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Role of innate immunity in prevention of Health care associated infections in critically ill children- A prospective study

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ABSTRACT

Introduction

Despite the advances in the critical care management, Health care associated infections (HAI) are leading to increased mortality and morbidity in Paediatric intensive care unit (PICU).Presently acquired immunity is measured routinely and not innate immune response. This study aims to measure Lipopolysaccharide (LPS) induced Tumour necrosis factor (TNF) α to determine innate immune response in sick children admitted to PICU and association with subsequent development of HAI.

Materials and Methods

A hospital based prospective and observational study was conducted wherein total of 63 children admitted to PICU requiring ventilation or indwelling central line for more than 2 days were included. Whole blood assay of TNF α levels using ELISA were measured after stimulation with Lipopolysaccharide on day 1,3 and 7 of ICU stay.Statistical analysis of the outcome variables was done by Mann Whitney U test and Wilcoxon signed rank test, ROC curve analysis for relation of TNF α with HAI and mortality

Results

TNF α is a significant predictor for HAI on day 1 estimation (p = 0.015). ROC curve showed Day 1 TNF α levels more than 247 pg/ml and Day 3 TNF α levels less than 203pg/ml as predictors of HAI and mortality respectively. There is statistically greater reduction in TNF α between Day 3-Day 7 than Day 1-Day 3 in non survivors (p=0.027). Children who had VAP (p=0.018), HAI (p=0.025), and such reduction was also present in children with HAI caused by multi drug resistant organisms (p=0.0047), gram negative organisms (p=0.0001) and MRSA (p=0.023). This reduction was absent in children who survived and did not develop HAI.

Conclusion

LPS induced TNF α levels are useful to predict likelihood of development HAI and mortality in critically ill children.

Keywords: *Health care associated infections (HAI), Ventilator associated infection (VAP), central line associated blood stream infection, Lipopolysaccharide (LPS) induced TNF α , Methicillin resistant Staphylococcus aureus (MRSA).*

INTRODUCTION

Health care associated infections are defined as infections contracted during course of treatment in hospital or health care facility and should be absent or should not be in incubation period on the day of admission. Contributing factors for development of HAI include indwelling devices, prolonged duration of broad spectrum antibiotics and prolonged hospital stay. [1]

Sepsis is an inflammatory response of the host to life threatening infectious stimuli. Phases of immunological response include initial hyper-inflammatory and late anti- inflammatory phase. Monocyte deactivation to maintain immune homeostasis occurs during this late phase in which reduced production of cytokines like TNF- α increases risk of mortality and developing secondary infections by replication of microbes and invasion into bloodstream through breached epithelium which may occur during placement of devices. Critically ill patients have reduced capacity to produce TNF – α in response to LPS hence monitoring these parameters helps to stratify the sick children into hyper or hypo inflammatory state and predict outcome.The gold standard for measuring innate immune response is measurement of cytokine production by monocytes in response to ex- vivo stimulation with LPS. [2]

Paediatric Intensive Care Units do not routinely analyse innate immune response as currently no guidelines for monitoring the immune status are available. There is a need for research to assess the innate immune response and role of immunotherapy in these children.

METHODS

A hospital based prospective observational study was conducted in Paediatric Intensive Care unit. Necessary approvals including Institutional Ethics Committee approval was obtained. A total of 63 critically ill children who had been ventilated and / or had central line in-situ for more than 2 days were included in the study. Infants/children with history of administration or immunosuppressive drug, haematological malignancy, diagnosed case of immunodeficiency diseases or who had been recently treated in intensive care elsewhere prior to our hospital admission were excluded.

Children were recruited after obtaining informed consent from parents/ relatives. A semi structured proforma was prepared to record the demographic, clinical, laboratory and other details. Subjects were enrolled on Day 1 of placement of device and blood sampling was done for stimulated TNF α assay on day 1, day 3 and day 7 of device placement during ICU stay.

Preparation of bacterial Lipopolysaccharide (LPS)

Bacterial Lipopolysaccharide Sigma Aldrich USA, commercially available as lyophilized powder form obtained by detoxification of *E.coli* 0111: B4 strain through chromatographic purification and delipidization by alkaline hydrolysis. This was stored at 2 -8^{o}C.

For cell culture use, LPS solution was prepared by serial dilution in phosphate buffer. A stock solution of 1 µg/mL strength was prepared. From the stock 0.1 mL was taken and was made up to 100 mL by adding 99.9 mL of phosphate buffer. This was stored in refrigerator for one month.

