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# Identification of marker proteins in preterm and term birth: Relationship of rankl and HSP70 in the cord blood

**Baris Uzunok[1], Selcuk Gurel[2]**

*[1]University of Usak, School of Medicine, Department of Physiology, Usak, Turkey*
*[2]Usak Oztan Hospital, Department of Pediatricians, Usak, Turkey*

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**Abstract**

The goal of this research was to investigate at the expression profiles of heat shock protein 70 (HSP70) and receptor activator of nuclear factor-kappa B ligand (RANKL) in term and preterm infants. Preterm birth (n=13) and term birth (n=13) cord blood samples were studied. In the cord blood serum protein expression levels of the HSP70 and RANKL were measured by ELISA. When compared to the term birth group, preterm birth had greater RANKL concentrations. When comparing the preterm birth study group to the term birth study group, protein expression of HSP70 and RANKL was not shown to be associated. These results indicate that HSP70 and RANKL levels in cord blood may affect both prenatal and postnatal treatment approaches.

**Keywords:** RANKL, HSP70, preterm birth, term birth

## Introduction

According to recent statistics data, the rate of preterm delivery has grown by one-third in the last 25 years. Preterm birth (PB) is the main cause of newborn morbidity and death, defined as delivery before 37 weeks of pregnancy [1,2]. Premature birth may be due to anatomical, physiological and biochemical, genetic reasons originating from mother or infant. Therefore, anthropometric measurements of a term-born infant and a preterm infant result in proportional and functional differences in organ functions and body fluids. This creates differences and difficulties in the approach, both causal and outcome. On a clinical level, it is important to show the compatibility or incompatibility of the data from women who have preterm labor and the data collected from the newborn. Response to PB may vary markedly between babies: while some babies may show an initial response or stable profile, in others response may be delayed- a process that requires the interaction of numerous molecules. Several immunological molecules play essential role in pathophysiological mechanisms in PB. Of these molecules, various cytokines, heat shock proteins (HSP) have been upregulated by means of various stimuli against the stress or inflammation. These stress proteins are evolutionary conserved peptide structure and express in the all cells [3,4]. For these reasons, some of immune-related molecules for example, HSP70 and receptor activator of NFKB ligand (RANKL) have been proposed. HSP70 is one of the most elevated protein that synthesized against the cellular stress and acts as a molecular chaperons function in cellular homeostasis and transport repair process. The heat shock response, which is characterized by increased production of heat shock proteins (HSPs), is triggered when cells and tissues are exposed to extreme environments that cause acute or chronic stress. HSPs, acting as molecular chaperones, is involved in regulating cellular homeostasis and promoting survival. If the exposure is too severe, this activates signals that lead to apoptosis, which is programmed cell death, thus striking a delicate balance between survival and death. In addition to external stimuli, HSPs are induced during normal cellular growth and development by a variety of conditions other than stress [5]. RANKL is a tumor necrosis factor family transmembrane protein that is produced on many cells, tissues and organs and mainly essential role in osteoclastogenesis and bone resorption [6,7]. RANKL binds to its receptor RANK on

***Corresponding Author:** Selcuk Gurel, Usak Oztan Hospital, Department of Pediatricians, Usak, Turkey
E-mail: gurelselcuk@gmail.com

the osteoclast precursor and activate RANK signaling cascades [8]. The physiological mechanisms of HSP70 and RANKL between the term and preterm birth have not been clarified.

We hypothesized that if there is a difference between the results obtained from cord blood, it can be used between the markers of preterm birth as a marker in the follow-up of preterm babies. The goal of the study is to correlate HSP70 and RANKL profiles in cord blood with pregnancy-related features and newborn problems induced by preterm delivery.

## Materials and Methods

### Sample Collection

Cord blood samples (26) were randomly collected by direct aspiration from the umbilical vessels at the delivery room of the Department of Obstetrics and Gynecology, Medical Park Hospital, Usak, Turkey and subsequently used for serum separation. The current study was confirmed by Ethical Committee of Usak University of Medical Sciences (Reference Number: 214-08) and written informed consents were obtained from all participant's parents.

