

## DECREASED HUMAN MILK IMMUNITY IN DOUBLE LIFE MOTHERS

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### ABSTRACT

*Double life mother (DLM) is expressing condition of stress that can decrease the quality of human milk immunity. This research was aimed to study of influence of DLM to decrease the quality of human milk immunity at double life mother and which is non double life mother. This cross-sectional study on the influence of stress to human milk immunity used 28 healthy subjects of DLM and 23 healthy subjects of non-DLM. The samples criteria were age of mother, period of breastfeeding, first child and area of the samples. This research used psychoneuroimmunological concept based on dependent variables in blood (cortisol, CD4 and CD8) and in human milk (IgG and IgA). The results of statistical analysis showed that biological condition was not different between DLM and non-DLM. Manova analysis of dependent variables was not significant ( $p > 0.05$ ), with clustering showed four sub-groups (DLM1 = 82.14%, DLM2 = 17.86%, non-DLM1 = 78.26% and non-DLM2 = 21.74%) was not significant ( $p < 0.01$ ). Discriminant analysis on dependent variables in four sub-groups revealed that cortisol, CD4 and IgA served as discriminant variables. In conclusion, there is a decrease of human milk immunity in double life mothers.*

**Keywords:** psychoneuroimmunology, mucosal immunity, stress

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### INTRODUCTION

Infant mortality rate (IMR) is one indicator of health level in a country. According to Indonesian Health Profile 1992, the infant mortality rate in Indonesia was 60 per 1000 living birth and underfives mortality rate was 84.4 per 1000 underfives. SUSENAS data in year 1999 showed that infant and underfives mortality rates remained high. Infants receiving human milk also remained showing problems in the aspect of infectious diseases morbidity (Mestecky et al. 1991; Kakai et al. 1995; Subiyanto 1997; WHO 1985), which could result from the reduction of immunity (Hamosh et al. 1996). Infectious disease morbidity in human milk-receiving infants was higher in those of breastfeeding mothers who were double life mothers as compared to those who were not (Subiyanto 1997).

Poor nutritional condition is related to low immunological status, either cellular or humoral. Individuals who are having stress often have loss of appetite that may affect an individual's nutritional condition, so that correlation between stress and immune function can serve as a background that leads to nutritional deficiency (Robinson et al. 2002). Human milk is a dietary source for infants, reliable both in its quality and quantity aspects. Human milk quantity is reflected from production capacity of mammary gland,

while the human milk quality is reflected in the composition of nutrients, vitamins, and electrolites, as well as the components of immunity (Cruz et al. 1982; Hamosh et al. 1996; WHO 1985). Human milk quantity and quality depend on mother's condition. In several areas in Indonesia, mothers also work to support fulfilling the needs of the family, or, in other words, play a double life, even though they are within the breastfeeding period. The number of double life mothers in lactating period indicates an increase from one year to another (Sanjaya 1988; Soemilah 1979).

Stress may decrease immunity (Robinson et al. 2002), while double life reflects such condition more (Riordan & Nicholas 1990). Breastfeeding mothers who work outside their homes may indicate human milk quantity and quality reduction resulting from fatigue or stress (Dermer 1995; Riordan & Nicholas 1990). However, the difference of the effect of stress condition on human milk immunity in breastfeeding double life and non-double life mothers was unclear. Human milk is a biochemical defense that has a role in natural immunity, in which it can protect the body from various gram-positive bacteria, since it contains nutrients, including lactooxidase and neuraminic acid that are antibacterial against *E. coli* and *staphylococcus*. In addition, human milk also contains several elements of immunity, including cellular, humoral components, cytokines and

cortisol (Hamosh et al. 1996; Hanson et al. 1994; Sirota et al. 1995; Zheng et al. 1995). Types of immunoglobulin detected in human milk are sIgA, IgA, IgG and IgM (Hamosh et al. 1996). Human milk contains several cytokines, such as interleukin-1 (IL-1); IL-6, IL-8, IL-10, TNF-alpha and macrophage growth stimulating factor (M-GSF). The presence of cortisol within human milk is suggested to have correlation with stress in mothers (Hamosh et al. 1996). All elements of immunity may help overcoming the development of infectious process in infants' body (Zheng et al. 1995).

Infant morbidity rate resulting from infectious disease remains high, particularly in double life mothers as compared to those who are non-double life. One of the factors in human milk quality that is reflected in human milk immunity. It has been recognized that stress may decrease immunity, in which double life reflects stress condition more. This study investigated the difference of stress effect on human milk immunity (using the parameters of IgA and IgG level as well as CD8, CD4 lymphocytes, and cortisol in blood) in breastfeeding mothers who experienced stress (breastfeeding double life mothers) and non-stress (breastfeeding non-double life mothers).

