for categorical ones. The Wilcoxon signed-rank test was used to compare related quantitative variables and the Mann Whitney U test for unrelated ones. Receiver operating characteristics (ROC) curve analysis was performed to evaluate the discriminate role of genes expression and the optimum cutoff values in predicting ALI. The area under the curve (AUC) and its 95% CI were used to studies their diagnostic efficiencies. Spearman's correlation was used to evaluate the association of the variables. Kaplan–Meier survival function was generated to explore the survival pattern and the log-rank test was used to evaluate the significance. A p-value below 0.05 was considered as the cutoff for significance. Statistics analysis was performed using SPSS 17 software **(SPSS Inc., IL, USA)**.

3. Results

The demographic, clinical characteristics and respiratory parameters were presented. As well as, the disease severity assessment and the outcome measures of the ALI patients were described (Table 1).

On day 1, the peripheral blood expression levels of TLR-4 and SCT-1 genes were significantly lower in patients with ALI compared to controls *($P < 0.0001$)* (Fig. 2). The ROC curve analysis showed significant predictive value *($p < 0.0001$)* for peripheral blood TLR-4 and SCT-1 expression to differentiate between the presence and absence of ALI (Fig. 3). The diagnostic performance criteria of TLR-4 and SCT-1 gene expression in the peripheral blood were demonstrated in Table 2. SCT-1 has higher sensitivity and specificity in ALI detection.

Spearman correlation study showed a significant positive linear relationship between the baseline TLR-4 and SCT-1 genes expression in the peripheral blood *($r = 0.67$, $p < 0.0001$)*. A significant negative correlation between SCT-1 gene expression and LISS was observed *($r = -0.54$, $p = 0.003$)*. But TLR-4 gene expression showed an insignificant

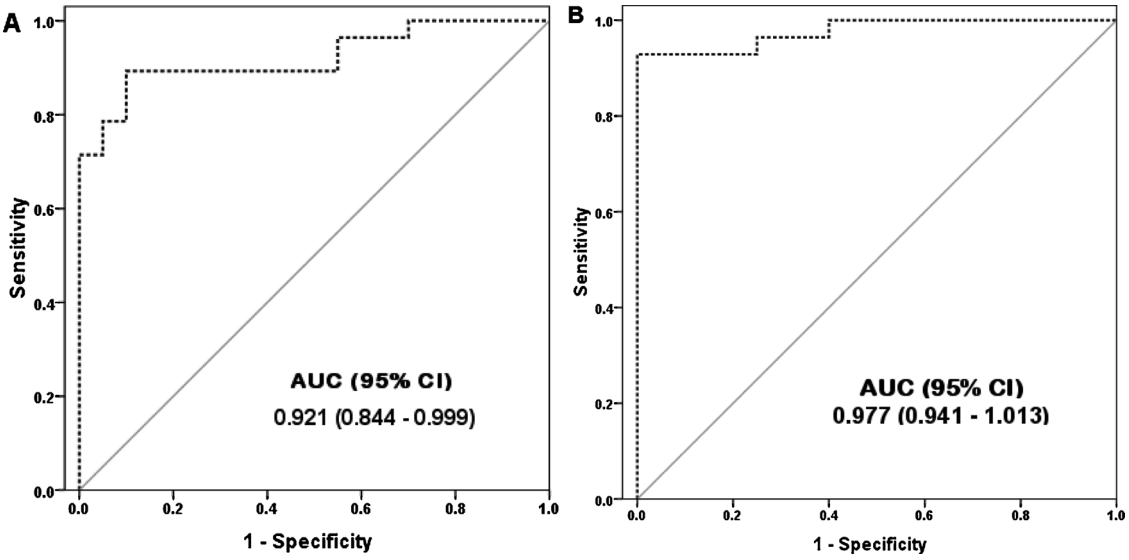

Fig. 3. ROC curve (A) TLR-4 (B) SCT-1 for the presence and absence of ALI.