### Protein measurement by ELISA

RANKL and HSP70 serum protein levels were analyzed using an ELISA (USCN Life Science, China) for preterm birth (n=13) and term birth (n=13) cord blood samples using the manufacturer's protocol. Absorbance readings were performed at 450 nm as well as 630 nm as reference wavelength on Microplate spectrophotometer (Thermo Fisher Scientific, Finland). Concentrations of unknown samples were determined from a standard curve obtained with the standards. According to the kit protocol, the sensitivities were 1.27 pg/ml and 1.33 ng/ml; intra-assay variations CV<10% inter-assay variations CV<12% for RANKL and HSP70 respectively.

### Statistical Analysis

The SPSS statistics software version 17 was used to do the statistical analysis (SPSS, Chicago, USA). The mean and standard deviation are used to represent the data (SD). The Kolmogorov-Smirnov test was used to assess the normality distribution of the collected data, and the Mann-Whitney U test was performed to calculate the mean difference between two groups. For correlation of two groups, the Nonparametric Spearman's test was applied. P-values of less than 0.05 were considered significant. For all comparisons with a statistically significant minimum P<0.05 level, ef- fect sizes and post hoc power analysis were performed in G* Power software. In the study, the minimum and maximum effect sizes were calculated as 0.400-0.780. The strength of the test was calculated as minimum 62% and maximum 89%

## Results

To determine the differences of HSP70 and RANKL between the preterm and term birth cord blood samples. The concentrations of these target molecules were determined by ELISA. The concentrations of studied proteins were observed in the preterm and term birth subjects. The protein expression level results of HSP70 and RANKL for control term and the study preterm group were represented in table 1 as a median [min-max]. Although the results of this study were shown in Figure 1A for HSP70 expression which was statistically significant higher in the preterm birth compared to term birth, as represented in Figure 1B, the levels of RANKL were not statistically significant for two groups. In addition to, there were no significant correlation was found between HSP70 and RANKL expression in term control and preterm study groups.

To address this issue, the importance of mechanical stress markers in pathophysiology was supported by the results of our study groups. HSP70 and RANKL levels in cord blood will affect both prenatal and postnatal treatment approaches.

**Table 1.** Serum Hsp 70 and RANKL concentrations (ng/ml; pg/ml) in umbilical cord blood, presented as Median [min-max]

| | Preterm Study Group (n=13) | Term Control Group (n=13) | P Value |
|---|---|---|---|
| **HSP70 (ng/ml) Median [Min Max]** | 45.0 [15.7-83.3] | 21.3 [9.5-173.1] | <0.05 |
| **RANKL (pg/ml) Median [Min-Max]** | 187.4 [115.9-343.6] | 142.5 [107.5-414.8] | >0.05 |

