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Comparison study between commercial and modified grains mix WHO formula F-100 to weight, serum amylase and lipase in children aged 1-3 years

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Abstract

Introduction: *children with cow´s milk allergy require special formulas contain ingredients that doesn´t cause allergies or intolerances. Ready-to-use modified grains mix formula at Dr. Soetomo hospital based on modified gain mix WHO formula F-100, while commercial grain mix products fortified with micronutrients and amylase enzyme can be used as alternative if it is not available in the hospital. This study aims to compare the differences of weight gain, amylase and lipase enzyme levels between commercial (CGMF) and modified grain mix WHO formula F-100 (CGMF) in children aged 1-3 years.* ***Methods:*** *the study were quasi-experimental trials randomized controlled design, subjects were children aged 1-3 years who drink soy formula with cow´s milk allergy at outpatient clinic Dr. Soetomo hospital divided into commercial grain mix (CGMF) and modified grain mix WHO formula F-100 (MGMFW) groups. The evaluation of weight gain, levels of serum amylase and lipase were done before and after of formula consumption. Statistical analysis including Chi-square test, independent sample t-test, paired sample t-test, and Anacova using SPSS ver. 17.0 (IBM, US).* ***Results:*** *a total of 17 subjects in CGMF and 16 subjects in MGMFW groups. There are significant difference in body weight gain, serum amylase and lipase level before and after the administration of CGMF and MGMFW (p<0.05), but the difference between both treatment groups were not significant (p>0,005).* ***Conclusion:*** *commercial formula is equivalent to modified grains mixed in weight gain, serum amylase and lipase levels so that it can be used as an alternative formula in the nutritional management of cow´s milk allergy in children aged 1-3 years old to ensure optimal growth.*

Introduction

Children with special conditions such as cow´s milk allergy, lactose intolerance, required a special formula that not contain ingredients that cause allergies or intolerances. Extensively hydrolyzed protein formulas and amino acids are formula for children used to treat cow´s milk allergies but have bad taste. Children over 1 years old of age who were already familiar with family foods tend to refuse this formula, that will reduced the calorie intake, lead to growth retardation. Fortified soy formula generally used to treat the children with cow´s milk and lactose free have been recommended by American Academy of Pediatrics since 1976 [1]. But there are some concern in the use of this formula: the content of phytoestrogens may interfere with the immune, thyroid and reproductive system and has low content of methionine and cystine amino acids. Modification formula as alternative formula made by Dr. Soetomo hospital refers to modified grain mix WHO formula F-100 (MGMWF), is often not available when needed and should be made specially. Currently there are commercial products as artificial food industry packaged fortified with micronutrients as well as enzymes amylase, lipase and protease that can be used as an alternative if not available in the hospital. The advantage in preparing patient meals not only depend on the hospital´s own homemade food. The use of a commercial product is sometimes necessary in nutrition care, because it is practical and efficient, requires no time for setting up, although requires a higher cost. The CGMF were supplemented with protease, lipase and amylase enzyme to optimize nutrient´s metabolism. Fortification of amylase enzymes improves the quality of food, energy density and tastes but the effect on growth is still debated, such as study conducted by Hossain MI *et al.* [2] showed significant improvement of weight gain in the subjects receiving fortified rice flour with amylase for 6 weeks compare to the subjects receiving rice flour without amylase fortification group aged 6-24 months, but other study showed there was no difference in body weight and length [3]. This study aimed to analyze the difference between the provision of commercial and modified grains mix WHO formula F-100 to the weight gain, amylase and lipase levels in children aged 1-3 years diagnosed with cow´s milk protein allergy.

Methods

Subjects: subjects are patients with cow´s milk allergy aged 1-3 years and has beed consuming soy formula when the study were conducted in the Outpatient Unit of Child Health, Hospital for the provision of treatment Dr. Soetomo Surabaya and Kedung Doro Laboratory for laboratory examination. The study was conducted in January 2014 - March 2014. The inclusion criteria were: patient who already drink soy milk at least 1 week before starting the study, already not breastfed at least 1 week when the study started, like or want to drink milk, follow the research by signing an informed consent by the parents. The exclusion criteria are patients with overweight/ obesity (Z-score >2 SD), suffering from congenital heart disease, malignant disease, kidney and liver disease, chronic infections (tuberculosis, HIV).