Table 2
Performance characteristics of TLR-4 and SCT-1 in ALI diagnosis.

Parameters	TLR-4	SCT-1
Cutoff (fold change)	< 0.91	< 0.81
Sensitivity	85.7%	92.9%
Specificity	90%	100%
PPV	92.3%	100%
NPV	81.8%	90.9%
Accuracy	87.5%	95.8%

TLR: Toll-like receptor; STC: Stanniocalcin; PPV: Positive predictive value; NPV: Negative predictive value.

Table 3
Respiratory parameters, ventilator settings and genes expression in survived patients.

Parameters	ALI day 1 (No. = 9)	ALI day 10 (No. = 9)	p
Tidal volume (mL/ kg PBW)	6.5 [6.22 – 6.5]	7.1 [7.1 – 7.3]	0.008*
Respiratory rate (c/min)	25 [25 – 28]	18 [17 – 18]	0.008*
Total PEEP (cmH2O)	8 [6 –11]	6 [5 – 6]	0.007*
Plateau pressure (cmH2O)	10 [10 – 13]	20 [20 – 21]	0.008*
Tidal compliance (mL/ cmH2O)	38 [32 – 44]	45 [41 – 45]	0.008*
FiO2	0.4 [0.4 – 0.41]	0.3 [0.24 – 0.26]	0.008*
PaO2/FiO2	240 [220 – 245]	37.69 [346.2 – 404.2]	0.008*
PaCO2 (mmHg)	40 [40 – 41]	36 [35 – 38]	0.007*
PH	7.32 [7.32 – 7.33]	7.43 [7.42 – 7.44]	0.011*
TLR-4	0.9 [0.8 – 1.1]	1 [0.85 – 1.1]	0.012*
STC-1	0.5 [0.43 – 0.8]	0.9 [0.8 – 0.94]	0.024*

Data are expressed as median [Interquartile range].
ALI = acute lung injury; PBW: predicted body weight; PEEP: Positive end expiratory pressure; PaO2/FiO2: Partial pressure of arterial oxygen to fraction of inspired oxygen; TLR: Toll-like receptor; STC: Stanniocalcin.
Plateau pressure was measured during a 1 s end-inspiratory pause.
Total PEEP: total positive end-expiratory pressure was measured during a 5 s end-expiratory pause.

negative correlation *(r= −0.34, p = 0.08)*.

On day 10, ALI patients had a significant improvement in their respiratory parameters. Peripheral blood expression levels of TLR-4 and SCT-1 were increased in ALI patients compared to their levels of the 1st day *(p = 0.012 and 0.024, respectively)* (Table 3).

Survival analysis illustrated in Fig. 4. The mortality pattern was higher in ALI patients with low levels of TLR-4 gene expression compared to patients with high levels of TLR-4 *(p = 0.014)*. SCT-1 gene expression levels were not related to the survival pattern *(p = 0.056)*.

4. Discussion

ALI is a life-threatening disorder; its clinical presentations are hypoxemia and bilateral lung infiltrates (Chambers et al., 2018). Apoptosis of different types of alveolar cells occurs during ALI. Lung endothelial integrity and survival are important determinants of the severity of lung Injury (Kawasaki et al., 2000).

In humans, the STC-1 gene is widely expressed in multiple tissues. SCT-1 regulates the immune responses as it enhances cell survival and inhibits inflammatory processes. (Ohkouchi S et al, 2012). STC-1 inhibits the activated innate immunity and also has antioxidant effects (Zhang et al., 2019). TLR-4 regulates the acute inflammatory signal transduction pathways and the inflammatory cytokines production (Zhou et al., 2011). TLR-4 protects lung structural cells against hyperoxic damage (Zhang et al., 2013) and plays a role in maintaining lung integrity as its expression was decreased in human COPD lungs (Lee et al., 2012; Speleta et al., 2009).

