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Level of pro-inflammatory cytokines in patients with transfusion-related acute lung injury - Multiple comparisons between patients, controls and donor

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ABSTRACT

Transfusion-related acute lung injury recently regarded as the leading cause of death after transfusion. Several pro-inflammatory cytokines TNF, IL6 and IL8 have been linked to the pathogenesis of TRALI, supported by the findings of increased their serum levels in recipient patients. This is a prospective case-control study, twenty-five patients with a diagnosis of TRALI after transfusion of blood products were included and compared to another 25 transfused patients. Serum was obtained after the onset of TRALI in patients and controls. Other samples were obtained from the saved donor transfused bag or segments. All samples were utilized for cytokines assay. The intubation rate among TRALI patients was 48%. No difference was found in the regarding the type of transfusion and the cytokine level for each specific type of blood or blood component transfused between TRALI and controls. The overall TRALI associated mortality was 4%. Results revealed significantly increased TNF alpha IL-6 levels in sera of TRALI patients as compared with control and donor unit for patients with TRALI. Serum levels of IL-8 were significantly higher in patients with TRALI (mean 42.11 pg/ml) as compared with sera of control and donor unit for TRALI patients. Serum level of TNF, IL-6 and-8 in patients with TRALI was significantly higher in patients with longer incubation time. Serum cytokines assay in patients with TRALI may add the significant advantage of assessing the severity, associated mortality and predicting the time of ventilator support.

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INTRODUCTION

With recent laboratory sophistication of blood donor selection and cross-matching, Transfusion Related Acute Lung Injury (TRALI) has replaced the incompatibility reactions and blood born infections as the commonest cause of transfusion-related morbidity and mortality (Holness *et al.,* 2004). TRALI has been found as the leading cause of death after transfusion with an incidence up to 1/323. Recently the mortality from TRALI found to be much higher approaching 5-10% of transfused patients, increased to 40% in the critically ill patient (Wallis *et al.,* 2003; Renaudier *et al.,* 2004). TRALI as a clinical entity was first described by Popovsky (Popovsky *et al.,* 1985). Most of the TRALI cases are diagnosed within the 1st 6 hours after transfusion with a large proportion of them occur in the first one or two hours, however recently it has been found that symptoms of TRALI can be delayed up to 24-72 hrs (Fisk *et al.,* 2004). Clinically, TRALI presents as an acute onset of dyspnea, tachypnea, rising pulse rate and body

temperature, while cyanosis and hypotension were inconsistent findings and limited to severe cases. Bilateral diminished breath sound and basal crepitation are evident on chest auscultation. Hypoxemia is clinically evident with PaO2/FiO2 less than 300mm Hg. Radiographic features of bilateral pulmonary homogenous infiltrate compatible with pulmonary edema. In 2004 the European Haemovigilance Network (EHN) and the Canadian Consensus Conference proposed criteria for the diagnosis of TRALI. Criteria for the clinical diagnosis of transfusion-related acute lung injury (TRALI). TRALI Consensus Conference Committee 2004 and European Haemovigilance Network (Popovsky *et al.,* 2001).

- Acute respiratory distress.
- Bilateral lung infiltrations in the chest radiograph.
- Occurrence during or within 6-h after completion of transfusion.
- No evidence of transfusion-associated circulatory.
- overload/cardiogenic lung oedema.
- Hypoxaemia (PaO2/FiO2 <300 mmHg or O2 saturation<90%.

In contrast to ARDS from other causes, patients with TRALI typically recover with a resolution of pulmonary infiltrates within 96 hours. Pathologically the impaired arterial oxygenation result from the pronounced alveolar capillary permeability culminating in protein-rich pulmonary edema. The importance of neutrophils as a common final target in the pathogenesis of TRALI been suggested by the finding of the increased number of neutrophils in the broncho-alveolar lavage and histological samples in patients with TRALI and they closely correlate with mortality. Several models have been proposed for the pathogenesis of TRALI; the antibody-mediated theory suggests that the infusion of donor anti HLA antibodies or anti-granulocytes antibodies will attack the cognate antigens on the neutrophils, activating them on the pulmonary endothelial cells with subsequent sequestration with consequent release of toxic products which damage the endothelial and vascular integrity. The resultant leakage of protein-rich fluid in the alveolar spaces is the hallmark of TRALI (Silliman *et al.,* 2003). The two hit theory of TRALI has been proposed according to the observation of TRALI cases that are mediated by biologically active substances that appear after blood storage including the bioactive lipids and various cytokines (Wallis *et al.,* 2003). The initial event (first hit) that has been suggested to induce neutrophils priming in the pulmonary capillaries includes recent surgery, sepsis, and other stressful stimuli. Once primed, neutrophils can be activated by the transfused biological

