

## MASSAGE STIMULATION REDUCES TUMOR NECROTIC FACTOR-ALPHA AND INTERLEUKIN-6 IN PRETERM, LOW BIRTH WEIGHT WITH APPROPRIATE GESTATIONAL AGE NEWBORNS

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### ABSTRACT

Preterm newborn is susceptible from various stresses such as infections or non infections. During stress, immune response is activated by synthesizing and releasing of cytokines from activated immune cells into the circulation. One of the efforts to overcome the stress is massage stimulation. Several studies have been carried out to find out the benefit of massage stimulation. The objective of this study is to find out the effect of massage stimulation to reduce of TNF- $\alpha$  and IL-6 levels on preterm, low birth weight, appropriate to gestational age newborn. This was a randomized controlled trial. Subjects of the study were all preterm newborns with a stable medical condition, birth weight 1,500-2,499 gram, appropriate to gestational age. The study was carried out on October-December 2011, in neonatology ward, Sanglah General Hospital, Vali-Indonesia. The number of cases was 35 subjects and the number of controls was 36 subjects. The examination of TNF- $\alpha$ , IL-6 levels and birth weights before and after massage stimulation were performed on both groups. The level of TNF- $\alpha$  and IL-6 was examined by applying ELISA method. The level of confidence was  $\alpha = 0.05$ . All statistical analysis was performed by computer programme. There was different level of cytokine pro-inflammation TNF- $\alpha$  ( $p=0.025$ ) and IL-6 ( $p=0,001$ ) comparing before and after condition massage stimulation. Outcome of body weight were also different at before and after massage stimulation, with  $p$  value 0.042. The conclusion of this study is that massage stimulation can reduce the level of TNF- $\alpha$ , IL-6 and increasing of birth weight on preterm, low birth weight appropriate gestational age newborn. Based on this study, in the future, massage stimulation can be done in newborn with stable medical conditions by parents or trained staffs.

**Keywords:** massage, stimulation, preterm, TNF- $\alpha$ , IL-6

### INTRODUCTION

The advance of technology in perinatal care has caused greater numbers of survived preterm infants. However the preterm infants have a higher risk in disturbance in growth and development. Preterm infants are infants who were born on gestational age below 37 weeks, with birth weight under 2,500 grams. Preterm birth rate and preterm mortality rate is increasing. Preterm and low birth weight mortality rate

at Sanglah Hospital Denpasar on 2006 and 2007 were 97/540 (17.9%) and 41/439 (93%) respectively. The survival rate of preterm infants increase significantly. Preterm infants often suffered from infection, chronic respiratory problems, central nervous system such as cerebral palsy, neurodevelopmental and behavioural problems, visual, motor and language deficits.<sup>1</sup> These developmental problems can persist for longer term until adolescent in form of neuropsychology such as lower *Intelligence Quotient* (IQ), articulation problems, concentration disorder, learning disorder, emotional and behavioural disorder, motor disability, and other developmental disorders.<sup>2,3</sup>

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Preterm infants were susceptible suffering from various stresses such as infections or non infections which were caused by environmental overstimulation or invasive procedures that were given to the infant. Stress involved neuro-immune-endocrine systems. Infection that was occurred in preterm infants increased the synthesis of TNF- $\alpha$  and IL-6. Stress also increases the level of stress hormones.<sup>1,4</sup>

One of the stimulations that was given to preterm infants was massage stimulation. Massage stimulation is one of touch stimulation which is a physical contact from one person to other.<sup>5</sup> There are many advantages of massage stimulation, such as reduces the level of stress hormones, increases immunity, repair the blood circulation and respiration, induces intestinal function, increases body weight, reduce depression and tension, increases awareness, increases better sleep, reduces pains, strengthens bonding between parents/caregiver and infants, gives perception that the parent is a trusted person, and gives sense of secure.<sup>5,6</sup>

Massage stimulation provides stimulus that affect neuro-immuno-endocrine systems. Hypothalamus reduces corticotropin-releasing factor (CRH) production, thus the adrenocorticotropin hormone (ACTH) production by anterior hypofise also decreases. The activity of serotonin neurotransmitter increases which cause the increasing of glucocorticoid receptor-binding capacity. As the result, adrenal glands reduce production of glucocorticoid like adrenaline or other stress hormones such as epinephrin, norepinephrin, and cortisol. Finally, the release of pro inflammatory cytokine will decrease associated with macrophage inhibition.<sup>4</sup>

