INDONESIAN JOURNAL OF
CLINICAL PATHOLOGY AND MEDICAL LABORATORY
Majalah Patologi Klinik Indonesia dan Laboratorium Medik

PAGE 107
2019 Nov; 26(1): 1-128
p-ISSN 0854-4263 e-ISSN 2477-4685
Available at www.indonesianjournalofclinicalpathology.org

INCREASED INSULIN-LIKE GROWTH FACTOR-1 AND ANTHROPOMETRIC STATUS IN PREMATURE INFANTS WITH BREAST MILK

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ABSTRACT

Massage stimulation has consistently led to greater anthropometric measures in the preterm infant by increasing IGF-1 which plays an important role in promoting growth by stimulating cell growth, multiplication and inhibiting apoptosis. This research aimed to analyze the effect of massage stimulation on IGF-1 and anthropometric measures in breastfeeding the preterm infant. A randomized control trial was conducted on a preterm infant with gestational age less than 37 weeks between February – May 2018 in nursery of Dr. Soetomo Hospital. Fifty infants in the nursery room were randomly divided into group with massage stimulation and control group. Massage stimulation were given three times for 15 minutes everyday for 10 days. Serum Insulin Growth Factor-1 was measured on day 1 and 10. Data were analyzed by statistical software using t-test and spearman correlation. The average increase of IGF -1 in the massage group and control group were 4.8 (SD 4.41) and 3.1 (SD 3.57), respectively. The average increase of body weight in the massage group and control group were 252.2 (SD 208.55) and 137.9 (SD 69.78), respectively. The average increase of body length in the massage group and control group were 2 (0.68) and 1.1 (0.33), respectively. The average increase of head circumference in the massage group and control group were 1.5 (SD 0.82) and 0.9 (0.28), respectively. The positive correlation between the mean increase of IGF-1 and body length was 0.347. There were IGF-1 and anthropometric increase in both groups, but the massage group has a significantly higher mean. There was correlation between increase of IGF-1 and increase of body length.

Key words: preterm infant, massage stimulation, insulin-like growth factor-1, anthropometry, breast milk

INTRODUCTION

Preterm birth is at a greater risk than term infants for mortality, health, and developmental problems. Preterm infants are infants who were born on gestational age under 37 weeks, with birth weight less than 2,500 grams.[1] Preterm infants were susceptible to various stresses, especially infection/inflammation caused by environmental overstimulation or invasive procedures that were given to the infant.[1,2] Breast milk contains numerous immune-related compounds with potential immune effects. For example, growth factors and IGF-1 of breast milk are important modulators of growth hormone in linear growth.

One of the stimulations that was given to preterm infants is touch stimulation. Touch stimulation is one of stimulations which is a physical contact from one person to another.[3] Touch therapy has consistently led to greater anthropometric status in the preterm infant by increasing insulin-like growth factor-1 (IGF-1) which plays an important role in promoting growth by stimulating cell growth, multiplication and inhibiting apoptosis. However, IGF-1 is notably lower in preterm infants and significantly correlated with birth weight, body length, and head circumference.[2]

The study about the benefits of touch stimulation in preterm infants' immunity and low birth weight in Indonesia has not been reported. The purpose of this study was to determine the effects of touch stimulation to increase IGF-1 value and anthropometric status in preterm infants with breast milk.[3-5]

METHODS

This randomized controlled trial study was aimed to determine touch stimulation effects on increasing of IGF-1 and anthropometric status in preterm infants with breast milk. Preterm infants included in this study was born in the Dr. Soetomo Hospital Surabaya with gestational age less than 37 weeks. This study was conducted between February–May 2018 in nursery room of Dr. Soetomo Hospital and approved by the ethics committee of Dr. Soetomo Hospital with number of 703/Panke.KKE/XI/2017.

Parental written informed consent was obtained prior to data collection.

After the preterm infants were transferred to the nursery room, they were randomly divided into touch stimulation and control group with total of fifty infants. Preterm infants were specifically excluded if they had major congenital anomalies, surgery procedure, severe condition, seizure; need ventilator support, cardiovascular, and respiratory disturbance; mother with diabetes mellitus, HIV, and malnutrition.