Study design: the study were quasi-experimental trials randomized controlled design. Subjects were divided 2 group: commercial grains mix formula group, abbreviated as CGMMF group and MGMWF, abbreviated as MGMWF. Evaluation of anthropometric and laboratory were performed before study (day 0) and end of study (week 6) so it is also called pre-post control group study. Commercial grains mix formula (CGMMF) is a plant protein-based formulas (CEO baby® manufacturer), made up of rice, brown rice, oats, wheat, barley and soy, contain 100 kcal/100 cc and 4 g of protein, fortified by amylase, lipase and protease enzymes and other nutrients, given as 50% of total RDA (Recommended Dietary Allowances) based on age and weight. Modified drain mix WHO formula F-100 (MGMWF) made from rice, oats and soybeans by section pediatric nutrition Dr. Soetomo hospital with calorie content of 100 kcal/100 cc and 4 grams of protein, without fortification, given as 50% of the total RDA based on age and weight. Weight gain is calculated by subtracting the body weight of 6 weeks (end of study) with body weight before study. Body weight was measured using GEA® digital scales, on children who can´t stand up and SMIC ZT-120® scale in children who could stand up, with the nearest 0.01 kg. Body weight was measured with minimal clothes or without clothes or without diapers, expressed in grams. The investigation conducted by the enzymatic calorimetric method in serum of amylase (normal levels <60 U/L) and lipase (normal levels <53 U/L).

Recall of food intake: food intake obtained by telephone 24-hour recall by a trained dietician every day after the initial recruitment to the caregivers of the subject. Food intake as a confounding factor in this study. Food intake data were collected and analyzed using Nutrisurvey for windows software.

Statistical analysis: test of normality (Kolmogorov-Smirnov and Shapiro-Wilk) were performed for all data with significant value of >0.05 determined as normal distribution and equality of variance (Levene´s Test) to determine the homogeneity of data, if P-value >0.05 the data is homogen. Subject´s characteristics between two groups were analyzed using Chi-squared test (significant if p<0.05). Differences of weight gain, amylase and lipase levels before treatment were analyzed using independent sample t-test (P-value of test of normality >0.05 or normal distribution, if P-value<0.05 Mann-Witney Test were enrolled). Weight gain, amylase and lipase levels before and after study were analyzed by paired sample t-test to analyse the difference in both group after the administration of formula (if p value>0.05 of Test of Normality, if p value<0.05 Wilcoxon Signed Rank Test were enrolled). Differences of weight gain, amylase and lipase levels after study account food intake as a covariate were analyzed by Anacova test. While anthropometric status of the subjects (weight-for-age z-score or WAZ, length-for-age Z-score or LAZ, weight-for-height z-score or WHZ, head circumference or HC and muscle arm circumference or MUAC) were analyzed using Chi-square Test, significant if p<0.05. All statistical analysis enrolled via SPSS 17.0 ver (IBM, US).

Ethical clearance: before the study was performed, a pre-eliminary study was enrolled to ensure the safety and patient´s tolerance of both formulas.

The study were registered with ethical clearance number 49/Panke.KKE/I/2014 issued by Ethic Committee of Dr. Soetomo Hospital Surabaya. Before the study enrolled, parents must fills inform for consents. The researchers explain the risk of intervention in detailed to the parents.

Results

Subject characteristics: sampling was done by consecutive sampling, total of 33 patients conducted this study, divided to 17 patients in CGMF group and 16 patients in the MGMWF group by simple randomization. The characteristic feature of the subject can be seen in Table 1, including variable for age, sex, parental income, parental education, parental employment, parenting and breastfeeding history which is distributed normally and homogenous between two groups (p>0.005).

Food recall: the mean amount of food intake during the observation of CGMF group were 421.29±144.08 kcal/day, whereas in the group of MGMWF were 399.77±150.55 kcal/day. Food recall were performed because it determined as confounding variable, and there is no significant difference of food recall between two group (p=0.678), distributed normally (Kolmogorov-Smirnov and Saphiro Wilk p>0.05) and homogen (Levene´s Test, p>0.05). The mean intake of CGMF were 529.41±77.17 kcal/day, whereas in the group of MGMWF were 534.37±76.85 kcal/day, and there is no difference in the number of the formula obtained in both groups (p=0.854).