Tumour necrotizing factor- $\alpha$  cytokine as a non specific respone which is stimulated in condition where infection or non-infection stressor exist have a dominant role to enhance further inflammation responses and responsible to body metabolism condition. On high level, TNF- $\alpha$  acts as catabolism mediator (cytotokine *chacexin*) which affect the body weight. IL-6 cytokine as a marker of proinflammatory nonspecific and specific mediators, plays role to the infection and non infection stressors, whether inflammation condition still going further or not.<sup>7-10</sup>

Many studies found that massage stimulation in the form of tactile and kinesthetic stimulation in preterm infants gave positive results. However, massage stimulation were not common in all hospitals especially those who had intensive care for preterm infants. The study about benefits of massage stimulation in immunity preterm infants and low birth weight is not present in Indonesia. We found limited studies about the effect of

massage stimulation to the immune level of low birth weight and preterm infants. Based on that, the researcher will study the change of TNF- $\alpha$  and IL-6 invivo in preterm, low birth weight, and appropriate with gestational age infants.

## METHODS

This study is a randomized controlled trial. The target population was all preterm, low birth weight, and appropriate with gestational age infants. Accessible population was preterm, low birth weight, and appropriate with gestational age infants who were born and hospitalised in Neonatology division, west Cempaka and east Bakung ward, Department of Pediatrics Faculty of Medicine Udayana University Denpasar. Intended simple was preterm, low birth weight, and appropriate with gestational age infants who fulfilled the inclusion and exclusion criteria. Inclusion criteria were preterm infants (gestational age < 37 weeks) with stabile medic condition or had mild stress according to The Neonatal Infant Stressor Scale,<sup>11</sup> birth weight 1,500-2,499 grams and appropriate with gestational age(kurva Lubchenco), and the parents agree to participate in the study. Exclusion criteria were preterm infants with other diseases, preterm infants with mayor congenital anomaly, preterm infants with asphyxia, and preterm infant with mother suffered from immune deficiency diseases. Sample who fulfilled the criteria were included consecutively until the expected number was fulfilled.

The researcher had conducted initial study to find the mean value of TNF- $\alpha$  and IL-6 in preterm, low birth weight, and appropriate with gestational age infants. Based on the initial study to 10 subjects, the sample size for each group was calculated 32 subjects. This study used block randomization technique with block of 4 (using 4 letters in a block).

The variables that identified in this study was the independent variable : massage stimulation, and dependent variables:level of TNF-  $\alpha$ , IL-6, body weight, and controlled variables: length of hospitalization, invasive procedures, maternal immune status, nutrition, birth history, gestational age, other diseases, mayor congenital anomaly, asphyxia, and neonatal infections.

Subjects were collected by resident as research coordinator. Subjects were allocated in to 2 groups, treatment (group who got massage stimulation) and control (group who didn't get massage stimulation) groups by block randomization. The researcher divide the nursery room for each groups, the treatment group was placed at west Cempaka ward and the control group was placed at east Bakung ward. There were no differences in usual treatment nor facilities in both

wards. The blood collection was performed in both groups (1cc for each specimen) to determine the initial level of TNF- $\alpha$  and IL-6, before treatment was given. Massage stimulation were performed 3 times daily with 6 hours interval (8 AM, 2 PM and 8 PM) for 5 days. At 8 AM on day -6 or 12 hours after the last treatment, the blood specimen for final level of TNF- $\alpha$  dan IL-6 after treatment were taken.

Massage stimulation were performed by 6 trained nurses. Blood sample collection was done by experienced laboratory worker. The blood taken from each subject was 1 cc, and divide in to two saving tubes. The blood specimens were brought and saved in the Clinical Pathology Laboratorium of Sanglah Hospital according to the standard procedure of blood saving, and measured at the same time. During observational period, body weight were measured twice, before and after the treatment with schedule before blood sample were taken. Nutritions that given to the subjects on demand per oral could be breast milk, formula milk or combinaton of those.

Study subjects who had procedures that was not appropriate with study line, discharged by parents initiatives, discharged with other reasons, or moving to other nursery rooms noted as drop out and analysed with intention to treat. There were no involvement of the researcher, whether in randomisation, treatment procedures, observational body weight or blood analysis of TNF- $\alpha$  and IL-6.

#### **Data Analysis**

Baseline characteristic data of the study was presented descriptively by comparison of two groups table, the massage stimulation and control group. The difference of changing in TNF- $\alpha$ , IL-6 and body weight among preterm, low birth weight, and appropriate with gestational age infants before and after treatment in both groups was analyzed with several steps of statistical analysis tests.

