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Major Histocompatibility Complex Class I-related Chain A and B Gene Expression in Sepsis Patient

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ABSTRACT Major histocompatibility complex class I-related chain A and B (MICA/B) function in the regulation of protective responses due to stress induced expression. One of the major causes of the morbidity is sepsis. The objective of this study was to measure the mRNA levels of these genes in the control and sepsis patient groups. Data showed increases in mRNA concentrations of MICA and MICB in the Patient Group in contrast to the Control Group. However this level was found higher than MICA mRNA concentration in the patient group. The current study is the first report according to the researchers' knowledge representing the significant increases in the MICA and MICB expressions in the sepsis. This might provide a useful data for emphasizing these molecules as sensitive biomarkers. Further research is needed to enlighten the detail mechanisms of the MICA/B roles and to develop new treatment approaches.

INTRODUCTION

Major histocompatibility complex (MHC) class I genes encoding polymorphic peptide-binding chains mediate the intracellular antigen recognition by cytotoxic T cells. MI A functions in presentation of antigen or recognition of T cell (Bahram et al. 1994). Wang et al. (2016) indicated that MICA and MICB may broadly regulate protective responses due to stress induced expression and recognition of MICA and MICB. It was reported that MICA and MICB also increased in the patients who had chronic liver disease and hepatocellular malignancy (Chen et al. 2006; Kahraman et al. 2010).

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Sepsis and also related syndromes are a major and common cause of morbidity and mortality in intensive care units (Cherian et al. 2007). The inflammatory response involving leukocytes and other inflammatory cells activation leads to oxidative stress in critical illness. Previous studies demonstrated a link between several postulated molecular mechanisms of sepsis generation and reactive oxygen species imbalance through antioxidant systems. However, there is not much research about sepsis and MICA and MICB gene expression in the related field.

This study aimed to investigate the response of MICA and MICB to evaluate the potential of these genes as sensitive biomarkers and provide fundamental data for developing the new treatment approaches in relation to sepsis.

MATERIAL AND METHODS

The curent study was carried out in line with the decision of Ethic Committee of Çukurova

University at Balcali Hospital. Individuals were seperated into two groups as control and sepsis patients. All groups were examined physically and controlled for several laboratory parameters.

Blood specimens (3 mL) from patients were collected from the venous blood into EDTA containing tube. For isolation of WBCs, plasma and cells were separated by centrifugation (4000 U min^{-1}, 20°C, 5 min) immediately after blood collection. After centrifugation the supernatant above the pellet was removed and the cell pellet was treated with red blood cell lysis buffer to lyse contaminating erythrocytes. The pellet was then washed twice using PBS-Dulbecco® (Gibco, Paisley, UK) and centrifuged at each step (13000xg, 30sec). The final pellet was dissolved in 2 mL of buffer from the RNA isolation kit. RNA isolated from WBCs of the patients and controls was stored frozen at -80°C, and gene expression analysis was performed. mRNA was isolated from WBCs using the High Pure RNA Isolation Kit (Roche Diagnostics, Mannheim, Germany). Quantitative PCR was performed using the LightCycler® instrument with 4 µL of Light-Cycler Taqman master mix, 0.2 mM of oligonucleotide primers and 5 µL of cDNA solution. Amplification was performed for 10 minutes at 95 °C, followed by 40 cycles of 10 minutes at 95 °C, 30 seconds at 60 °C, and 1 seconds at 72 °C. Melting curve data were collected from 95 °C to 60 °C, at 72 °C steps. Relative gene expressions were calculated from the threshold cycles in relation to housekeeping gene, to untreated controls or healthy donors, respectively. PCR primers were as follows (Mica forward 5'-GTA TTG GGA CCG GAA CAC AC-3', Mica reverse 5' -ATG CTC TGG AGG GTG TGA GA-3', Micb forward 5'-TGC CAT GAA GAC CAA GAC AC-3', Micb reverse 5' –GGG GCA CTG TTC TCC TGA T-3', SDHA forward 5'–ACA CAG ACC TGG TGG AGA CC-3', SDHA reverse 5' – CAA AGG GCT TCT TCT GTT GC-3') The Real-Time PCR was performed using the following cycle parameters: initial enzyme activation at 95 °C for 10 min; followed by 45 cycles of 95 °C for 10 s, 60 °C for 20 s, and 65 °C for 30 s. Following the amplification phase, a cooling step was performed at 40 °C for 10 s (ramp rate of 1.5 °C/s).