The touch stimulation protocol developed by field *et al* was used in this study.[4] Touch stimulation were given three times for 15 minutes everyday for 10 days. The first, the second, and the third treatment were given approximately 1 hour after morning feeding, one and half hour after midday feeding, and 45 minutes after the second treatment session. Vital signs were observed before, during, and after stimulation.[4,5] Each session consisted of tactile stimulation for 5 minutes, followed by kinesthetic stimulation for 5 minutes, and ended with another 5 minutes of tactile stimulation.[4-6] The stimulations were given to the infant inside an isolette or a crib. The therapist warmed his/her hands before the start. The tactile stimulation segment was given when infant was in a prone position.[7,8] Moderate pressure stroking was given with the flats of the fingers of both hands. Each area (the head, shoulders, back, legs, and arms) was stroked for six 10-second strokes for a total of 5 minutes. During the kinesthetic phase, the infant was placed in a supine position. The limbs were moved into flexion and extension 6 times (10 secs each movement) in the following sequence: right arm, left arm, right leg, left leg, and both legs simultaneously for a total of 5 minutes. The tactile segment was then repeated with the infant in a prone position.[6,9,10] Anthropometric data (weight, height, head circumference) were recorded daily.

Serum Insulin-like Growth Factor-1was measured on day 1 and 10. The 1-2 cc blood sample was taken from heelstick by the phlebotomist. Serum IGF-1 (ng/mL) was assayed using commercial kits and sandwich ELISA. This ELISA kit applies to the in-vitro quantitative determination of Human IGF-1 concentrations in serum, plasma, and other biological fluids. The minimum detectable concentration of Human IGF-1 was 0.938ng/mL (the sensitivity or lowest detectable limit (LDL) of this assay was defined as the lowest protein concentration that could be differentiated from zero, with limit of 1.563-100ng/mL). This kit recognizes natural and recombinant Human IGF-1 with no significant cross-reactivity or interference between Human IGF-1 and analogs was observed.[11] The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to IGF-1.[11] Standards or samples were added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for IGF-1 and Avidin-Horseradish Peroxidase (HRP) conjugate were successively added to each microplate well and incubated. Free components were washed away. The substrate solution was added to each well. Only those wells that contained IGF-1, biotinylated detection antibody and Avidin-HRP conjugate would develop blue color.[12] The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color turned yellow. The Optical Density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value was proportional to the concentration of IGF-1.[11,12] The concentration of IGF-1 in the samples was calculated by comparing the OD of the samples to the standard curve. Samples should be clear and transparent and centrifuged to remove suspended solids. Samples were allowed to clot for 2 hours at room temperature or overnight at 4^0C before centrifugation for 15 minutes at 1000×g. The supernatant was collected and the assay was carried out immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.[11,12] All reagents and samples were brought to room temperature before use. The sample was centrifuged again after thawing before the assay. It was recommended that all samples and standards be assayed in duplicate. 100µL of Standard, Blank, and Sample were serially added to each well. The blank well was added with reference Standard & Sample Diluent. Solutions were added to the bottom of the micro ELISA plate. The plate was then covered with sealer and incubated for 90 minutes at 37^0C.[12] The liquid of each well was removed without washing. 100µL of Biotinylated Detection Ab working solution was immediately added to each well and plate was covered with the plate sealer. The plate was gently tapped to ensure thorough mixing and plate was then incubated for 1 hour at 37°C.[12] Each well was repeatedly aspirated and washed three times. Each well was washed with approximately 350µL Wash Buffer (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer were used).[12] Complete removal of the liquid at each step was essential. After the last wash, the remaining Wash Buffer was removed by aspirating or decanting. The plate was inverted and

patted against thick clean absorbent paper.[11] 100µL of HRP Conjugate working solution was added to each well and plate was covered with the Plate sealer. Plate was incubated for 30 minutes at 37^{0}C.[12] The wash process was repeated for five times. 90µL of Substrate Solution was added to each well and plate was covered with a new Plate sealer. Plate was incubated for about 15 minutes at 37^{0}C and protected from light. The reaction time might be shortened or extended according to the actual color change, but not more than 30 minutes. The reaction was terminated after an apparent gradient appeared in standard wells. 50µL of Stop Solution was added to each well leading to immediate color change into yellow. The stop solution was added in the same sequence as the substrate solution. The OD value of each well was determined at once using a microplate reader set to 450 nm. The microplate reader was opened, preheated, and testing parameters were set in advance.[11,12]

Data were analyzed by statistical software using t-test and Spearman correlation. Processing and analysis of research data were carried out by computerization using the SPSS program ver. 21. Descriptive analysis was carried out to determine the description and distribution of samples, followed by statistical analysis by calculating the mean value and standard deviation or change of each variable before and after treatment. If the data distribution is normal, the independent t-test was used between groups, while the paired t-test is used for before and after treatment. If the data distribution was not normal, the Mann-Whitney U test was used for data between groups, while the Wilcoxon Sign Rank test was used before and after treatment.