Those steps were: data selection by editing, coding, and tabulation, normality test was performed in interval data like TNF- $\alpha$ , IL-6 and body weight before and after treatment in both groups with significancy level  $\alpha = 0,05$ . In this study, the data was found not distributed normally, so the non parametric test was performed. Wilcoxon non parametric test was used to compare the level of TNF- $\alpha$ , IL-6 and body weight before and after treatment in both group with significance level  $\alpha = 0,05$ . Other variables which was predicted as confounding factors that can not avoided by exclusion criteria were tested by ancova analysis. The significance level used was  $\alpha = 0.05$ .

#### **RESULTS**

During study period, there were 89 low birth weight, and appropriate with gestational age infants as eligible subjects. Seventy two (72) out of 89 became the study subject.

Comparison levels of TNF- $\alpha$ , IL-6 and body weight before and after stimulation in both groups (table 2), the results of Wilcoxon test statistic, obtained the difference in levels of TNF- $\alpha$ , IL-6 and body weight before and after stimulation massage, which differ significantly statistically significant (table 3).

There were several conditions other than massage stimulation that can affect the level of plasma TNF- $\alpha$  dan IL-6 and subject's body weight. Analysis of relationship between several variables that suspected to have effect (gender, gestational age, nutrition) on studied outcome (TNF- $\alpha$ , IL-6 and body weight) was performed by ancova test.

Respectively, in Table 4, 5 and 6 show the effect of stimulation of massage treatment on levels of TNF- $\alpha$ , IL-6 and body weight after stimulation treatment and was statistically significant compared with other variables. Analysis of the outcome variable plasma levels of TNF- $\alpha$ , IL-6 and body weight after stimulation massage with some of the variables thought to have influence, with a significance value of  $p = 0.011$  (TNF- $\alpha$ );  $p = 0.037$  (IL-6) and  $p = 0.019$  (body weight).

#### **DISCUSSION**

Infant who were born in low birth weight (LBW) or preterm condition need a good and continous adaptation, external environmental conditions, caring, good attention and nutrition (especially breast milk) to help the adaptation process. A good adaptation will result a better homeostatic of organ systems, clinically seen in a stabile preterm infants, including the immune system and increase of body weight.<sup>12-13</sup>

This study showed the comparation of given massage stimulation and not given massage stimulation in stabile preterm infants, resulted in primary outcome on immune system condition which represented by pro inflammatory cytokine TNF- $\alpha$  and IL-6 and body weight as secondary outcome.

Plasma TNF- $\alpha$  level before treatment in massage stimulation group was 4.04-32.15 pg/ml and in control group was 3.25-43.21 pg/ml. The normal level on aterm normal value in preterm and aterm infants showed that preterm condition, immune system like pro infant is 2.05-7.23pg/ml, preterm infants with normal range for preterm infants.

Table 1  
 Characteristics of Study Subjects Effect of Massage Stimulation in Preterm Infants

Characteristics	Massage stimulation (n= 35)	Control (n=36)
<b>Gender</b>		
Male, n (%)	19 (54.3)	16 (44.4)
Female, n (%)	16 (45.7)	20 (55.6)
Birth weight, grams, median ( <i>range</i> )	2050 (1500-2450)	2050 (1500-2450)
Gestational age, weeks, median ( <i>range</i> )	34.0 (32-36)	34.0 (32-36)
<b>Nutritional support</b>		
Breast milk, n (%)	16 (45.7)	21 (58.3)
Breast milk + formula milk, n (%)	9 (25.7)	6 (16.7)
Formula milk, n (%)	10 (28.6)	9 (25)
<b>Mother's education</b>		
Graduated from elementary school, n (%)	10 (28.6)	7 (19.4)
Graduated from junior high school, n (%)	6 (17.1)	5 (13.9)
Graduated from high school, n (%)	16 (45.7)	21 (58.3)
Graduated from university, n (%)	3 (8.6)	3 (8.3)
<b>Mother's occupation</b>		
Not working, n (%)	11 (31.4)	10 (27.8)
State employee, n (%)	1 (2.9)	1 (2.8)
Private's employee, n (%)	20 (57.1)	18 (50)
Others, n (%)	3 (8.6)	7 (19.4)
<b>Father's education</b>		
Graduated from elementary school, n (%)	2 (5.7)	5 (13.9)
Graduated from junior high school, n (%)	10 (28.6)	5 (13.9)
Graduated from high school, n (%)	18 (51.4)	23 (63.9)
Graduated from university, n (%)	5 (14.3)	3 (8.3)
<b>Father's occupation</b>		
Not working, n (%)	2 (5.7)	3 (8.3)
State employee, n (%)	29 (82.9)	24 (66.7)
Private's employee, n (%)	4 (11.4)	9 (25.0)