Statistical Analysis

Statistical analysis of data (Mean ± Standard error) were performed by SPSS Statistics 15.0 (SPSS, Chicago, IL) package programs. Homogeneity of variance was controlled between the groups to evaluate the data distribution before the analysis. Differences between control and patient group were compared statistically by both One way ANOVA and paired Student t ($P<0.05$).

RESULTS

Individuals were aged <18 years and >60 years in both groups and half of individuals were female. MICA and MICB gene expressions were negative in the all individuals of control group. In the patient group MICA gene expression were only negative in 9th patient. Negative gene expressions were found in MICB in three patients (individual 7, 9 and 13). Nevertheless, positive MICA and MICB gene expressions were recorded in the other patients except these (Table 1). Therefore, three patients were eliminated during the statistical analysis for being out of the normal distribution.

Table 1: Gene expression response of control and sepsis patient groups

Individuals (N=22)	*Control group*		*Sepsis patient group*	
	Mica	*Micb*	*Mica*	*Micb*
1	Negative	Negative	Positive	Positive
2	Negative	Negative	Positive	Positive
3	Negative	Negative	Positive	Positive
4	Negative	Negative	Positive	Positive
5	Negative	Negative	Positive	Positive
6	Negative	Negative	Positive	Positive
7	Negative	Negative	Positive	Negative
8	Negative	Negative	Positive	Positive
9	Negative	Negative	Negative	Negative
10	Negative	Negative	Positive	Positive
11	Negative	Negative	Positive	Positive
12	Negative	Negative	Positive	Positive
13	Negative	Negative	Positive	Negative
14	Negative	Negative	Positive	Positive
15	Negative	Negative	Positive	Positive
16	Negative	Negative	Positive	Positive
17	Negative	Negative	Positive	Positive
18	Negative	Negative	Positive	Positive
19	Negative	Negative	Positive	Positive
20	Negative	Negative	Positive	Positive
21	Negative	Negative	Positive	Positive
22	Negative	Negative	Positive	Positive

The mRNA concentration of MICA was measured as $4.91x10^{-7}\pm0.55x10^{-7}$ in the control group though its concentration was 0.978±0.175 in the

sepsis patient group. There were observed an $2x10^6$ fold increase in the patient group ($P<0.001$) (Table 2).

MICB mRNA concentration was observed as $2.06x10^{-7}\pm0.60x10^{-7}$ in the control group whereas it was $2.75x10^5\pm0.48x10^5$ in the group of sepsis patients. It was found as $1.4x10^{12}$ fold increase in the MICB mRNA concentrations of sepsis patient group ($P<0.001$) (Table 2).

DISCUSSION

Septic shock caused by a Gram-negative bacteria infection results in death generally. A specific response corresponding to stress and stimulus type is induced by several factors impairing the homeostasis. Patient can cope with this by releasing various agents to eliminate or restrict the injury development. Thus, it was also emphasized that the significance of evaluation of selected systems which participate in neutralizing the stress inducers should be taken into account (Madej et al. 2007). In addition, it is also important to evaluate the evidence of relation between sepsis and stress biomarkers. On this basis, there were a lot of studies demonstrating the reactive oxygen species as relevant mediators in sepsis and septic shock in both animals and humans (Tsuji et al. 1996; Kwiecien et al. 2016). Increased level of thiobarbituric acid reactive substances and decreased levels of antioxidants are reported indicating the lipid peroxidation resultant by oxidative stress in the plasma of patients with septic shock (De Winter et al. 2005). In addition, Kapoor et al. (2006) suggested the increased antioxidant enzyme activities (superoxide dismutase, catalase and glutathione peroxidase) in the neonates with septicemia. Authors concluded that patients defense mechanisms deteriorated against free radicals.

Although there are a lot of data showing the close relationship between oxidative stress biomarkers and sepsis, there are no studies investigating the function of MICA and MICB gene expression in sepsis. The present study is a significant step in the direction to demonstrate the MICA and MICB gene expression increasing as a cellular stress in sepsis.

Polymorphic stress-regulated molecules Human MHC class I chain-related MICA and MICB are known as cell stress sensors. They have been characterized as the human ligands for NKG2D (Nielsen et al. 2015). MIC molecules expressed on a wide range of transformed epithelial cell surface are sensitive to immune responses mediated by NK and T cells. Despite their absence in normal adult tissue, their expressions are found in epithelial, endothelial and activated CD4+ and CD8+ T cells in the condition of stress veya stress situations (Andresen et al. 2007; Jordanova et al. 2008).