Many animal models studies have shown that TLR-4 involved in the pathogenesis of ALI (Hoth et al., 2009; Villar et al., 2010; Liu et al., 2014). SCT-1 expression in the animal's lung tissue was regulated by TLR-4. SCT-1 has roles in pulmonary and endothelial protection against oxidant-induced lung injury (Zhang et al., 2019). So, this study aims to delineate the relation between TLR-4 and STC1 genes expression in peripheral blood of ALI patients.

This study showed that the baseline peripheral blood expression levels of TLR-4 and SCT-1 genes were significantly lower in patients with ALI compared to controls. The down expression of TLR-4 in blood mononuclear cells of ALI patients was also observed (Ramírez et al., 2004). This was consistent with Zhang et al. (2019) who detected lower expression levels of STC-1 and TLR-4 in the murine lung endothelial cells. The diagnostic performance criteria of peripheral blood TLR-4 and SCT-1 expression in the prediction of ALI were evaluated. The peripheral blood SCT-1 expression has higher sensitivity and specificity in ALI detection.

This study showed a significant positive linear relationship between TLR-4 and SCT-1 genes expression in peripheral blood. This was in

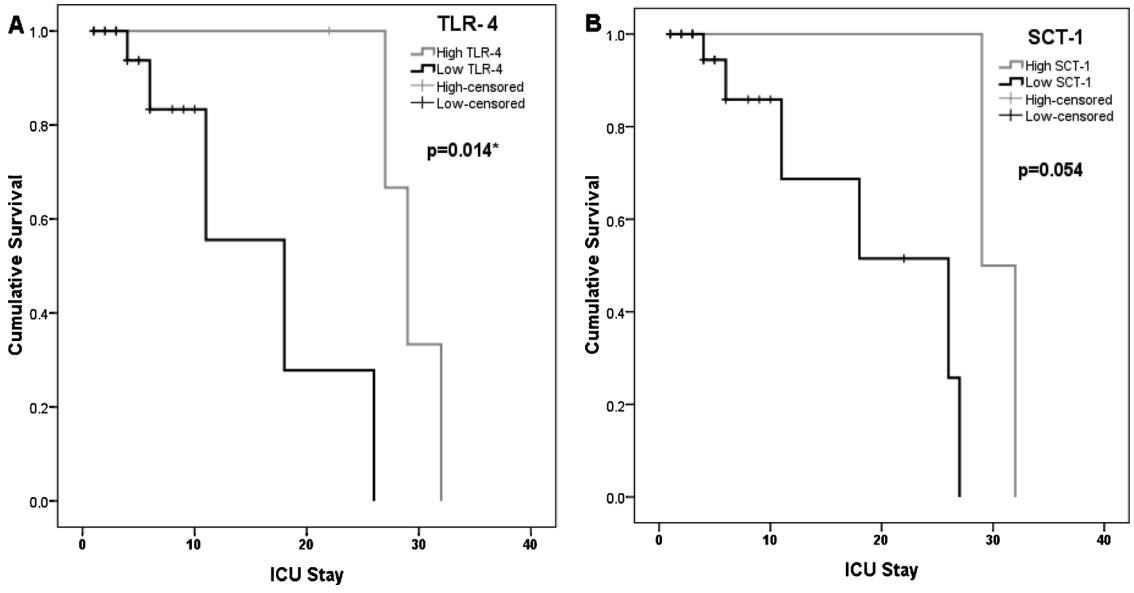

Fig. 4. Kaplan–Meier curve showed cumulative survival curve of patients relative to genes expression (A) TLR-4 and (B) SCT-1.

accordance with Zhang et al. (2019) who reported that STC-1 expression was regulated by TLR4. The SCT-1 gene expression, not the TLR-4 gene showed a significant negative correlation with LISS. This finding supports the findings of Ohkouchi et al. (2015) who found that inhalation of STC-1 reduce the severity of lung injury and promote recovery by decreasing the intracellular Reactive oxygen species.