elements (second hit) they undergo pulmonary sequestration and adherence and subsequent damage of the pulmonary endothelium (Khan *et al.,* 2006). Although supported by many studies, the two hits theory fail to explain why TRALI do occur in patients who have no initial trigger. Recently a new model has been described, which suggest merging the two after mentioned theories and termed the threshold model in which the strong potential to TRALI may need no priming events to reach the threshold of TRALI after transfusion (Bux and Sachs, 2007). Following major stressful extrapulmonary insult including sepsis and operative trauma the systemic endothelial and /or epithelial injury and stimulation of immune cells is induced by the release of cytokines among which (tumor necrosis factor alpha) TNF α, (interleukin) IL-1, and IL-6, thus accelerating the epithelial apoptosis which is in combination with cytokine releases regarded as strong stimulus for neutrophils recruitment in the pulmonary alveolar capillary interface (Steinberg, 2004). In addition, many stored blood components like antibodies, cytokines and active lipid molecules also induce activation of endothelial cells and of already primed neutrophils by the primary priming insult. It has been found that the primed neutrophils become activated and perform its damaging effect within 24 hrs of original priming insult, recent surgery was found to be common initial priming factor (Sachs *et al.,* 2006). Other stimuli that are involved in neutrophils priming and pulmonary sequestration are those originating from the endothelial cell including TNF α, IL8 which has the additional effect of neutrophils adherence. TNF α and IL-1 are known to produce local activation of macrophages, fibroblast and endothelial cells to releases their pro-inflammatory cytokines including IL-8 which is a potent chemoattractant for pulmonary neutrophils recruitment. A variety of chemokines released by endothelial and immune cells responsible for the neutrophilic infiltration of the lung, among them the alveolar macrophages are the major source of these chemokines. and produce IL-8. The relevant pronounced flux of neutrophils into the alveolar spaces is thought to be brought about by the high level of IL-8. Furthermore, it has been found that IL-8 bound to some antibodies to form a complex which can be detected in the broncho-alveolar lavage fluid, levels of these complexes were found to correlate with onset, severity and prognosis of TRALI (Bernard *et al.,* 1994). Many researchers highlighted the importance of several proinflammatory cytokines among them TNF- α and IL-6, their major role in unopposed inflammation in ALI had been well established. Elevated serum Levels of IL-6 is found to correlate well with disease severity in ALI in critically ill patients IL-8 is considered as a very potent neutrophils attracter, and found to be elevated

in both serum and bronchoalveolar lavage in patients with ALI, and can predict mortality. The role of IL-8 in the pathogenesis is also explained by the formation of anti IL8 autoantibodies and IL8-complexes. Dong and Yuan Found that the IL6 and TNF α were increased in the mice serum when exposed to LPs (Dykes *et al.,* 2006). Recent researches found a reasonable correlation between the storage time of red blood cells and the occurrence of TRALI through the findings of storage lesions (the membrane lipids and reactive oxygen species) in the stored blood, which have been found to have potential role in lung injury immediately after transfusion, the effects are directly proportional to the storage time. Two possible sources of an elevated cytokines in TRALI patients has been suggested, patient endogenous cytokines as a result of the profound inflammatory reaction or exogenous source in the transfused blood either from the altered stored leukocytes and platelet or a preformed incubated cytokine from donor who suffer a subclinical illness (Parsons *et al.,* 1985).

PATIENTS AND METHODS

Study design: This is a prospective case-control study conducted in al Diwanyia teaching hospital, college of medicine, Al Qadissyia university, during a period of 5 years from July 2013 to September 2018. The study approved by the local scientific comity of the college.

Patients: Twenty-five patients with a diagnosis of TRALI after transfusion of blood or blood products were included in the study and compared to another age and sex-matched 25 transfused patients without evidence of TRALI. All patients were adults (>18 years) underwent major surgery or a victim of trauma who were admitted in intensive care or did so if ventilator support is needed.

Sampling: Five ml blood was drawn within a median time of 6 hours after the onset of TRALI in patients and a similar amount obtained within a median time of 12 hours after transfusion in controls. Other samples were obtained from the saved donor transfused bag or segments. Samples were centrifuged, and the measured amount was utilized for cytokines assay.

Assay method: Cytokine concentrations measurements were established using the commercial microarray kit, ProteoPlex Human Cytokine Array manufactured by Novagen (EMD Biosciences, Inc, Madison, WI). The slide chip is an ELISA based. The measured cytokine included TNF alpha, IL-6 and IL-8.

