THE EFFECT OF MASSAGE STIMULATION TO REDUCTION OF *TUMOR NECROTIC FACTOR-ALFA (TNF-α)* AND *INTERLEUKIN-6* (IL-6) IN PRETERM, LOW BIRTH WEIGHT APPROPRIATE WITH GESTATIONAL AGE INFANTS

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ABTRACT

Preterm condition gives a new stressful environment to infants. This condition needs a good and gradual adaptation. External environmental conditions, treatment, care and good nutrition are needed. Massage stimulation in preterm infants is a method that can prepare preterm infants' adaptation through the critical post-birth period. A good adaptation of the system will provide better organ homeostasis including the immune system.

There are three paths of mechanisms that can explain how massage stimulation can regulate pro-inflammatory cytokines TNF-α and IL-6. The first: the hypothalamus pathway, massage stimulation act as a comfort stimulation that can control (inhibit) the release of pro-inflammatory cytokines TNF-α and IL-6 by macrophages; second: through activation of vagus nerve, massage stimulation act as a tactile stimulation which activates vagal system and the immune system; third: the hypothalamic-pituitary pathway, massage stimulation act as a kinesthetic stimulation that activates the hypothalamic-pituitary to release cortisol, GH (Growth hormone) and IGF-1 (Insulin-like Growth Factor 1). A randomized controlled trial about massage stimulation on preterm infants was performed in Sanglah Hospital Denpasar on 2011. The study revealed a different level of cytokine pro-inflammation TNF-α (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.

Mechanisms of massage stimulation affect the immune system of preterm infants through regulation of inhibition the release of pro-inflammatory cytokine TNF-α, IL-6 by macrophages, through three main pathways: the hypothalamic; activation of the vagus nerve and the hypothalamic-pituitary-adrenal axis.

Keywords: massage stimulation, preterm, TNF-α, IL-6

INTRODUCTION

The advance of technology in perinatal care has bring 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.[1] Preterm birth rate and preterm mortality rate is increasing. Preterm and low birth weight mortality rate in 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 nerveous system such as cerebral palsy, neurodevelopmental and behavioural problems, visual, motor and languange deficits.[2] These developmental problems can persist for longer term until adolescent in form of neuropschology such as lower *Intellegence Quotient* (IQ), articulation problems, concentration disorder, learning disorder, emotional and behavioural disorder, motor disability, and other developmental disorders.[3,4]

Preterm infants were susceptible suffering from various stresses especially infection/inflammation which were caused by environmental overstimulation or invasive procedures that were given to the infant. Stress involved neuro-immune-endocrine systems. Infection that was occured in preterm infants increases the synthesis of TNF-α and IL-6. Stress also increases the level of stress hormones.[2,5]

One of the stimulations that was given to preterm infants is massage stimulation. Massage stimulation is one of touch stimulation which is a physical contact from one person to other.[6] 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.[6]

Massage stimulation will provide stimulus that affect neuro-immuno-endocrine systems. Tumor necrotizing factor-α cytokine as a nonspecific response 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-α acts as catabolism mediator (cytotokine *chacexin*) which affect the body weight. Interleukin-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.[7,8,9,10]

The study about benefits of massage stimulation in preterm infants' immunity and low birth weight is not present in Indonesia. The purpose of this study was to know the effect massage stimulation on the change of TNF-α and IL- 6 invivo in preterm, low birth weight, and appropriate with gestational age infants.

METHODS

Research Design

This study was a randomized controlled trial that performed in west Cempaka and east Bakung ward, Department of Pediatrics Faculty of Medicine Udayana University Denpasar, Sanglah Hospital Denpasar on 2011. Sample were preterm, low birth weight, and appropriate with gestational age infants who fulfilled the inclusión and exclusión 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[11], birth weight 1,500-2,499 grams and appropriate with gestational age (Lubchenco curve), 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 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- α, 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- α 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- α and 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 will be 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, dicharged with other reasons, or moving to other nursery rooms will be noted as drop out and will be 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- α and IL-6.