The current study demonstrated the alterations in the levels of MICA and MICB gene expression in comparison between sepsis patient group and controls. MICA and MICB are transcribed in endothelial cells, keratinocytes, fibroblasts, monocytes, gastrointestinal epithelial tissues and epithelial cell lines in humans under normal conditions. Responsive induction of MIC molecules were shown on different cell surfaces after several condition such as heat shock (Groh et al. 1998), oxidative stress (Yamamoto et al. 2001), and infections (Groh et al. 2001). Low levels of MICA expression in normal cells might be a protective mechanism to prevent the NK cell-mediated MIC-dependent cytotoxicity (Molinero et al. 2006). This was accompained with the researchers' data which the MIC A and MIC B expressions were very low in the control group in contrast to patients with sepsis. Wang et al. (2008) recorded that IFN-γ upregulated the MIC molecule expression on the surface of monocyte in the human PBMC population. Several responses were reported including decreases and increases in MICA and MICB gene expresion in the literature. For instance, Jinushi et al. (2005) studied the role of MIC A showing elevated transcripts in patients with hepatocel-

Table 2: mRNA concentrations of MIC A and MIC B in the sepsis oatient and the control group. Each data point represents the mean±standard error (n=22). Asterisks indicates the significant difference ($P<0.001$) between control and the patient group

mRNA concentration	*Control group*	*Sepsis patient group*	*Fold increase*
MIC A	$4.91x10^{-7}\pm0.55x10^{-7}$	$0.978\pm0.175^*$	$2x10^6$ ($P<0.001$)
MIC B	$2.06x10^{-7}\pm0.60x10^{-7}$	$2.75x10^5\pm0.48x10^{5*}$	$1.4x10^{12}$

($P<0.001$)

lular carcinoma. In the healthy inidividuals and patients who had autoimmune hepatitis, primary sclerosing cholangitis and primary biliary cirrhosis, MIC A/B expression was also examined (Holdenrieder et al. 2007). Kahraman et al. (2010) demonstrated that the induction of MIC A/B in stress conditions may function as a mechanism for the damaged, infected, or malignantly transformed cells determination. The exact mechanisms remain to be clarified by further studies. Therefore, assessment of MIC A/B serum levels could be identified as a novel index to assess. MIC A/B contribution to disease severity in patients with nonalcoholic steatohepatitis (NASH) was the first study suggesting the significant function of MIC A/B in liver injury conducted by Kahraman et al. (2010). In addititon, their results has a provision for further research on the basis of investigation the mechanisms of MIC A/B expression associated with NASH, as well as their potential importance as antigen-presenting molecules for activating hepatic NK cells. This study also found the higher expression rate in MICB than MICA in the patient group which was also in accordance with the study showing higher expression levels of MICB in the patients with NASH (Kahraman et al. 2010).

It was indicated that stress (such as ischaemia-reperfusion (I/R) injury) related MIC gene expressions are observed in epithelial cells. Zou et al. (2017) also suggested a substantial increased expression in MICA in pulmonary epithelial cells resultant by scald indicating the importance of MIC A that might reflect the level of organ injury related with burns. In addition, Sconocchia et al. (2009) investigated the relationship between MIC A/B expression and innate inflammatory infiltrate in renal cell carcinoma (RCC) and they demonstrated the possible reason of expression of these molecules in most RCC cells might expose them to NK cell recognition. Furthermore, the significance of MICA expression in the regulation of macrophage/NK cell crosstalk during inflammation was also stated due to the correlation between proinflammatory cytokine secretion and the level of MICA upregulation on macrophages (Eissmann et al. 2010). Similarly, an induction of MIC expression was also observed during cytomegalovirus infection (Nielsen et al. 2015). It is concluded that the over expressed MICB gene in the patients with multiple sclerosis is an significant criterion of this depending upon the interaction between MICB and its receptor on CD8+T or NK cells (Nielsen et al. 2015).

CONCLUSION

The significant increases observed in the expressions of both MICA and MICB in the sepsis patient group is the first report according to the researchers' knowledge. Therefore, the current study might provide useful data for emphasizing these molecules as sensitive biomarkers in sepsis. Nevertheless, there is a need for further research to enlighten the detail mechanisms of the MIC A and MIC B roles in sepsis and also to develop new treatment approaches in this area.

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