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Role of Gene Polymorphism in TNF Alpha Expression and Outcome in Surgical Patients

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Authors' contributions

This work was carried out in collaboration among all authors. Author EVR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VS, RM, AQH, GRP and GS managed the analyses of the study. Author EVR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: An acute phase response to tissue injury leads to release of pro inflammatory and anti inflammatory cytokines. TNF alpha is an early pro inflammatory cytokine that released in SIRS and largely responsible for clinical manifestation of sepsis. The release of TNF alpha is influenced by messenger RNA transcription of TNF alpha gene. In patients with severe sepsis genomic polymorphism with in the TNF locus found to be associated with TNF alpha production and outcome.
Objectives: To evaluate genetic polymorphism of TNF alpha gene at c 850 t locus, influence on TNF alpha expression and on outcome.
Materials and Methods: A prospective cohort study conducted at our institute between June 2007 to 2009 in 100 cases. Serum TNF alpha levels measured by using ELISA .TNF alpha polymorphism done at c850t locus in 100 patients and were compared with 70 controls who were normal subjects. By using MEDCALC software mean and standard deviations were calculated, continuous variables were compared using t-test. ROC curves were used to determine the predictive capability of the variables.

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Results: The most common polymorphism observed was CT in 51 patients. The significant different TNF alpha level expression between the three groups were observed. Significant Tallele was observed in cases (100) when compared with controls (70), p= 0.0002.

Conclusion: Genetic polymorphism of TNF alpha gene may play critical role in stress response and outcome of the patient but it needs to be validated in large number of population.

Keywords: Tumor necrosis factor alpha; gene polymorphism; major surgery.

1. INTRODUCTION

Response to trauma is part of the integrated, organized systemic reaction that encompasses a wide range of endocrinological, immunological, inflammatory and hematological components. The inflammatory response to injury and activation of cellular process are inherently designed to restore tissue function and eradicate invading micro organism. In response to tissue injury acute phase proteins are released by liver, stimulated by cytokines which is known as acute phase response. IL-1, IL-6, IL-8 and TNF alpha are the pro inflammatory cytokines released in SIRS among which TNF alpha is released early [1]. Binding of TNF alpha and IL-1 to their cellular receptors induces member of secondary messengers such as G-protein, Adenylcyclase, Phospholipase A2 etc. Nuclear factor kappa beta plays a critical role in the transcription induction of pro inflammatory mediators Which is activated by endotoxins, viruses, oxidants and cytokines [2,3]. Transcription of TNF alpha is regulated by nuclear transcription factor such as kappa beta and is suppressed by short lived repressors. The genetically determined capacity of cytokine production and release will contribute to a wide range of clinical manifestations. The messenger RNA transcripts of TNF alpha gene may potentially influence the response of TNF alpha after a microbial challenge. In patients with severe sepsis genomic polymorphism within a TNF locus has been found associated with TNF alpha production and outcome [4]. TNF alpha plays a central role in the pathogenesis of acute inflammatory response and in some studies high levels of TNF alpha correlates with severity of disease [5]. Single nucleotide polymorphism at the TNF alpha loci within the MHC on chromosome 6 have been well charecterised. Genomic information may be used to identify the groups of patients with a high risk of developing sepsis, multi organ dysfunction and determining which patient will benefit from anti mediator strategies. Because of their genetic determination to high cytokine release in the inflammatory response will be the subject of future trial.Among those genetic variations i.e single nucleotide polymorphisms in the TNF alpha gene is of major interest with respect to genetically determined differences in the response to surgery or trauma.

2. MATERIALS AND METHODS

A prospective cohort study conducted at Kamineni hospital. LB nagar Hyderabad, India between June 2007 to June 2009 in hundred cases. Inclusion criteria was thoraco abdominal major surgeries and thoraco abdominal trauma cases. Exclusion criteria was preexisting organ failure. Serum TNF alpha levels were measured using ELISA. Minimal detectable value 3 pg/ml and significant value is considered as >39 pg/ml.Serum samples collected immediately after surgery(in 1hr) in elective cases, in trauma cases, samples collected in casuality within 1 hour of arrival. TNF alpha polymorphism evaluated at c850t locus by DNA isolation, PCR, Restriction digestion and Electrophoresis. Results were analyzed as CC Homozygous ,TT Homozygous, CT Heterozygous. Seventy normal subjects were taken as controls and TNF alpha genetic polymorphism evaluated at same locus and were compared with cases.