Statistical analysis

Baseline characteristic data of the study presented descriptively by comparison of two groups table, the massage stimulation and control group. Normality test was performed in interval data like TNF-α, IL-6 and body weight before and after treatment in both groups with significancy level α =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-α, IL-6 and body weight before and after treatment in both group with significance level α = 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 α= 0,05. Data analysis were performed using computer programme.

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.

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

Characteristics	Massage stimulation (n= 35)	Control (n=36)
Gender		
Male, n (%)	19 (54.3)	16 (44.4)
Female, n (%)	16 (45.7)	20 (55.6)
Birth weight, grams, *range* (min-max)	950 (1500-2450)	900 (1500-2400)
Gestational age, weeks, *range* (min-max)	4.0 (32-36)	4.0 (32-36)
Nutritional support		
Exclusive 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)
Mother's education		
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)
Mother's occupation		
Not working, n (%)	11 (31.4)	10 (27.8)
Country's 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)
Father's education		
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)
Father's occupation		
Not working, n (%)	2 (5.7)	3 (8.3)
Country's employee, n (%)	29 (82.9)	24 (66.7)
Private's employee, n (%)	4 (11.4)	9 (25.0)

(Min-max) = minimal-maximal value

Comparison levels of TNF-α, 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-α, IL-6 and body weight before and after stimulation massage, which differ significantly statistically significant (Table 3).

Table 2

Outcomes before and after massage stimulation in both Groups

Indicators	Massage stimulation (n=35)	Control (n=36)
TNF-α level before massage stimulation (pg/ml), *range* (min-max)	28.11 (4.04-32.15)	39.96 (3.25-43.21)
TNF-α level after massage stimulation (pg/ml), *range* (min-max)	23.67 (2.28-25.95)	41.75 (3.05-44.80)
IL-6 level before massage stimulation (pg/ml), *range* (min-max)	38. 17 (3.74-41.9)	30.72 (5.18-35.90)
IL-6 level after massage stimulation (pg/ml), *range* (min-max)	33.16 (2.18-35.34)	34.78 (1.63-36.42)
Body weight before massage stimulation (grams), *range* (min-max)	950 (1500-2450)	900 (1500-2400)
Body weight after massage stimulation (grams), *range* (min-max)	900 (1650-2550)	1125 (1400-2525)

Table 3

Difference of median value for TNF-α, IL-6 and body weight before and after massage stimulation

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

Wilcoxon's non parametric test

There were several conditions other than massage stimulation that can affect the level of plasma TNF-α; 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-α, IL-6 and body weight) was performed by ancova test.

Table 4

Effect of massage stimulation, gender, gestational age, and nutrition to level of TNF-α 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	0.004*
Gender	0.20	0.316
Gestational age	0.54	0.462
Nutrition	0.22	0.637

*$p<0.05$ with ancova test

Respectively, in Table 4, 5 and 6 show the effect of stimulation of massage treatment on levels of TNF-α, 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-α, 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-α); p=0.037 (IL-6) and p=0.019 (body weight).

DISCUSSION

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-α and IL-6 and body weight as secondary outcome. Plasma TNF-α 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 infant is 2.05-7.23 pg/ml, preterm infants with gestational age 32-36 weeks is 9.7-20.7 pg/ml.[12] The difference of TNF-α normal value in preterm and aterm infants showed that preterm condition, immune system like pro inflammatory cytokine have been functioned 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.[12]

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.[12] Pro inflammatory cytokine of TNF-α and IL-6 in both groups were within normal range for preterm infants. These results showed that preterm infants in both group were at stable condition appropriate with the normal level of TNF-α and IL-6 in each group.

During this study, after having massage stimulation 3 times daily for 5 days, found difference in pro inflammatory cytokine TNF-α (p=0,025) and IL-6 (p=0,001) before and after treatment. There were no study reports, reference review nor theory to explain the pathophysiology mechanism of those phenomena.