Table 2  
 Outcomes before and after massage stimulation in both groups

Indicators	Massage stimulation (n=35)	Control (n=36)
TNF- $\alpha$ level before massage stimulation (pg/ml).	15.94 (4.04-32.15)	17.18 (3.25-43.21)
TNF- $\alpha$ level after massage stimulation (pg/ml).	13.26 (2.28-25.95)	15.73 (3.05-44.80)
IL-6 level before massage stimulation (pg/ml).	10.00 (3.74-41.9)	11.00 (5.18-35.90)
IL-6 level after massage stimulation (pg/ml).	6.47 (2.18-35.34)	10.96 (1.63-36.42)
Body weight before massage stimulation (grams).	2050 (1500-2450)	2050 (1500-2450)
Body weight after massage stimulation (grams).	2200 (1650-2550)	2000 (1400-2525)

The values indicate in median (*range*)

Table 3  
 Difference of median value for TNF- $\alpha$ , IL-6 and body weight before and after massage stimulation

Indicators	Difference of median	Z	p value
TNF- $\alpha$ level before massage stimulation (pg/ml)	-2.32	-2.245	0.025
TNF- $\alpha$ level after massage stimulation (pg/ml)			
IL-6 level before massage stimulation (pg/ml)	-1.14	-3.782	0.001
IL-6 level after massage stimulation (pg/ml)			
Body weight before massage stimulation (grams)	0.00	-2.034	0.042
Body weight after massage stimulation (grams)			

Wilcoxon's non parametric test

Table 4  
 Effect of massage stimulation, gender, gestational age, and nutrition to level of TNF- $\alpha$   
 After massage stimulation

Variables	F	p value
Massage stimulation	6.81	0.011*
Gender	5.21	0.126
Gestational age	0.857	0.358
Nutrition	0.12	0.721

\* $p < 0.05$  with ancova test

Table 5  
 Effect of massage stimulation, gender, gestational age, and nutrition to level of IL-6  
 after massage stimulation

Variables	F	p value
Massage stimulation	4.53	0.037*
Gender	0.02	0.884
Gestational age	0.33	0.564
Nutrition	1.78	0.187

\* $p < 0.05$  with ancova test

Table 6  
 Effect of massage stimulation, gender, gestational age, and nutrition to body weight  
 after massage stimulation

Variables	F	p value
Massage stimulation	5.74	0.019*
Gender	0.20	0.652
Gestational age	0.54	0.462
Nutrition	0.22	0.637

\* $p < 0.05$  with ancova test

The difference of TNF- $\alpha$  gestational age 32-36 weeks is 9.7-20.7 pg/ml.<sup>15</sup> In form of means, the level of TNF- $\alpha$  on both groups were within inflammatory cytokine have been functionated and worked. Higher level in aterm infants as a clue that preterm infants suffers from stresses that can be seen in increasement of pro inflammatory cytokine.<sup>15</sup> Similar condition seen in level of pro inflammatory cytokine IL-6. The reffered value for aterm infants is 12.6–116 pg/ml, while in preterm infants with gestational age 32-36 weeks is 4.1-102.5 pg/ml. Initial data of interval IL-6 level in treatment group was 3.74-41.9 pg/ml and in control group 5.18-35.90 pg/ml. The mean levels of IL-6 in both groups were within normal range for preterm infants. Pro inflammatory cytokine of TNF- $\alpha$  and IL-6 in both groups were within normal range for preterm infants. These results showed that preterm infants in both group were at stabile condition appropriate with the normal level of TNF- $\alpha$  and IL-6 in each group.<sup>15-19</sup>

During this study, after having massage stimulation 3 times daily for 5 days, found difference in pro inflammatory cytokine TNF- $\alpha$  ( $p=0.025$ ) and IL-6 ( $p=0.001$ ) before and after treatment. We have done

electronic and library browsing to explain that phenomena, and found there were no study report, reference review nor theory to explain the pathophysiology mechanism of that phenomena. We come with several theory concepts and evidences reports to support the new theoretical concept that can explain the mechanism of massage stimulation can affect pro inflammatory cytokine regulation, especially plasma TNF- $\alpha$  and IL-6 in preterm infants.

There are 3 main ways mechanism of massage stimulation can regulate pro inflammatory cytokine TNF- $\alpha$  and IL-6. The first way is through hypothalamus, where massage stimulation can produce sense of comfort which can control (inhibit) the release of pro inflammatory cytokine TNF- $\alpha$  and IL-6 from macrophage cell; second way:through activation of vagus nerve, where massage stimulation is a tactile stimulus that activate vagal system (vagus nerve) and immune system; third : through hypothalamus-hypofise, where massage stimulation act as kinesthetic stimulus that activite hypothalamus-hypofise to release cortisol, GH (*Growth hormone*) and IGF-1 (*Insulin like Growth Factor-1*) (Figure 2).