2.1 Statistical Analysis

Continuous variables were expressed are mean ± one standard deviation. continuous variables between survivors and nonsurvivors were compared using t test. To determine the effect on final outcome(mortality)of the studied variables univariate regression, logistic regression were used. To determine predictive capability of the same variables and to derive criterion values (value above which there would be hundred percent mortality i.e., 100% specificity of variables in predicting mortality) ROC Curves were used.

Software used: MEDCALC.

3. RESULTS

100 patients who were admitted to Kamineni Hospitals between the period of June 2007 and June 2009 with major trauma and after major surgery were analyzed. The average age of the patients was 39.3 ± 16.9 years in the present study. 69%were males and 31% were females. Out of 100 patients 67 patients underwent surgery and 33 patients were post trauma. 28 patients of the trauma group underwent surgery while 5 were managed conservatively. At the end of the study period 89% of the patients were alive and 11 patients died. On t Test comparison between survivors and nonsurvivors TNFalpha, ofsurvivors (28.9±69) was not significantly different from that of nonsurvivors(11.7±10.0)with p=0.4.Using univariate regression TNF alpha, found to have no significant effect on outcome (p=>0.002).When ROC curve was applied TNF Alpha found to be not predictive of out come (AUC-0.487,CI-0.38-0.58,p=0.89) (Fig.1). The polymorphism observed were CC(10), CT(51),TT(39). T allele observed were 129 and 71 C allele were observed (Fig. 2). Significant different TNF alpha expression between three groups were observed <0.03(b/w CC and CT), <0.001(b/w CT and TT) (Table 1). The different TNF alpha levels expression in various polymorphism were CC(13.3 pg/ml) CT(21.7 pg/ml) and TT(37.3 pg/ml).Patients with T allele have a 1.28(28%) higher chance of mortality than those with C allele but was not statistically significant.Significant T allele was observed in cases(100) when compared with controls(70) p=0.0002 (Table 2).

4. DISCUSSION

TNF alpha has been highly conserved across mammalian species during evaluation underscoring its critical role in innate immunity with approximately 80% aminoacid identity between human, Musmusculus, Bostaurus and canisfamiliaris TNF alpha proteins.TNF alpha is proinflammatory cytokine i.e important in defence against infection and is produced in large amount in response to stress. An increasing clinical experience with antibodies to TNF alpha in conditions including Crohns, RA and sepsis underscores the risks the lower availability of TNF Alpha. Many studies have suggested a link between TNF alpha promoter polymorphism and infections [6]. Moreover the functional significance of TNF alpha-308 SNP in transcriptional regulation is recognized and the 308A allele in the TNF alpha has been shown to be associated with higher levels of TNF alpha protein by number of studies [7,8,9,10].

Table 1. TNF alpha (levels) expression between each polymorphisms

	CC Homozygous(n-10)	CT Heterozygous(n-51)	TT Homozygous(n-39)
Avg	13.3	21.7	37.3
Std deviation	17.1	47.7	89.4
p value	<0.03(b/wCC &CT)		<0.001(B/W CT & TT)