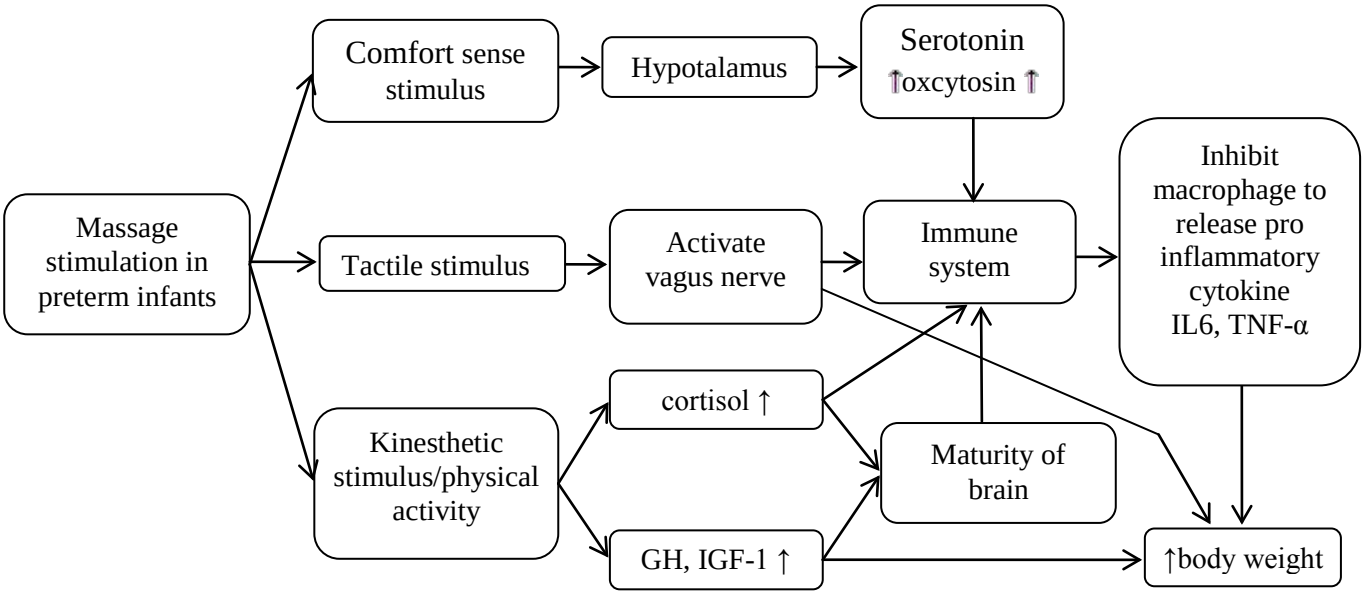

Figure 1. The Three main ways mechanism of massage stimulation can regulate pro inflammatory cytokine TNF-α and IL-6

There are 3 main ways mechanism of massage stimulation can regulate pro inflammatory cytokine TNF-α 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-α 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 hypotalamus-hypofise to release cortisol, GH (*Growth hormone*) and IGF-1 (*Insulin like Growth Factor-1*). (**Figure 1**).

Mechanism massage stimulation through hypothalamus way to inhibit the release of TNF-α and IL-6 from macrophage

The reduction of pain sensation, theoretically associated with reduced of serotonin level.[13] Serotonin is a natural pain killer from the body. Serotonin's benefit are to stimulate sense of comfort, reduce depression and anxiety, which are the important effect of massage

stimulation. Serotonin declared and biochemistry proven can reduce P substance (pain chemical matter) in a complex biochemistry interaction of massage stimulation.[13]

Plasma level of serotonin was reported increased, simultaneously with dopamine after massage stimulation in preterm infants. Massage stimulation have positive biochemistry effect, that is increasing the level of serotonin and dopamine, both are neurotransmitters in central nervous system which can give sense of comfort, reduce depression, anxiety and stress condition. Serotonin and oxytocin productions will decrease in physiologic condition.[14]