Procedure

Venous blood samples were collected under aseptic precautions in EDTA anticoagulant tubes.50 µl of whole blood was added to 500µl of highly standardized stimulation solution containing 500 pg/ml of LPS from E. coli 0111.B4 detoxified [Sigma Aldrich] within 30 minutes of collection of the sample. Stimulated samples were incubated in water-bath for 4 hours at 37 degree Celsius and Supernatants were collected and stored at - 20 degree Celsius for the analysis of TNF alpha levels by ELISA[3]done in Department of Biochemistry. Subjects were prospectively followed for the development of HAI (namely ventilator associated pneumonia, Central line associated blood stream infections) and mortality. Data collected was entered on excel spread sheet.

Statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 16. Data was expressed as median and Interquartile range. Groups were compared using Mann Whitney U test and Wilcoxon signed rank test. p value <0.05 was considered as statistically significant. A receiver operating characteristic curve with area under the curve was used to analyse the relationship of TNF- αlevels with HAI and mortality.

The sample size was calculated using the formulae below

Sample size (n) based on sensitivity

$$\frac{Z^2_{1-\alpha/2} \times S_N \times (1-S_N)}{L^2 \times \text{Prevalence}}$$

Sample size (n) based on specificity

$$\frac{Z^2_{1-\alpha/2} \times S_P \times (1-S_P)}{L^2 \times (1-\text{Prevalence})}$$

Where n=required sample size, S_N=anticipated sensitivity, S_P =anticipated specificity,α =size of the critical region(1- α is the confidence level),1-α/2 =standard normal deviate corresponding to the specified size of the critical region(α) and L= absolute precision desired on either side(half width of the confidence interval) of sensitivity or specificity. With anticipated sensitivity of LPS induced TNF α levels in predicting HAI as 82% sensitivity. Sample size calculation has 10% precision, 95% confidence interval, 10% non-response error. Non random sampling method was applied.

RESULTS

Data was expressed as median and interquartile range. TNF α is a significant predictor for HAI on day 1 estimation (p = 0.015). Serial measurements of TNF α levels in non survivors on day 1,day 3, day 7 were 227.7 pg/ml, 197.7 pg/ml and 155.6 pg/ml whereas in those with non- HAI survivors were 214.9 pg/ml, 321.9 pg/ml and 157.5 pg/ml. There was no statistical significance between these two groups. p value for statistical difference between these groups on day1 ,3 and 7 were 0.937, 0.684 and 0.963 respectively.

ROC of TNF α levels showed a cut off of more than 247 pg/ml on Day 1 with sensitivity of 68% and specificity of 60% in predicting HAI (Fig.1) and a cut off of more than 203 pg/ml on Day 3 with sensitivity of 77 % and specificity of 55 % in predicting mortality in critically ill children(Fig.2).

TNF α levels analysed in sick children showed higher reduction of innate immune response between Day 3-Day 7 than Day 1-Day3 in non survivors (Table I), VAP, HAI (Table II) and also those who were secondarily infected with Multi drug resistant organisms, gram negative organisms, and MRSA (Table III).Non –HAI survivors did not have such significant decrease in innate immune response.

p value less than 0.05 is considered significant.

Table I Relation of reduction of TNF α in non survivors

TNF α	Group A (n= 24)	Group B (n= 29)	p value
D1-D3Reduction	19pg/ml (-51- 124pg/ml)	-29.5 pg/ml (-197 - 35 pg/ml)	0.544
D3-D7Reduction	133 pg/ml (-11 – 584.8pg/ml)	10.25 pg/ml (-66 - 104 pg/ml)	0.911
p value	**0.027**	0.201	

*****Group A – non- survivor, Group B – non HAI survivors**.** # Statistical analysis for reduction in TNF – α level in each group was done by Wilcoxon signed rank test and between the groups was by Mann Whitney U test. Greater reduction in levels of TNF α from D3-D7 compared to D1-D3 in group A **(p = 0.027)** and such reduction was absent in group B.

Table II Relation of reduction of TNF – α levels in children with Health care associated infections

TNF α	Group A (n= 25)	Group B (n= 29)	p value
D1-D3Reduction	14pg/ml (-119 - 146pg/ml)	-29.5 pg/ml (-197 - 35 pg/ml)	0.759
D3-D7Reduction	123.8pg/ml (3–363.9pg/ml)	10.25 pg/ml(-66 - 104 pg/ml)	0.51
p value	**0.025**	0.201	

***** Group A – children with HAI, Group B – non HAI survivors**.** # Statistical analysis for reduction of TNF – α level in each group was done by Wilcoxon signed rank test and between the groups was by Mann Whitney U test. There was significant reduction in TNF α levels between D3-D7 than D1-D3 in Group A **(p = 0.025)** and such reduction was absent in group B.