RESULTS AND DISCUSSION

There were 50 preterm infants who met inclusion and exclusion criteria and were involved in this study. After analyzed by Mann-Whitney U test, there was insignificant difference of sex in each group of the infants had the same characteristic of sex, by using. The mean initial IGF-1 level before a massage in the treatment group and the control group were 5.1 (SD 4.59) ng/mL and 4.8 (SD 2.23) ng/mL, respectively. Based on the results of Mann-Whitney U test, there was no significant difference of the mean initial IGF-1 level between the two groups with p=0.181. After analyzed by the Wilcoxon signed ranks test, there was significant increase of the mean final IGF-1 level in the treatment group compared to the initial value with P <0.001. Furthermore, there was significant increase of mean final IGF-1 level in the control group compared to the initial value with P <0.001 (Table 1).

In this study, male and female sex in the control and treatment groups were not significantly different. In a previous study, higher value of IGF-1 levels were found in females, probably due to major fat deposits in females.[13,14] The gestational age of subjects in this study was not significantly different. IGF-1 levels were able to be affected by gestational age; the younger the gestational age, the lower IGF-1 levels will be found. It was previously suggested that IGF-1 levels began to increase at 33 weeks of gestation with 2 to 3-fold increase.[15] The type of delivery in this study did not differ significantly, consisted with no studies showing that IGF-1 levels were affected by labor.[10] In this study, the results of anthropometric data were significantly different in the two groups, with significant mean increase of body weight, body length, and head circumference. Both term and premature babies will lose weight 10-15% at 1 week of life. The target for weight gain and head circumference are 10-20 grams/kg/day and 0.5-1 cm/week,respectively. The recovery time to birth weight was expected to be achieved within 14-21 days.[2,15]

Based on Table 2, there was increase of IGF-1 levels and its significant difference in both groups. Touch stimulation increases weight by increasing insulin and IGF-1 leading to conversion of glucose to glycogen and fat. Moreover, IGF-1 increases growth through stimulation of cell growth, multiplication, and preventing apoptosis. In premature infants, stress would disrupt the regulation of glucose metabolism including hyperglycemia and insulin resistance.[4,13,15] Touch stimulation could reduce stress in premature babies and increased calmness in babies through parasympathetic response.[4,15] Study by Tiffany in 2008 showed that massage in premature infants was able to significantly increase the insulin hormone and IGF-1 in 5 days.[3] Giving a massage to premature babies was not merely able to increase IGF-1 levels, massage is one of the early stimulations to also increase attachment/bounding known as emotional relationship with parents or the closest person who cares for the baby.[3,16]

In this study, the results were significantly different in the two groups, with a significant mean increase of body weight, body length, and head circumference (Table 3). Both term and premature babies would lose their weight 10-15% at 1 week of life. Recovery to birth weight ranges from 10-14 days

Table 1. Basic characteristics of subjects

Characteristics	Touch(n=25)	Control(n=25)	P (one tail)
Sex, n (%)			
boy	13 (52)	12 (48)	0.388
girl	12 (48)	13 (52)	
Gestasional age (weeks) n (%)			
<28	0 (0)	0 (0)	0.384
28- < 32	10 (20)	8 (16)	
32- < 37	15 (30)	17 (34)	
Delivery, n (%)			
Spontaneus	9 (36)	10 (40)	0.385
Cesarean section	16 (64)	15 (60)	
Asphyxia, n(%)			
Yes	2 (8)	3 (12)	0.320
No	23 (92)	22 (88)	
Chronological age when stimulation started Median (min-max)	9 (4-43)	7 (4-45)	0.151
Anemia, n(%)			
Yes	3 (12)	0	0.038
No	22 (88)	25 (100)	
Breast fed n(%)			
Fully	14 (28)	13 (26)	0.500
Dominantly	11 (22)	12 (24)	
Birth weight (gram)			
Mean (SD)	1461,0 (366,54)	1647,6 (377,19)	0.041
Birth weight (gram), n(%)			
<1000	3 (12)	2 (8)	0.140
1000-1500	9 (36)	3 (12)	
1500-2000	12 (48	12 (48)	
>2000	1 (4)	8 (32)	
Birth Length (cm), n(%)			
Mean (SD)	39.9 (3.12)	41,6 (4,82)	0.019
Birth Head Circum (cm), n(%)			
Mean (SD)	28.9 (2.39)	29.9 (2.19)	0.045
Weight at first start (gram) Mean (SD)	1544.3 (309.72)	1675.4 (316.32)	0.07
Length at first start (cm) Median (min-max)	42 (34-46)	43 (27-50)	0.052
Head circum at first start (cm) Median (min-max)	30 (25-34)	31 (27-33)	0.059