Pressure from the stressor will affect the release of both substances from hypothalamus and will increase their level, thus give effect that associated with reduction of hypothalamus-hypofise-adrenal (HPA) axis.[15,16,17,18] Oxytocin is an nonpeptide hormone and act as neurotransmitter that sythetized in paraventriculer (PVN) and superoptic (SON) nucleus of hypothalamus.[15,19] Oxytocin is related in immune system through its anti-inflammation activity, in reducing the release of TNF-α cytokine, and protecting the organ injury because of inflammation process.[14,20,21] Whereas oxytocin deficiency in study of mice showed hyperactivated response from HPA axis. Given oxytocin will give temporary reduction or further reduction of endotoxin induction to immune system, increase ACTH in plasma, cortisol, and influence productions of procalcitonin and TNF-α, IL-1, IL-6, cytokines from macrophage.[14,22,23]

Other mechanism that can explain effect of massage stimulation in pain syndrome is Gate Theory.[24] According to it, pain will induce short and less myelinated (or less isolated) nerve fiber so the time needed by the stimulus to reach the brain is longer than pressure stimulus that brought by isolated nerve fiber and able to send stimulus faster. Information from pressure stimulus will reach the brain before the pain information and “close” the gate from pain stimulus. This is a metaphoric of chemical and electric changing

that possible happened, generally used to explain the habit to massage/press spontaneously the hit location to reduce pain effect after got hit.[24]

Mechanism of massage stimulation through vagal activity way inhibit release of TNF-α and IL-6 in macrophage

Massage stimulation with medium pressure can induce vagus nerve which lead to impact in increased of vagal activity, like increased gastric motility (intestinal gastrointestinal tract) and release of insulin and growth hormone (GH and IGF-1). The mechanism of this hypothesis based on study which showed increased vagal activity[25,26] and insulin level[27] in preterm infants after massage stimulation.

In that study, significant increasement to vagal activity occurred through stimulation of mechanic/pressure in dermal and subdermal that innervated by afferent vagus nerve fibre. Afferent vagus nerve bring the sensory information to lymbic system in brain including in hypothalamus structure that involved in autonomic nerve system regulation and cortisol secretion. The mechanism of this way is supported by few evidences. First, anatomical studies showed that baroreceptors, and in the lower level, mechanoreceptors of muscle tissue and under the skin (Pacinian corpuscle) send the afferent sensory signal to vagal nucleus. The sensory information transferred to ambigus nucleus that processed and transformed in to efferent signal through dorsal motor neuron (DMN) of vagus nerve.[28] Second, collection of several studies, massage stimulation can reduce blood pressure.[29,30,31] Recent evidences showed vagal acitivity mechanism or parasimpathy role in inhibiting release of pro inflammatory cytokine and control inflammation response (anti-inflammation cholinergic pathway)[32] (**Figure 2**). Acetylcholine neurotransmitter inhibits the release of inflammatory cytokine: TNF-α, IL-1, IL-6, and IL-18 from endotoxin induction (lipopolysaccharide) in human macrophage. Those processes are not mediated by production mechanism of anti-inflammatory cytokine

IL-10. Other than its role as neurotransmitter and neuromodulator in central nerve system, perifer autonomic (ganglion), and somatic neurotransmitter, acethylcholine, is a main neurotransmitter of parasymphatic neuron/vagal postganglionic. In mice models, endotoxins (as a natural stimulator in release of pro inflammatory cytokine) and electric energy stimulation to vagus nerve (represents the efferent nervous response from dorsal motoric neuron), will release acetylcholine neurotransmitter to the immune system cells (acetylcholine macrophage receptor α7nAchR dan mAchR), inhibits the release of serum pro inflammatory cytokine, were proven by reduction in level of TNF plasma and liver (**Figure 2**). Inhibition mechanism of vagal activity to inflammation response is not mediated by synthesis of anti-inflammation cytokine (IL-10), differs with hypothalamus autonomic nervous response, other than cortisol release will be followed with synthesis of anti-inflammation cytokine.[32]