Table III Relation of reduction of TNF A in children with HAI due to Multi resistant organisms, gram negative organisms and MRSA

TNF α	Group A (n=7)	Group B (n=2)	Group C (n=4)	Group D (n=4)
D1-D3Reduction	19 pg/ml (-225- 73 pg/ml)	78pg/ml	1 pg/ml (-441 - 188pg/ml)	36.5pg/ml (-164 - 216pg/ml)
D3-D7Reduction	41.2pg/ml (-11- 135 pg/ml)	-	358.9 pg/ml (35.5 - 805.5 pg/ml)	15 pg/ml (-1653 -11.5pg/ml)
P value	**0.0047**	Not significant	**0.0001**	**0.023**

*****Group A –children with HAI due to multidrug resistant organisms, Group B - children with HAI due to non-multidrug resistant organisms, Group C- children with HAI due to gram negative organisms, Group D- children with HAI due to MRSA. Significant reduction between D3-D7 than D1-D3 in children with HAI due to multidrug resistant organisms (**p=0.047**), gram negative organisms (**p=0.0001**) and MRSA (**p=0.023**).

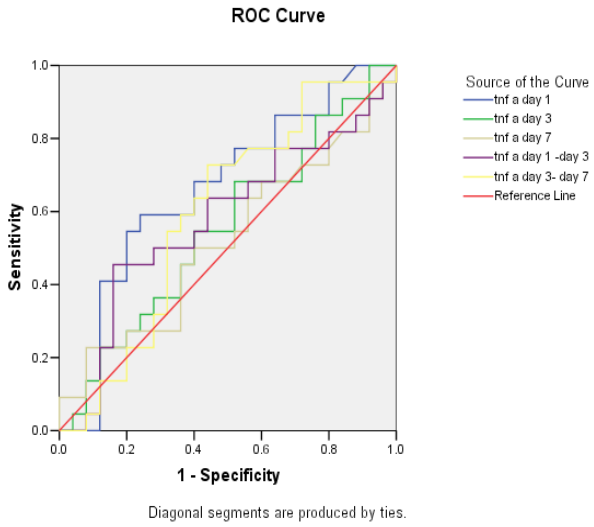

Fig.1 ROC analysis shows the comparison of TNF αvalues in predicting HAI in critically ill children. Area under the curve on day 1 was.661with **p value 0.059.**

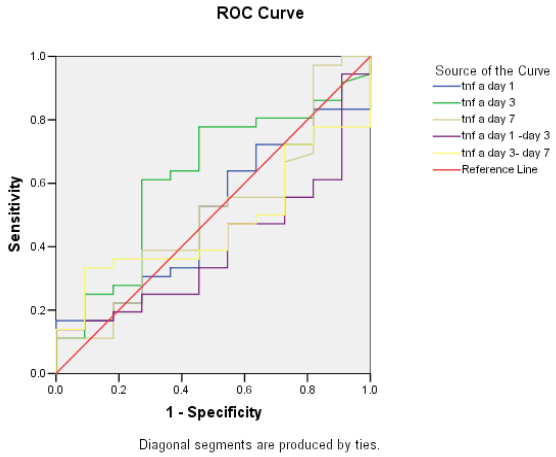

Fig. 2 ROC analysis shows the comparison of TNF α values in predicting mortality in critically ill children. Area under the curve was 0.622 with p value of 0.223

DISCUSSION

Health care associated infections being major causes of morbidity and mortality pose greater burden on the children and health care system. Predisposing factors to HAI include placement of devices, recumbent position, parenteral nutrition and malnutrition. [4] Host factors like innate immune status is one major contributing factor as well ,thus implementation of immune monitoring in routine clinical practice in critically ill children helps to identify the hyper and hypo-inflammatory states, as both states in extremes are deleterious to the child's health. Prolonged second phase of endogenous immunosuppression results in immunoparalysis and is characterized by reduced TNF α production capacity by monocytes.