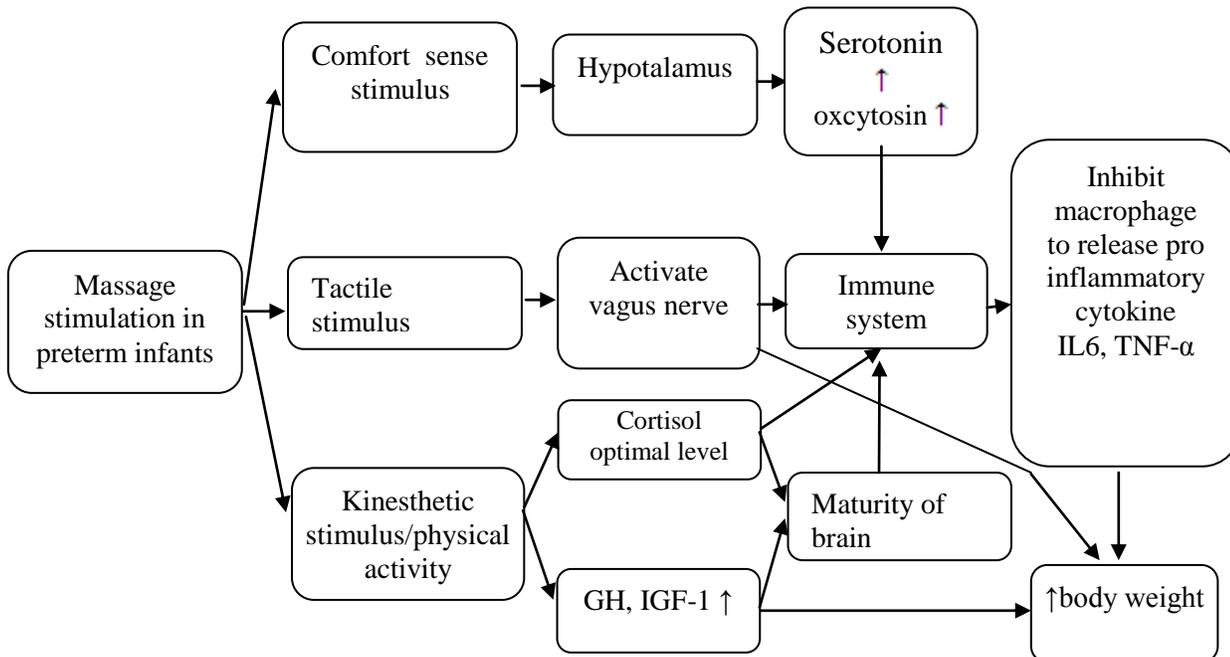


Figure 2  
 Three main ways mechanism of massage stimulation can regulate pro inflammatory cytokine TNF- $\alpha$  and IL-6

### Study novelties

Study effect of massage stimulation in preterm, low birth weight, and appropriate with gestational age infants that has been given 3 times daily, 10 minutes for each session, for 5 days, to the improvement of immune system has never been conducted.

The novelty of this study is the new concept of the mechanism theory of massage stimulation in regulating proinflammatory cytokine (TNF- $\alpha$  and IL-6). Mechanisms of massage stimulation can inhibits the release of pro inflammatory cytokine by macrophage through 3 mayor ways: hypothalamus way; vagus nervous activation way; and hypothalamus-hypofise-adrenal.

### Study limitations

The limitation of this study was evaluation of mediated variables (cortisol, serotonin, oxytocin, GH, IGF-1, insulin and dopamine) were not performed.

## CONCLUSIONS AND SUGGESTIONS

### Conclusions

Based on the results of the study and its discussion, we concluded the following:

- ✓ Massage stimulation reduce the level of TNF- $\alpha$ , in preterm, low birth weight, and appropriate with gestational age infants.
- ✓ Massage stimulation reduce the level of IL-6, in preterm, low birth weight, and appropriate with gestational age infants.

### Suggestions

Multi center study is needed to know the effect of massage stimulation for immune respon, growth and development of preterm, low birth weight, and appropriate with gestational age infants trial in other place or population.

Evaluation of mediated variables (cortisol, serotonin, oxytocin, GH, IGF-1, insulin and dopamine) should be done by the next researchers.

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