The ALI patients' assessment on day 10 showed a significant improvement in their respiratory parameters. Peripheral blood expression levels of TLR-4 and SCT-1 were significantly higher compared to the baseline levels. This is in accordance with Liu et al. (2017) found that peripheral blood cells expressed TLR-4 increased significantly in the survival of ALI patients than the death group on day 3 of the study. TLR-4 over-expression causes a significant increase in lung cells survival (Jiang et al., 2015). Also, Kim et al. (2019) found that TLR-4 keep lung homeostasis.

In this study, survival analysis demonstrated that the mortality pattern was higher in ALI patients with low TLR-4 gene expression levels compared to patients with high levels of TLR-4. In agreement with Ramírez et al. (2004) who revealed that TLR-4 expression dysregulation is a prognostic factor for ALI patient. Takyar et al. (2016) found that lung endothelial cells expressed TLR-4 increased mice survival by 30% reduction of apoptosis and lung injury.

ALI is a serious disorder therefore important to investigate its diagnostic and prognostic markers. This may help in designing new treatment strategies for ALI. So, further studies on a large number of participants are recommended to confirm the findings of this study.

5. Conclusions

The peripheral blood expressions of TLR-4 and STC-1 genes were decreased in ALI patients. Both genes expressions were increased with patients' recovery. SCT-1 had higher sensitivity and specificity in ALI diagnosis. The peripheral blood expressions of SCT-1 and TLR-4 genes seem to be diagnostic and prognostic markers in ALI.

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Declaration of Competing Interest

None.

References

Chambers, E., Rounds, S., Lu, Q., 2018. Pulmonary endothelial cell apoptosis in emphysema and acute lung injury. Adv. Anat. Embryol. Cell Biol. 228, 63–86. https://doi.org/10.1007/978-3-319-68483-3_4.

Cheung, O.Y., Graziano, P., Smith, M.L., 2018. 6- acute lung injury. In: Leslie, Kevin O., Wick, Mark R. (Eds.), Practical Pulmonary Pathology: A Diagnostic Approach, 3rd ed. Elsevier, pp. 125–146. https://doi.org/10.1016/B978-0-323-44284-8.00006-5. ISBN 9780323442848.

Ciencewicki, J., Trivedi, S., Kleeberger, S.R., 2008. Oxidants and the pathogenesis of lung diseases. J. Allergy Clin. Immunol. 122 (3), 456–470. https://doi.org/10.1016/j.jaci.2008.08.004.

Guo, C., Wu, T., Zhu, H., Gao, L., 2019. Aquaporin 4 blockade attenuates acute lung InjuryThrough inhibition of Th17 cell proliferation in mice. Inflammation 2019. https://doi.org/10.1007/s10753-019-01002-4.

Hoth, J.J., Wells, J.D., Brownlee, N.A., 2009. Hiltbold EM, Meredith JW, McCall CE, Yoza BK. Toll-like receptor 4 dependent responses to lung injury in a murine model of pulmonary contusion. Shock 31, 376–381. https://doi.org/10.1097/SHK.0b013e3181862279.

Huang, S.R., Ma, A.Y., Liu, Y., Qu, Y., 2017. Effects of inflammatory factors including plasma tumor necrosis factor-α in the clinical treatment of acute respiratory distress syndrome. Oncol. Lett. 13 (6), 5016–5020. https://doi.org/10.3892/ol.2017.6090.

Itoa, Y., Zemans, R., Correll, K., Yang, I.V., Ahmad, A., Gao, B., Mason, R.J., 2014. Stanniocalcin-1 is induced by hypoxia inducible factor in rat alveolar epithelial cells. Biochem. Biophys. Res. Commun. 452 (4), 1091–1097. https://doi.org/10.1016/j.bbrc.2014.09.060. 3.

Jiang, D., Liang, J., Fan, J., Yu, S., Chen, S., Luo, Y., Prestwich, G.D., Mascarenhas, M.M., Garg, H.G., Quinn, D.A., Homer, R.J., Goldstein, D.R., Bucala, R., Lee, P.J., Medzhitov, R., Noble, P.W., 2015. Regulation of lung injury and repair by toll-like receptors and hyaluronan. Nat. Med. 11 (11), 1173–1179. https://doi.org/10.1038/nm1315.