Failure of clinical trials in sepsis directed against the initial hyper inflammatory phase and increased occurrence of secondary infections during later stages had thrown light on existence of immunosuppressive phase in sepsis. Boosting the immune response during immunoparalysis might prevent the occurrence of new infections and subsequent death. [5]

Hence, identifying these patients by immune monitoring is essential for administering immunotherapy which is presently under research. Gold standard for detecting Immunoparalysis ex vivo LPS induced TNF α production.

Normally, Healthy children have robust capacity to produce TNF α in response to LPS i.e.900-2172 pg/ml[6] .In this study significant difference of TNF α levels in sick children with HAI (includes VAP and CLABSI) and survivor group without HAI in day wise measurements was absent. Significant difference of TNF α levels in non survivors and survivors without HAI in day wise measurements was absent. Therefore, one time estimation is not ideal way to depict innate immune response in critically ill children and is due to the complex and dynamic nature of sepsis, heterogeneity of clinical condition and individualized response to noxious insult. [7] Serial estimation of induced TNF α levels is required for identification of at risk children due to the lack of availability of cut off values in each clinical setting. Persistent endogenous immunosuppression over a period of time constitutes immunoparalysis. Hence, its recognition requires monitoring of immune status over a period of time, this is further substantiated with a study done by van Vught L A et al[8] reported lack of significant low levels of TNF α on day 1 of ICU admission in sick patients who developed HAI than those without HAI.

On contrary to the present study, Wolfgang Ertel et al [9] reported that subjects with severe sepsis on comparison with controls showed significant reduced TNF α production capacity on day wise measurements up to ten days. [48]

Receiver operative curve analysis of ex vivo LPS induced TNF α levels in predicting HAI on Day 1 showed sensitivity of 68% and specificity of 60% with cut off 247 pg/ml and was better than day 3 and day 7(Fig.1).Children with TNF α levels more than 247 pg/ml on day 1 are at high risk of developing HAI. This shows the fact that greater the severity of initial illness, higher will be the subsequent immunosuppression [7] and sample collection in early phase of sepsis might explain the high levels of induced TNF α levels in subjects with HAI.ROC analysis of ex vivo LPS induced TNF α levels in predicting mortality on Day 3 showed sensitivity of 77 % and specificity of 55 % with cut off 202.8pg/ml and was better than day 3, day 7 values.(Fig.2).Therefore, induced TNF α levels on day 3 below cut off 202.8 pg/ml is predictive of mortality in critically ill children. Early measurements of innate immune response do not indicate the risk of secondary sepsis in various clinical settings except stress and pattern of immune status from monitoring of innate immune response would reflect the risk of HAI.

Significant reduction of TNF α levels from D3-D7 than D1-D3 was noticed in children who developed HAI (Table II) ,VAP, those with HAI caused by multi drug resistant organisms, gram negative organisms and MRSA(Table III).Significant reduction of TNF α levels from D3-D7 than D1-D3 was noticed in non survivors (Table I). Survivors without HAI had no such significant reduction (Table I).

This is further substantiated by the following studies: Talita Freitas Manzoli et al [10] suggested monitoring the variations in innate immune response is a better way to predict the mortality in septic children. Non survivors had reduction in innate immune response in second sample which implies lack of recovery of immune status to normalcy and those with increased innate immune response are less vulnerable to death. Study conducted by Jian-Feng Wu et al [11] reported that

monitoring the reduction of innate immune responses over a period of time is better indicator of mortality in septic patients.

Serial monitoring of immune responses in septic patients better reflects the predictive value for mortality**.** Another study done by Lukaszewicz AC et al [12] had demonstrated poor recovery in innate immune response (i.e. persistent low levels) in subjects who developed HAI. Monneret et al [13] highlighted the importance of analysing the variations of innate immune response as it reflects the dynamic changes of sepsis. Subjects who fail to recover the normal immune status are vulnerable to acquire secondary infection and high risk of mortality.

Heterogeneous study population was the limitation in the study.

CONCLUSIONS

Immunoparalysis occurs in late stages of critical illness in children. TNF α levels on day 1 would be useful to identify high risk children who might develop HAI .Dynamic state of innate immune response in critically ill children is better reflected by serial analysis of innate immune response than day wise measurements as reducing trend of innate immune response are at high risk of acquiring HAI and vulnerable to death. Such trend was also observed in children with HAI caused by multi drug resistant organisms, gram negative organisms and MRSA. Children with minor reduction in innate immune response recovered without the development of HAI. Recognizing this immune response pattern helps in providing immune-stimulation therapy to improve the survival in sick children.

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