## MATERIALS AND METHODS

This study was focused on breastfeeding mothers to disclose the mechanism of the effect of breastfeeding double life mothers on the response of immunity in human milk. This study used cross-sectional design. Samples were taken from breastfeeding mothers population based on sample criteria in all peripheral working areas of Surakarta who gave consent to participate in this study. The sample criteria were as follows: breastfeeding mothers are mothers who breastfeed their own infants, mothers' age is breastfeeding age of 18-25 years, mature immunologically, breastfeeding period is from delivery to around 3-5 months, the first child of a breastfeeding mother is the child with her first husband, double life mothers are breastfeeding mothers who have routine work outside the home and do not take the infant to the place of work for more than 5 days a day and they receive salary from the company, non-double life mothers are breastfeeding mothers who have non-salary receiving work outside the home, sample areas are the areas where the breastfeeding mothers live at the periphery of the city of Surakarta, stress condition is the reflection of body biological response against the presence of (physical and biological) load indicated by blood cortisol, and, as additional criterion, the breastfeeding mothers are within normal range, either physically or laboratorically.

Sample size was determined according to the formula from Higgins and Klinbaum (1995). This study involved 28 double life mothers and 23 non-double life mothers. Cortisol level measurement was carried out using Fluorescence Polarization Immunoassay (FPIA) method by means of TDxFLx System instrument, while IgA and IgG measurement in human milk was undertaken using Turbidimetri (Turbiquant) method, and CD8 and CD4 lymphocytes were measured with immunofluorescent (ortho-mune) method. To find the difference in human milk immunity response in stress and non-stress breastfeeding mothers we used statistical tests, i.e., Manova test to find the difference of human milk immunity response in double life and non-double life mothers, and discriminant test to obtain discriminant variable used in explaining the mechanism of immunity reduction based on psychoneuroimmunological concept.

## RESULTS

Results revealed that the samples of breastfeeding mothers from peripheral areas of Surakarta had age of 22.7 +/- 2.01 years, the age of breastfeeding period was 3.9 +/- 0.79 months, either in double life (28 persons) or non-double life mothers (23 persons) who had a first child. From the result of homogeneity test on control variable data on double life and non-double life mothers, it was found that there was no difference between both groups ( $p > 0.05$ ). This indicated that those variables were not possible to provide effect on immunologic response of the body (CD4 and CD8 lymphocytes, IgG and IgA levels). Stress condition could be demonstrated by the increase of blood cortisol secretion (Dimsdale & Moss 1980). Human milk immunologic quality has an important role in humoral adaptive immune response. However, low homeostatic capability in infants since it has not been fully developed from the thymus. This may result in the susceptibility to infection, so that infants need protective power from their mothers through the human milk. The susceptibility in infants is a reflection of adaptive learning stage on the interaction with microorganism (Suharyono 1992). The result of this study indicating cortisol level, CD4 and CD8 lymphocytes in mothers' blood and IgA level in the human milk can be seen in Table 1.

The result of Manova indicated no significant difference between double life mothers and non-double life mothers ( $p > 0.05$ ), so that clustering was made, whose results can be seen in Table 2. From the result of clustering, discriminant test was carried out, and three discriminant variables, i.e. IgA, CD4 and cortisol, as can be seen in Table 3.

Table 1. Cortisol level, CD4 and CD8 lymphocytes in mother's blood as well as IgG and IgA in human milk

Variables	Double Life		Non-Double Life	
	mean	SD	mean	SD
Cortisol	14.012	5.651	12.893	5.510
CD4	0.900	0.186	0.778	0.269
CD8	0.897	0.220	0.835	0.275
IgA	78.698	69.709	119.165	135.989
IgG	91.678	37.552	86.826	41.846

Notes : CD4 = lymphocyte ratio CD4 : total lymphocyte; CD8 = lymphocyte ratio CD8 : total lymphocyte ; Cortisol ( $\mu\text{g/dL}$ ); IgA ( $\mu\text{g/dL}$ ); IgG ( $\mu\text{g/dL}$ )

Table 2. Immune response grouping, between double-life and non-double life

Clusters	Double Life	Non-Double Life
1	23 (82.14%)	18 (78.26%)
2	5 (17.86%)	5 (21.74%)

Table 3. Results of immune response discriminant test in double life and non-double life

Discriminant Variables	Wilk's Lambda	Significance
Ig A	0.26608	0.0000
CD4	0.20584	0.0000
Cortisol	0.19153	0.0000

The magnitude of human milk immunity response pattern based on psychoneuroimmunological concept can be seen in Table 4.

Table 4. Magnitude of human milk immunity response pattern based on psychoneuroimmunological concept

Variables	Double Life		Non-Double Life	
	G1	G2	NG1	NG2
Cortisol	4.48	2.1	3.819	0.171
Ig A	1.942	30.017	2.46	53.609
CD4	18.544	24.805	12.769	31.368

Notes: G1 and G2 = double-life sub-group  
NG1 and NG2 = non-double-life sub-group

The magnitude of the pattern indicates complementative contribution values between variables.