Statistical analysis: Data were analyzed using SPSS software system version 22. Chi-square test used for categorical data, and Kruskal Willis test

was used for skewed continuous variables of cytokine values.

Inclusion criteria for patients: any patient who develop signs and symptom of acute lung injury within 6 hours of transfusion, in whom the PaO2 less than 90, and a chest x-ray revealed bilateral pulmonary infiltrate, the existence of left atrial hypertension was excluded by clinical examination and echocardiography with no temporal relationship to an alternative risk factor for TRALI.

Inclusion criteria for control: patients who are recipients of blood or blood products, without clinical or radiological evidence of ALI.
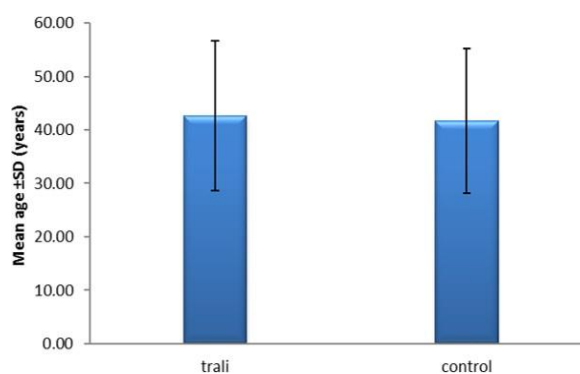
RESULTS

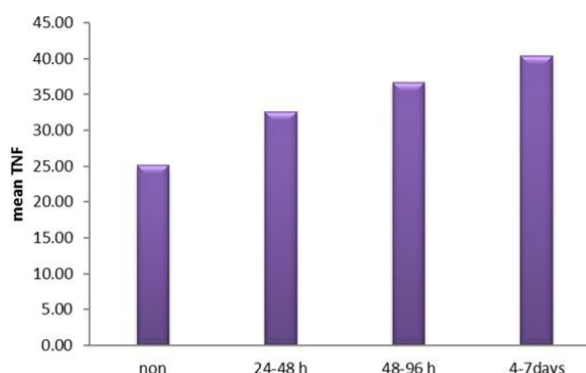**Figure 1: age of the study population**

Figure 2: The correlation of mean TNF level and intubation time

Among 50 patients there was 29 males and 21 females with male to female ratio of 1.38. The mean age of the total study population was 42.14 years, for patients with TRALI was 42.64 years and 41.64 years for control. (Table 1). The intubation rate among TRALI patients was 48%, no single control case required intubation. No significant differences were found between patients and control regarding the type of transfusion (table 2) and the cytokine level for each specific type of blood or blood component transfused (Table 3). The overall TRALI associated mortality was 4% (Table 4). Multiple data comparison analysis of serum cytokine

Table 1: Age and gender characteristics among TRALI and controls

			Gender		Total
			Male	Female	
TRALI	Age groups	18-30	3	2	5
		30-40	3	3	6
		40-50	4	2	6
		50-60	3	3	6
		Above 60	1	1	2
	Total		14	11	25
Control	Age groups	18-30	3	3	6
		30-40	2	3	5
		40-50	5	3	8
		50-60	3	1	4
		Above 60	2	0	2
	Total		15	10	25

Table 2: Types of transfused blood and blood components

Type of transfusion	TRALI	Control	Total	P value
Fresh blood	3	2	5	Reference
Whole stored blood	13	15	28	0.941
Fresh frozen plasma	6	6	12	1.000
Platelet concentrate	3	2	5	1.000
Total	25	25	50	

Table 3: Cytokine level for each specific type of blood or blood component

	Type of transfusion	N	Mean	Std. Deviation	P value
IL-6	Fresh blood	5	25.5	16.57	Reference
	Whole stored blood	28	19.5	13.19	0.376
	Fresh frozen plasma	12	21.5	9.95	0.551
	Platelet concentrate	5	24.2	13.14	0.894
tumour necrosis factor	Fresh blood	5	26.8	15.44	Reference
	Whole stored blood	28	20.2	11.13	0.256
	Fresh frozen plasma	12	22.2	13.57	0.549
	Platelet concentrate	5	23.0	15.85	0.707
IL-8	Fresh blood	5	37.3	22.38	Reference
	Whole stored blood	28	25.1	17.11	0.170
	Fresh frozen plasma	12	27.8	21.43	0.425
	Platelet concentrate	5	30.7	14.95	0.601

Table 4: The overall mortality

Mortality	TRALI	control	Total	P
Positive	1	0	1	
Negative	24	25	49	1.000
Total	25	25	50	