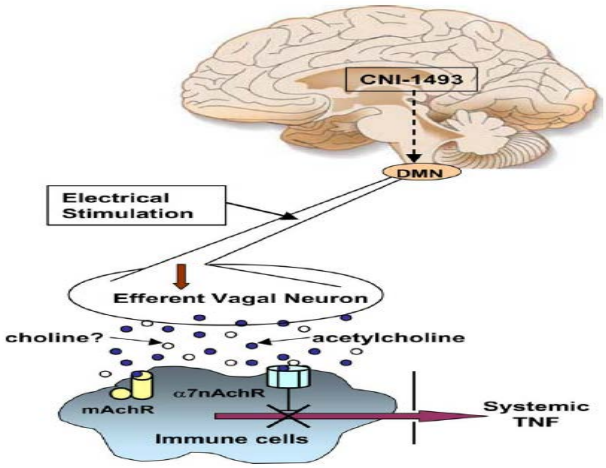

Figure 2. Regulation mechanism inhibition of TNF cytokine release from vagal activity in macrophage cell[32]

Mechanism of massage stimulation through hypothalamus-hypofise that inhibit the release of TNF-α and IL-6 in macrophage

Kinesthetic overstimulation or heavy physical exercise can cause injury in muscle and other tissues, thus can induce inflammation response which marked as pro inflammatory cytokine secretion, chemokine, and inflammatory mediated hormone. Physiologic

kinesthetic stimulation like massage stimulation can induce contra regulation (anti-inflammatory) through secretion of imunosupresan mediator, such as cortisol and anti-inflammation cytokine.

Massage stimulation as kinesthetic stimulation can regulate the level of cortisol, increase level of IGF-1 and oxytocin.[33] Cortisol is a final product of symphatic system that produced when body has stress and the level will decrease after relaxation like massage stimulation. Cortisol can reduce hypersensitivity and hyperreactivity of body immune because of its role as variable that affect the activity of effector immune cell.[34]

Gleeson (2007) reported that moderate and routin kinesthetic stimulation can influence the activity of macrophage cell. One of the pathophysiology mechanisms is through reduction of number and functional capacity of macrophage and circulated leucocyte.[35]

Field et al. (2008) found the elevation of insulin and IGF-1 level in preterm infants after massage stimulation given. Insulin increases glucose absorption and change it to glicogen storage. IGF-1 induces growth and cell multiplication. This mechanism is suspected plays role in acceleration of brain maturation in preterm infants. Brain maturation affect immune system regulation, hyperactivity and hyperreactivity of immune system can be avoided if brain maturation is reached rapidly.[27]

Massage stimulation that induced release of glucocorticoid from adrenal cortex assisted with hypothalamus-hypofise-adrenal (HPA) axis has important effect in metabolism and have anti inflammation and immunosuppressive effect that impacted to release of proinflammation cytokines from macrophage like IL-1, IL-6 and TNF-α.[36,37]

Mechanism of massage stimulation in increasing infant body weight

In general, neonate's body weight will decreases in early first week of life. In newborn, the amount of extracellular fluid is more than intracellular fluid, that cause evaporation is more likely to occur and in bigger volume.

There are few mechanisms that can be used to explain the increasement of body weight, the first: massage stimulation can enhance vagal activity. Study by Diego (2005) showed that preterm infants who had massage stimulation will showed increased activity of vagus. One of the branches of vagus nerve innervates gastrointestinal tracts that induces motility of gastrointestinal tracts, gastric emptying, and increase production of gastrin and insulin hormones. Second: vagal activity also inhibits the release of TNF-α, so the level become low. TNF-α has systemic effect as a pro cathabolic hormone. This can be proved in study of preterm infants who got massage stimulation had the lower basal metabolisme rate (BMR) than the control group. Third: massage stimulation in preterm infants also related with the release of Growth hormones (GH dan IGF-1) compared with control group with clinical manifestations as increased bone density an other anthropometri indicator.[25,27,33]

CONCLUSIONS

Massage stimulation reduce the level of TNF-α; IL-6, and can increase body weight in preterm, low birth weight, and appropriate with gestational age infants.

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