## DISCUSSION

Immunity is an aspect of the body serving the function of defense, homeostasis, and immune surveillance, through interconnection between cytokines and neurohormones. Mammary gland that produces human milk has been proved to be able to function as the nutritive and protective material, by which human milk can decrease morbidity rate in the first six months of infant's life (Sunoto 1992). Immunoglobulin can be measured in blood serum, intraperitoneal fluid, as well as in human milk, in which the level sIgA reaches the highest peak until breastfeeding age of one year. IgG and IgM are also found in human milk (Hamosh et al. 1996). There are facts indicating that stress may increase the incidence of cancer, autoimmune diseases, and infectious diseases. Psychoneuroimmunology paradigm with stress cell concept is a fundamental view on the problems in psychoneuroimmunology based on stress cell, requiring an understanding on the correlation between brain and immune system through hypothalamo-pituitary-adrenal (HPA) axis and autonomic nerve system (ANS) (Norris 1986).

Prior to interpreting the difference in immunity between double life and non-double life mothers, we conducted multivariate Manova differential test (Table 1). The results of the Manova test provided two possibilities, difference or no difference. First, if difference was found, the analysis was continued with discriminant analysis to obtain variables that serve as the strongest discriminant response. Furthermore, the interpretation of different immunity response was based on the pattern of immunity response arranged according to the discriminant variables. Magnitude indicated in a variable in such pattern was not the mean, but the mean multiplied by Fisher's function coefficient (Norris 1986). Second, if Manova results revealed no difference in response, cluster analysis should be undertaken to find sub-group response in double life and non-double life (Table 2). Furthermore, the subgroups were regarded as new groups and subjected to discriminant analysis (Table 3), which was manifested in a pattern like those in the first step of analysis.

It has been recognized that stress behavior in breastfeeding double life mothers can be reflected in the fluctuation of blood cortisol. Several types of immunoglobulin, such as IgA, IgM, and IgG, can be detected in human milk. In this study, only IgA and IgG were examined since both types could indicate mucosal (mammary gland) resilience and both were measurable (Hamosh et al. 1996). However, to find mechanical bridge of immunity response in human milk, measurement of CD4 and CD8 lymphocytes in blood was undertaken, although both could also be found

within human milk (Hamosh et al. 1996). Therefore, the concept of psychoneuroimmunology is a multivariate concept that may reflect response of the body complementatively.

Cortisol is a steroid hormone produced in adrenal gland. Its role is important in blood pressure and heart function regulation. Cortisol production would increase when the body is exposed to stress, either physically or psychologically. Pituitary gland produces ACTH. ACTH production stimulates adrenal gland to increase its production and secrete cortisol. From the hypothalamus, pituitary gland receives signal that presents as CRH (corticotropin releasing hormone) that may stimulate pituitary gland to release ACTH. Therefore, during stress, there will be an increase of ACTH and CRH, resulting in rapid increase of cortisol (Putra 2005). The result of this study revealed significantly different cortisol level between stress and non-stress breastfeeding mothers (Table 1 and 3). In this study, stress condition is reflected in breastfeeding double life mothers (Riordan & Nicholas 1990). This confirmed the suggestion that psychological burden higher than physical burden could lead to higher cortisol secretion (Dimsdale & Moss 1980). Since all leucocytes, including lymphocytes, have receptors for cortisol, cortisol is able to modulate immune response, and high cortisol level serves as immunosuppressor (Glaser & Kiecolt-Glaser; Putra 2005) that may result in the suppression of CD4 lymphocyte (Table 4) (Besedovsky & Rey 2006). This was indicated from the results of this study, where there were differences in Ig levels in each groups (Table 1). Nevertheless, the one that could be used as a discriminant between both groups was IgA level (Table 3). This confirmed the findings that breastfeeding mothers who work outside the home could show a reduction in human milk quantity and quality resulting from fatigue and stress (Dermer 1995; Riordan & Nicholas 1990).

Based on the multivariate psychoneuroimmunological concept, we could interpret a discriminant variable that could be used as an indicator of immunity response in stress (double life) and in non-stress (non-double life) breastfeeding mothers, so that it could also be employed to interpret human milk immunity mechanism. In this study, the immunity response pattern viewed from psychoneuroimmunological concept (Table 4) is apparent. Therefore, the findings in this study can be used as a basis to solve health problems in the clinic and in the community, particularly those related to nutritive and protective role of human milk in infants.

Socioeconomic problems, particularly in developing countries, compels a change in women's role as they must have double life, both as a housewife and as

salary-seeking women whose workplace is not infrequently far away from their homes (Soemilah 1979). Data from Central Bureau of Statistics (Badan Pusat Statistik, BPS) in 2003, total workers in Indonesia were 100,316,007, 64.63% of which were male and 35.37% were female. Female workers are demanded to improve their capability and working capacity maximally without leaving away their role as woman. The provision of human milk to infants is the best way for improving human resource quality as early as possible. Human milk is the best food for infants, as it contains high nutrients needed for the growth and development of nerve and brain (Pusat Kesehatan Kerja Depkes RI 2007; Putra 2005), provides immunity against several diseases (reduces the risk of diarrhea, airway infection, allergy, as well as other infections), and establishes emotional bound between the mother and the baby (Glaser & Kiecolt-Glaser; Putra 2005).

## CONCLUSION

Double life in breastfeeding mothers decreases the quality of human milk immunity.

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