Kawasaki, M., Kuwano, K., Hagimoto, N., Matsuba, T., Kunitake, R., Tanaka, T., Maeyama, T., Hara, N., 2000. Protection from lethal apoptosis in lipopolysaccharide-induced acute lung injury in mice by a caspase inhibitor. Am. J. Pathol. 157 (2), 597–603. https://doi.org/10.1016/S0002-9440(10)64570-1.

Kim, S.J., Shan, P., Hwangbo, C., Zhang, Y., Min, J.N., Zhang, X., Ardito, T., Li, A., Peng, T., Sauler, M., Lee, P.J., 2019. Endothelial toll-like receptor 4 maintains lung integrity via epigenetic suppression of p16INK4a. Aging Cell 12914. https://doi.org/10.1111/acel.12914.

Lee, S.W., Kim, D.R., Kim, T.J., Paik, J.H., Chung, J.H., Jheon, S., Huh, J.W., Lee, J.H., Lee, C.T., 2012. The association of down-regulated toll-like receptor 4 expression with airflow limitation and emphysema in smokers. Respir. Res. 13, 106. https://doi.org/10.1186/1465-9921-13-106.

Liu, C.H., Kuo, S.W., Ko, W.J., Tsai, P.R., Wu, S.W., Lai, C.H., Wang, C.H., Chen, Y.S., Chen, P.L., Liu, T.T., Huang, S.C., Jou, T.S., 2017. Early measurement of IL-10 predicts the outcomes of patients with acute respiratory distress syndrome receiving extracorporeal membrane oxygenation. Sci. Rep. 7 (1), 1021. https://doi.org/10.1038/s41598-017-01225-1.

Liu, W., Shan, L.P., Dong, X.S., Liu, Z., 2014. Toll-like receptor 4 implicated in acute lung injury induced by paraquat poisoning in mice. Int. J. Clin. Exp. Med. 7 (10), 3392–3397 PMID: 25419373.

Livak, K., Schmittgen, T., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262.

Máca, J., Jor, O., Holub, M., Sklienka, P., Burša, F., Burda, M., Janout, V., Ševčík, P., 2017. Past and present ARDS mortality rates: a systematic review. Respir. Care 62 (1), 113–122. https://doi.org/10.4187/respcare.04716.

Matthay, M.A., Zimmerman, G.A., 2005. Acute lung injury and the acute respiratory distress syndrome: four decades of inquiry into pathogenesis and rational management. Am. J. Respir. Cell Mol. Biol. 33 (4), 319–327. https://doi.org/10.1165/rcmb.F305.

Medzhitov, R., 2001. Toll-like receptors and innate immunity. Nat. Rev. Immunol. 1 (2), 135–145. https://doi.org/10.1038/35100529. 2.

Murray, J.F., Matthay, M.A., Luce, J.M., Flick, M.R., 1988. An expanded definition of the adult respiratory distress syndrome. Am. Rev. Respir. Dis. 4 (3), 720–723. https://doi.org/10.1164/ajrccm/138.3.720.

Ohkouchi, S., Block, G.J., Katsha, A.M., Kanehira, M., Ebina, M., Kikuchi, T., Saijo, Y., Nukiwa, T., Prockop, D.J., 2012. Mesenchymal stromal cells protect cancer cells from ROS-induced apoptosis and enhance the Warburg effect by secreting STC1. Mol. Ther. 20 (2), 417–423. https://doi.org/10.1038/mt.2011.259.

Ohkouchi, S., Ono, M., Kobayashi, M., Hirano, T., Tojo, Y., Hisata, S., Ichinose, M., Irokawa, T., Ogawa, H., Kurosawa, H., 2015. Myriad functions of Stanniocalcin-1 (STC1) cover multiple therapeutic targets in the complicated pathogenesis of idiopathic pulmonary fibrosis (IPF). Clin. Med. Insights Circ. Respir. Pulm. Med. 9 (1), 91–96. https://doi.org/10.4137/CCRPM.S23285.