Table 2. IGF-1 value before and after touch stimulation

IGF-1 Value	Touch	Control	P (one tail)
Before, mean(SD)	5.1 (4.59)	4.8 (2.23	0.181[a]
After, mean(SD)	9.9 (6.45)	7.9 (3.71)	0.303[a]
P	<0.001[b]	<0.001[b]	

a Mann-Whitney U test,b Wilcoxon signed ranks test

Table 3. Body weight before and after touch stimulation

Weight (gram)	Touch	Control	P (one tail)
Before, mean (SD)	1544,3 (309,72)	1675,4 (316,32)	0.072[a]
After, mean (SD)	1796,6 (318,45)	1813,4 (315,23)	0.426[a]
P	<0.001[b]	<0.001[b]	

a Independent sample t-test, b Paired sample t-test

Table 4. Body length before and after touch stimulation

Length (cm)	Touch	Control	P (one tail)
Before, mean (SD)	41.1 (2.98)	42.0 (5.18)	0.052[a]
After, mean (SD)	43.2 (2.94)	43.1 (5.21)	0.295[a]
P	<0.001[b]	<0.001[b]	

a Mann-Whitney U test, b Wilcoxon Signed Ranks test

with the rate of weight gain 15-20 grams/kg/day to reach 2000-2500 grams. The target for weight gain and head circumference are 10-20 grams/kg/day and 0.5-1 cm/week, respectively. The recovery time to birth weight is expected to be achieved within 14-21 days.[2,15] The mean final body weight after treatment between the two groups did not show a significant difference (p=0.426) with value of 1796.6 (SD 318.45) grams 1813.4 (SD 315.23) grams in the treatment and in the control group. However, birth weight in both groups showed a difference (p=0.041). Birth weight in the treatment group was lower than the control group. Most previous studies on the massage of premature infants did show the final weight in the larger group with massage.[14-17]

RCT studies in LBW infants in Yazd, Iran showed that touch stimulation 3 times a day by mothers for 14 days was proved to increase final weight. However, the study was carried out in late preterm infant with a gestational age of around 34 weeks.[9] Likewise, other studies provided the same results. Touch Research Institute in Miami Florida using the Field's protocol (average of 30 weeks' gestation) showed that weight gain was observed in 21-47% of babies .[4]

Meta-analysis with the same protocol showed a mean daily weight gain of 5.1 grams.[18] Weight gain also occurs after massage by parents. Pregnancy age must be considered as one of factors in assessing weight gain or final weight in premature babies who get a massage.[7] This was considered to cause no statistical difference in this study even though randomization has been carried out.

The mechanism of weight gain in infant massage was caused by increased vagal activity, gastric motility, IGF-1, and insulin.[5-16] Weight gain was observed in premature infants of both groups in this study. The mean increase in body weight after massage in the treatment group was 252.2 (SD 208.11) grams greater than the control group of 137.9 (SD 69.78) grams. Research by Field (1986) reported an increase of body weight in 40 premature infants after massage for 10 days.[6] There was average weight gain of 8 grams in infants given a massage, suggesting a greater weight gain than the control group from the hospital.[4] Touch stimulation requires large calorie consumption, whereas according to Field et al, premature babies who received massage did not consume more milk.[4] Massage also saves calories by increasing sleep time. Scafidi *et al*, infants who received massage were more alert and spent a lot of time in active conscious states, indicating that weight gain was not achieved with increased sleep time or reduced activity.[17]

The mean final body length of premature infants after receiving a massage was not different compared to control, with value of 43.2 (SD 2.94) cm compared to 43.1 (5.21) cm in the control. Body length cannot be assessed in a short time even though the initial body length in the two groups was not significantly different (Table 4). The results of this study were also similar to other previous studies. RCT studies in LBW infants in Yazd, Iran showed that infant massage 3 times a day for 14 days was proven unable to accelerate weight gain compared to controls in late preterm infants with a gestational age of around 34 weeks.[9]

Although the mean final body length of the two groups did not differ significantly, the mean increase of body length after the massage was higher than that of control with value of 2.0 (SD 0.68) cm and 1.1 (SD 0.33) cm with p <0.001, respectively. Massage for 14 and 31 days with coconut oil in premature babies (1500-2000 grams) and term infants of more than

Table 5. Head circumference before and after touch stimulation

Head Circumference (cm)	Touch	Control	P(one tail)
Before, mean (SD)	29.8 (2.06)	30.7 (1.70)	0.059[a]
After, mean(SD)	31.4 (2.18)	31.6 (1.73)	0.318[a]
P	<0.001[b]	<0.001[b]	

a Mann-Whitney U test, b Wilcoxon Signed Ranks test

Table 6. The correlation between final IGF-1 levels, body length, body weight, and head circumference in premature infants after receiving infant massage