Table 5: multiple comparisons of cytokine levels in the sera of patients with TRALI, control and the donor

Cytokine	Mean ± SD			
	Trali	Control	doner in trali	doner control
TNF	31.14±8.1	12.19 ± 7.8	10.46 ± 3.9	9.56 ± 3.1
IL-6	31.19±9.7	10.98±4.2	11.01±4.9	11.04±4.1
IL-8	42.11±13.8	12.98±7.5	6.28±1.9	6.19±2.0

levels between patients, control and cytokine levels in the transfused unit, revealed significantly increased TNF alpha IL-6 (mean 31.14pg/ml, 31.19 pg/ml respectively) levels in sera of TRALI patients as compared with control and donor unit for patients with TRALI. No significant difference found in TNF alpha and IL-6 levels between control patient's sera and donor unit. Serum levels of IL-8 were significantly higher in patients with TRALI (mean 42.11 pg/ml) as compared with sera of control and donor unit for TRALI patients, the level

Table 5: multiple comparisons of cytokine levels in the sera of patients with TRALI, control and the donor (Contd....)

| Cytokine | P value | | | |
	Trali vs control	Trali vs trali donor	Control vs donor control	Trali vs control
TNF	< 0.001	< 0.001	0.609	< 0.001
IL-6	< 0.001	< 0.001	0.987	< 0.001
IL-8	< 0.001	0.004	0.003	< 0.001

Table 6: Association between intubation time and cytokines level

		Intubation time	Mean	
	tumour necrosis factor	non	25.17	*P*
		24-48 h	32.60	
		48-96 h	36.70	<0.001
		4-7days	40.43	
		Total	31.14	
	IL-6	non	24.45	
		24-48 h	37.50	
Cytokine level		48-96 h	35.63	<0.001
		4-7days	43.75	
		Total	31.19	
	IL-8	non	31.40	
		24-48 h	57.40	
		48-96 h	51.34	<0.001
		4-7days	56.93	
		Total	42.11	

again was significantly higher in control than donor unit for control (table 5). Serum level of TNF, IL-6 and IL-8 in patients with TRALI was significantly higher in patients with longer intubation time. (Table 6).
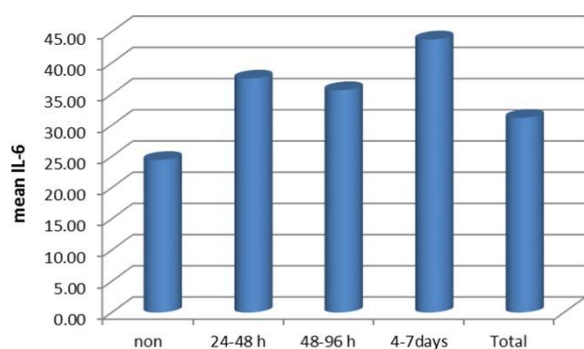
Figure 3: The correlation of mean IL-6 level and intubation time
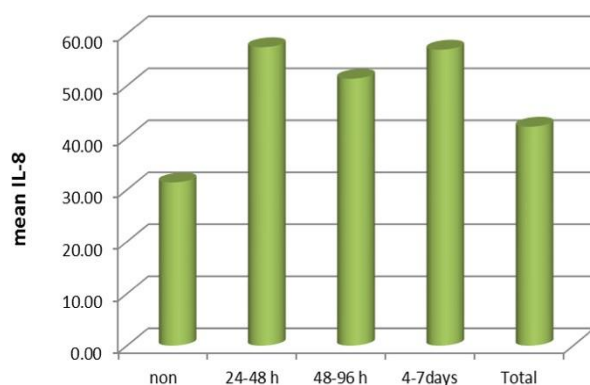
Figure 4: The correlation of mean IL-8 level and intubation time

DISCUSSION

TRALI represent the ARDS counterpart that is related to a transfusion event. The associated mortality is very variable between centers with transfusion practice and recently superseded the mortality due to other transfusion complications (Eder *et al.,* 2007). Many cases of TRALI passed

unnoticed preassembly due to lack of recognition as a consequence of great overlap with other pulmonary insults, and the mild forms do exist in which natural recovery is common sequel (Higgins *et al.,* 2007). The most recently accepted theory in the pathogenesis of TRALI is the threshold model (Dong, 2018). Which is a mating result of the two proposed antibody mediated (Wyman *et al.,* 2002), and the two hits theories (Janz *et al.,* 2013). Leukocytes priming, sequestration and activation with the subsequent pulmonary alveolar damage, was agreed as the common path that is shared by all proposed models (Sparrow, 2010). All transfused patients in this series including controls had undergone recent major surgery, which is regarded by many as the initial priming insult (Yazer *et al.,* 2004). The role of many cytokines among many blood-derived components has been extensively investigated as part in the pathogenesis of TRALI. The existence of IL-6 and TNF alpha as a pro-inflammatory cytokine and its role in inflammation has been proved together with the IL-8 as a potent chemokine, but its role in the pathogenesis in TRALI is only recently elucidated in many kinds of