Perl, M., Lomas-Neira, J., Venet, F., Chung, C.S., Ayala, A., 2011. Pathogenesis of indirect (secondary) acute lung injury. Expert Rev. Respir. Med. 5 (1), 115–126. https://doi.org/10.1586/ers.10.92.

Ragaller, M., Richter, T., 2010. Acute lung injury and acute respiratory distress syndrome. J. Emerg. Trauma Shock 3 (1), 43–51. https://doi.org/10.4103/0974-2700.58663.

Ramírez, C.N.E., Maldonado, B.C., Cuevas, U.M.L., Castañoń, J., López, M.C., Isibasi, A., 2004. Toll-like receptors: dysregulation in vivo in patients with acute respiratory distress syndrome. Rev. Alerg. Mex. 51 (6), 210–217 PMID: 15794413.

Sheikh-Hamad, D., 2010. Mammalian stanniocalcin-1 activates mitochondrial antioxidant pathways: new paradigms for regulation of macrophages and endothelium. Am. J. Physiol. Renal Physiol. 298 (2), 248–254. https://doi.org/10.1152/ajprenal.00260.2009.

Speleta, M., Merentiti, V., Kostikas, K., Liadaki, K., Minas, M., Gourgoulianis, K., Germenis, A.E., 2009. Association of TLR4-T399I polymorphism with chronic obstructive pulmonary disease in smokers. Clin. Dev. Immunol. 2009, 260286. https://doi.org/10.1155/2009/260286.

Takyar, S., Zhang, Y., Haslip, M., Jin, L., Shan, P., Zhang, X., Patty, J., Lee, P.J., 2016. An endothelial TLR4-VEGFR2 pathway mediates lung protection against oxidant-induced injury. FASEB J. 30 (3), 1317–1327. https://doi.org/10.1096/fj.15-275024.

Tang, S.E., Wu, C.P., Wu, S.Y., Peng, C.K., Perng, W.C., Kang, B.H., Chu, S.J., Huang, K.L., 2014. Stanniocalcin-1 ameliorates lipopolysaccharide-induced pulmonary oxidative stress, inflammation, and apoptosis in mice. Free Radic. Biol. Med. 71, 321–331. https://doi.org/10.1016/j.freeradbiomed.2014.03.034.

Villar, J., Cabrera, N., Casula, M., 2010. Mechanical ventilation modulates Toll-like receptor signaling pathway in a sepsis-induced lung injury model. Intensive Care Med. 36, 1049–1057. https://doi.org/10.1007/s00134-010-1799-3.

Wagner, G.F., Hampong, M., Park, C.M., Copp, D.H., 1986. Purification, characterization, and bioassay of teleocalcin, a glycoprotein from salmon corpuscles of Stannius. Gen. Comp. Endocrinol. 63 (3), 481–491. https://doi.org/10.1016/0016-6480(86)90149-8.

Zhang, Y., Shan, P., Srivastava, A., Li, Z., Lee, P.J., 2019. Endothelial STC1 maintains mitochondrial bioenergetics and prevents oxidant-induced lung injury via TLR4. Antioxid. Redox Signal. 30 (15), 1775–1796. https://doi.org/10.1089/ars.2018.7514.

Zhang, Y., Zhang, X., Shan, P., Hunt, C.R., Pandita, T.K., Lee, P.J., 2013. A protectiveHsp70-TLR4 pathway in lethal oxidant lung injury. J. Immunol. 191 (3), 1393–1403. https://doi.org/10.4049/jimmunol.1300052.

Zhou, B., Zhou, H., Ling, S., Guo, D., Yan, Y., Zhou, F., Wu, Y., 2011. Activation of PAR2 or/and TLR4 promotes SW620 cell proliferation and migration via phosphorylation of ERK1/2. Oncol. Rep. 25, 503–511. https://doi.org/10.3892/or.2010.1077.