Table 2. Odds ratio between cases and controls

Odds ratio	0.4311
95% CI	0.2772 to 0.6704
z statistic	3.735

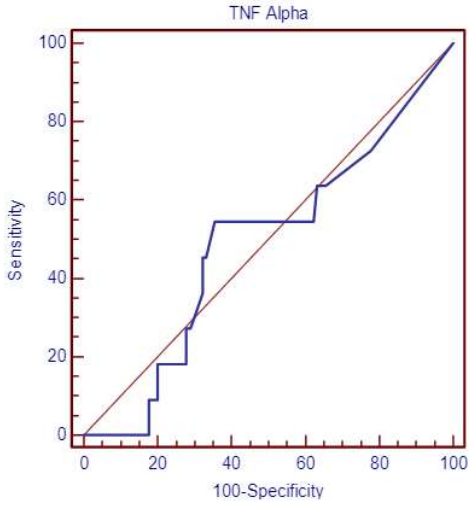

Fig. 1. ROC curve-TNF alpha vs mortality

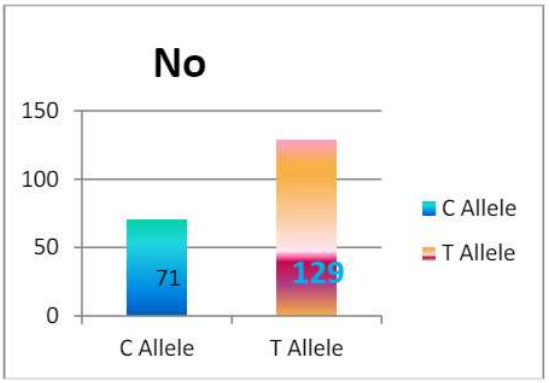

Fig. 2. Allele frequency

So initially we have studied polymorphisms of TNF alpha at 308 locus in the 15 patients and we have not observed any polymorphism at that locus. Hence another locusi.e C850T was studied and TNF alpha serum levels estimated. This study revealed significantly higher distribution of TNF alpha 850 CT genotype in a cohort of patients after major surgery and trauma and the significant T allele was observed in cases (100) when compared with controles(70). The study thus supports the hypothesis that inter individual variation in the TNF Alpha gene may impact on the stress following trauma or major surgery. In healthy individuals the difference in levels of expression of TNF alpha due to genotype in promoter region would be expected to be within physiologic limits in response to stress situations this, however, may not pertain to conditions of significant immune cell perturbations that may follow major injury which is often undertaken in patients already compromised immunologically by advanced age and comorbidities. In this scenario an impaired TNF alpha response to an insult might differentiate an adequate as oppose to a clinically relevant suboptimal response to stress. In this study significant different TNF alpha levels expression was observed in three groups and the patients with T allele have a higher chance of mortality than with C allele, but was not statistically significant. This was contradicted by some studies in which increased TNF alpha response [11,12] as well as poor prognosis [13,14] were observed in postoperative patients.

With this study we can state that a pattern emerged specifically in polymorphism of TNF alpha gene that supports the paradigm that inter individual variation of response may determine important clinical outcomes. It is not possible to identify all the genetics confounding variables that might predispose to infections; nevertheless in light of studies showing associations of significant expression of TNF alpha in stress and higher chance of mortality in patients with T Allele compared with C allele and presence of sinficant T allele in cases when compared with controles support the hypothesis that the presence of T allele(CT,TT) could be predictive factor for outmore after major surgery or trauma though it needs to be studied in larger numbers. Moreover by study with no pattern emerging for other cytokines,suggest that efforts to understand and apply polymorphism in TNF alpha within clinical trials and novel treatment approaches may have the greatest rationale in infection profile access in high risk surgical patients or trauma patients.

5. CONCLUSIONS

1. TNF alpha levels varied with CC Homozygous, CT Heterozygous, and TT Homozygous groups for TNF alpha c850t locus
2. T allele of TNF alpha had a higher chance of death
3. T allele of TNF alpha 850 was found significantly as compared with controls

6. LIMITATIONS OF THE STUDY

1. TNF alpha 850 locus has not been studied in general population.This is a prerequisite before it is used as background body type predictor for prognostication
2. The model of the study needs to be further fine tuned taking all confounding factors into consideration
3. Age, type of injury, time interval before presentation, organ dysfunction at admission need to be matched in future study
4. Single nucleotide polymorphism needs to be validated in large group of populations

CONSENT AND ETHICAL APPROVAL

The study was approved by ethical committee in kaminenihospitals, LB Nagar (KHL/DNB/SURG/oo1/12). Written informed consent was obtained from participants in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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