IGF-1	Weight	Body Length	Head Circumference
P (one tail)	0.018[a]	0.039[a]	<0.001[a]
Correlation (r)	+0.298	+0.252	+0.500

aSpearman's rho correlation test

Table 7. The correlation between the increase of IGF-1 levels,and the increase of body weight, body length, and head circumference in premature infants

IGF-1	Increasing of Weight	Increasing of Body Length	Increasing of Head Circumference
P (one tail)	0.386[a]	0.007[a]	0.295[a]
Correlation (r)	+0.042	+0.347	+0.078

a Spearman's rho correlation test

2500 grams have greatly increased bed length compared to placebo.[13,18] The absence of differences in the final body length in each study was inevitably influenced by initial body weight, length of observation and gestational age. Most studies did not evaluate body length but were focused on weight changes associated with the role of IGF-1 in glucose metabolism.

Premature babies have a high risk of osteopenia. Physical activity through massage can stimulate bone formation. Physical activity can be carried out through kinesthetic movements in premature infants with compression and extension/flexion in the lower and upper extremities.[17] Infants who got a massage showed a higher C-terminal propeptide of serum type I collagen a marker of the bone formation, while the control group showed decreased level ($p < 0.01$). Serum parathyroid hormone levels also increased in premature infants who got massage stimulation.[15,17]

The mean of final head circumference of preterm infants after receiving massage therapy in the treatment group was not different from control with value of 31.4 (SD 2.18) cm compared to 31.6 (1.73) cm, respectively. There was increase of final head circumference in both groups. However, the mean increase (delta change) of head circumference after massage was greater, with value of 1.5 (SD 0.82) cm compared to 0.9 (SD 0.28) cm, respectively. The mean increase of head circumference in the treatment group was greater than the control ($p = 0.001$ (Table 5).

The increase of final IGF-1 levels was associated with the increase of body length but was not related to weight gain and head circumference in premature infants (Table 6,7). Insulin-Like Growth Factor 1 plays an important role in maintaining homeostasis, increasing progenitor cells and inhibiting apoptosis.[15] Insulin-Like Growth Factor 1 has influence on insulin in terms of glucose metabolism and fat. In the fetus, IGF-1 and insulin increase the production of IGF-1 rather than GH, which is essential for growth. Insulin-Like Growth Factor 1 is mediated by the insulin-glucose axis, which results in a rapid response to nutritional fluctuations, and IGF-1 concentration increases in the middle of pregnancy to accelerate growth, which is normally observed in the third trimester of pregnancy.[15]

Direct measurements showed the dominant influence of IGF-1, IGF-1 levels in serum from week 15 to week 37 of pregnancy, correlating with bone weight and length.[15] IGF-1 levels in the umbilical cord reflect IGF-1 levels at birth, which also correlates with body weight. Low IGF-1 levels were found in premature infants and in the Intra Uterine Growth Restriction (IUGR) case. Insulin like-growth factor-1 is also found in the body tissues of the fetus since the first trimester, this illustrates the role of IGF-1 from

the beginning of fetal development and growth.[15,18] The increase of IGF-1 in the fetus blood during pregnancy until the end of the third trimester indicates that IGF-1 plays an important role in fetal growth.[15,19] Massage therapy can contribute to weight gain by increasing insulin and IGF-1. Insulin increases glucose conversion both for short and long term storage, and IGF-1 stimulates cell growth. Stress, which is often experienced by premature babies, can contribute to the dysregulation of glucose metabolism (hyperglycemia and insulin resistance). It proven that massage could reduce stress and sooth babies. Massage may also reduce cortisol levels and increase vagal activity. Furthermore, massage can increase body weight by reducing the effect of cortisol inhibition on insulin secretion.[15,18,19]

The limitations of this study were the different basic diseases and their different lengths as the characteristics of infants. Stress of nursing mothers also influenced the results of this study. Use of specific age of pregnancy was necessary; for example, late preterm pregnancy, very preterm or other gestational age. Initial weight must also be considered.

CONCLUSION AND SUGGESTION

Insulin like growth factor-1 and anthropometric status increased in both groups, but the touch stimulation group had a significantly higher mean increase. An increase of IGF-1 levels correlated with an increase of body length. Another study to observe progress of anthropometric status and IGF-1 levels in long term period was required. It was necessary to perform a study on healthy premature babies to prevent many confounding factors.

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