literature (Looney *et al.,* 2014). It has been published that the type and age of transfused blood or blood fractions has great influence upon the incidence of TRALI, the older the blood and the plasma containing products are associated with more risk, however in our study transfused unit was less than 14 days old and no significant differences found between controls and TRALI patients, however, a wide range of controversy has been published regarding this issue. Wallis et al. reported an increased incidence with plasma containing transfusion, in contrast, Eder et al. published contrary results. However, the storage of RBCs for a prolonged period has been linked to increased transfusion-related mortality; however, Kor found no difference in the outcome between old and fresh blood transfusion. We found that neither the age nor the type of transfused unit significantly influences the serum cytokine levels. Trail associated mortality exhibit wide range in the published data, however in our results, the mortality was in the lower limit probably because most TRALI cases was of mild to moderate severity (Wallis *et al.,* 2003), moreover Higher mortality rates were reported by Gajic et al. in TRALI critically ill patients. Results in this study were relevant to the significantly elevated mean serum levels of IL-6 and TNF and IL-8 in patients with TRALI as compared to controls. This finding is compatible with the finding of Calfee *et al.,* Pearl and Clifford**,** Vlaar et al.

To run the question, that the accumulated and or generated cytokine in the blood or blood fraction stored unit can significantly alter the serum level after transfusion and hence alter the susceptibility to TRALI after transfusion. We found that the mean IL-6 and TNF alpha levels were higher in the donor blood bag than normal range serum level. The increased levels of these inflammatory cytokines in stored blood and blood fraction has been found to be reduced by leukoreduction Weisbach *et al.,* Jacobi et al. and Seghatchian, however, Biffl et al. found that leukoreduction does not affect the incidence of trail (Weisbach *et al.,* 1999). Whether the elevated cytokines level is generated in the stored bag or it is donor related who suffer a subclinical illness at the time of donation, a question is not yet answered. Patients with TRALI express a significantly higher serum IL-6 and TNF alpha and IL-8 level s than their measurable transfused bag corresponding cytokines, and further, levels of IL-6 and TNF alpha were not statistically different between sera of the controls and cytokines levels in their corresponding transfused bag , this finding was not observed for the levels of IL-8 in which significantly higher in control sera than their corresponding transfused blood bag. These observations were contrary to findings published by Weisbach et al. and Jacobi (Jacobi *et al.,* 2000) who

document that many cytokines including IL-8 are elaborated into the stored blood. This may be denoted that the cytokines level was endogenously released in patients with TRALL as part of the distinctive inflammatory process.In addition, IL-8, unlike another cytokine, does not attain higher levels during storage and this support the endogeneity of this chemokine in the pathogenesis of TRALI. Many kinds of the literature suggest that the serum pro-inflammatory cytokines to be released from the circulating monocytes, it takes 20 hours, a fact which is incompatible with a time of TRALI development following transfusion (Seghatchian *et al.,* 2000). The aim of treatment in TRALI is to ensure a sufficient pulmonary gas exchange while the pulmonary function recovered, this may be accomplished by mask or nasal administration of oxygen or in severe cases endotracheal intubation, and ventilatory support is needed during the recovery period , in general the more severe the ALI (, the more intense the inflammatory process), the longer the intubation time. In our series, the intubation rate for TRALI patients was 48% as compared with Looney who states that 87% of patients required ventilatory support however this difference might be explained by the threshold for intubation, and many milder forms of our patients responded to the oxygen supplement. We found that the mean level of the three measured serum cytokines level was significantly higher in patients with longer intubation time. This finding suggests that serum levels of these blood biomarkers may be an indicator of the severity of ALI (Chen *et al.,* 2012; Biffl *et al.,* 2004).

CONCLUSION

Recent major surgery is the most common initial trigger for TRALI following transfusion. Serum levels of pro-inflammatory cytokine in patients with TRALI are generally elevated, their levels have a significant association with the severity of the condition marked by the time needed for endotracheal intubation and ventilation support. Type of the transfused blood and blood fractions has no significant effect on the incidence of TRALI, however inconclusive results were obtained about the effect of old stored blood on the incidence of TRALI.

Conflict of interest: The authors have disclosed no conflicts of interest.

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