Data are presented as median [Min–Max]; p value <0,05 is significant

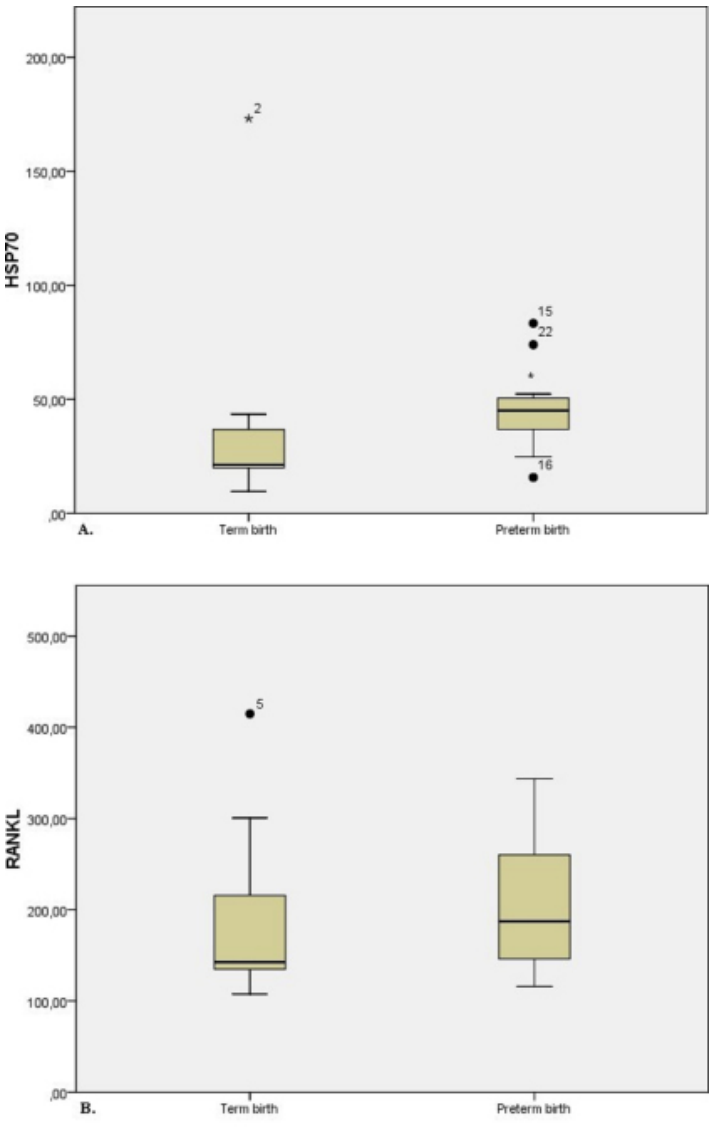


**Figure 1.** A. Box plots of HSP70 concentrations in blood serum samples in term control and preterm study groups. B. Box plots of RANKL concentrations in blood serum samples in term control and preterm study groups

## Discussion

Premature birth is one of the leading causes of infant death and morbidity. The majority of preterm births are thought to be caused by cytokines generated for various causes. Premature newborns with an insufficient congenital immune response may be more susceptible to infection [9]. It has been conducted in some studies that immunogenic molecules such as cytokine levels differ in preterm birth compared to term birth. There are multiple immunogenic molecules causes and factors associated with preterm delivery [2,6,10]. There are multiple immunogenic molecules causes and factors associated with preterm delivery. Our study has been conducted in preterm birth study group to find out the association of HSP70 and RANKL expression. The HSP70 levels were found to be high in preterm birth cases who were resistant to treatment. According to this study, it was predicted that HSP70 could be a biomarker in evaluating the curative effects of treatment for preterm labor [11]. In another study, it was emphasized that the difference in HSP70 level showed pathophysiological differences between early and late-onset preeclampsia patients [12]. It has been reported that HSP70 serum level is significantly higher in newborns with asphyxia compared to newborns without asphyxia [13]. In addition to, it was shown that the rate of HSP70 in cord blood in cesarean and vaginal births was significantly lower than in patients with gestational diabetes by Mrkaic et al. [14]. The second point that RANKL, Mitsuhashi et al. [15] reported that HSP70 has a modulating effect on RANKL. It has been reported that differences in RANKL levels which might be related to lower bone turnover was detected in pregnancies complicated by preeclampsia compared to the control pregnancy [16]. In preterm newborns, oxidative stress is a pathogenic stress factor. Premature newborns are especially prone to oxidative stress because the oxidant/antioxidant equilibrium condition begins before birth [17]. This state of stress causes changes in some mediator activity as well as inflammation. It is known that cytokines play a role in the immune system as well as in placental development and birth. Although there were similar studies with other mediators, there was no such study with HSP70 and RANKL [9,18].

## Conclusion

The discovery of a new biomarker molecule that may be used to assess the degree of mechanical and psychological stress experienced during preterm birth should help with the evaluation of the next step. Stress indicators in cord blood have been found to be biomarkers of mechanical and/or psychological stress, according to research [13]. In addition, the link between HSP70 and RANKL has been studied previously. HSP 70 is presently recognized a sensitive and effective indicator for the evaluation of psychological stress as a result of these studies. The goal of this study was to identify markers that may be used to assess preterm and term births. We evaluated for indicators of both mechanical and psychological stress, and we found HSP70 and RANKL levels in cord blood from preterm and term infants. The link between these variables and the stress caused by preterm birth was examined. The significance of this finding might be supported in larger studies.

**Conflict of interests**

*The authors declare that they have no competing interests.*

**Financial Disclosure**

*All authors declare no financial support.*

**Ethical approval**

*The current study was confirmed by Ethical Committee of Usak University of Medical Sciences (Reference Number: 214-08) and written informed consents were obtained from all participant's parents.*

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