Anthropometric status of the subjects: anthropometric status of the subjects (weight-for-age z-score or WAZ, length-for-age Z-score or LAZ, weight-for-height Z-score or WHZ, head circumference or HC and muscle arm circumference or MUAC) all distributed normally (Kolmogorov-smirnov and Shapiro-Wilk, p>0.05) and homogen (Levene´s test, p>0.05) between both group. Table 2 shows the mean z-score of the patient before the administration of CGMF and MGMWF. Based on the z-score of weight for age, there was not differences significantly in both groups (p=0.219). The mean z-scores height for age, head circumference for age was not significant differences between both groups (p>0.05). There were significant differences according to z-scores MUAC for age between both groups with mean -0.49±1.30 in CGMF group and -1.77±1.60 in MGMWF group (p=0.018). Subjects with malnutrition (z-score <-2 SD) before administration of CGMF and MGMWF are shown in Table 3. Growth parameter investigation before treatment (weight-for-age, length-for-age, weight-for-length z-score) showed that 29.4% of patients in the CGMF group were underweight, 23.5% were stunted and 17.6% were wasted. Meanwhile 18.8% patients in the MGMWF group with underweight, 25% patients were stunted and severely stunted, 30% with wasted and severely wasted. There is no significant difference in all growth parameters in both groups (p-value respectively were 1.000; 0.11; and 0.484).

Differences of weight gain after administration commercial and modified formula: differences weight gain in this study account of the daily food intake of the patient obtained in addition to the provision of the formula. Anacova test analyzes there is differences in the body weight gain after administration of the formula, account food intake as a covariate factor. Table 4 shows the mean body weight before and after administration of commercial and MGMWF. The difference of body weight before administration in both groups was not significant statistically (p=0.130). After the intervention for 6 weeks, there is no difference of body weight between the CGMF group and MGMWF (p=0.144). Value of food intake as a covariate was 0.001, means that the food intake was a covariate influencing the final body weight. There is a significant enhancement of weight gain in the subjects in the CGMF group before and after administered of the formula (p<0.001, paired sample t-test). Similarly, weight gain of patients in the MGMWF group were significant (p<0.001, paired sample t-test).

Changes in serum amylase levels: changes in serum amylase levels in the CGMF group and

MGMWF group were shown in Table 5. The mean of serum amylase levels before administration of CGMF was 18.04±6.80 U/l, and 24.81±8.51 U/l after the administration of CGMF for 6 weeks, the enhancement of this level were significant statistically (p<0.001). Meanwhile the mean serum amylase levels before administration of MGMWF was 14.4±6.92 U/l. After administration of a MGMWF for 6 weeks, serum amylase levels were 20.27±6.81 U/l, significant statistically (p<0.001). But there is no significant difference of amylase levels before the intervention before (p=0.135) and after (p=0.088) additional of both formulas in both groups or after treatment, the difference type of formula and food intake given to the subjects does not affect serum amylase levels.

Changes in levels of serum lipase: changes in serum lipase levels in the CGMF group and MGMWF shown in Table 6. Serum lipase levels ranged from 17.10 to 91.50 (median 28) U/l before administration of CGMF. After administration of commercial vegetable formula for 6 weeks, serum lipase levels ranged from 23.30 to 76.59 (median 26.25) U/l, this increase was significant statistically (p = 0.022). Serum lipase level ranged from 12.30 to 65.10 (median 26.25) U/l before administration of MGMWF. After administration of a MGMWF for 6 weeks, serum lipase levels ranged from 17-70 (median 32.30) U/l, the enhancement was significant statistically (p=0.001). There is no difference in lipase levels before the intervention in both groups (p = 0.358) and after observation for 6 weeks (p = 0.460) (**Table 6**). Changes in lipase levels in CGMF group had ranges -15 to 23 U/l, with a median of 2.8 U/l, whereas in the MGMWF groups had range -2.10 to 15.2 U/l with a median of 4.9 U/l. There were no differences in changes of lipase levels between both groups (p = 0.15).

Intolerance symptoms: during the 6 weeks obtained intolerance symptoms such as nausea, vomiting, diarrhea without dehydration and bloating. Intolerance symptoms arising in the MGMWF group is 31.2% larger than the of CGMF group of 23.5%.

Discussion

Average food intake recall showed that only about 50% of the total daily intake meets RDA needs (approximately 950.700±187.57 and 934.14±174.72 kcal in respective group, whereas the recommended daily allowance of children aged 1-3 years is 100 kcal/kg/day. Average body weight of the subjects was 10 kg, RDA is about 1,000 kcal/day) [4]. With the same method found that the average daily intake of the patients with cow's milk allergy were under the RDA [5] reported that the intake of cow's milk replacement formula either soy or protein hydrolyzate smaller than healthy children. The subjects were supplemented with 450-600 ml (450-600 kcal) of formula, which meets 50% RDA needed by the subjects. Daily food intake recall in this study using 24-hour recall method which record the type and amount of food consumed during the past 24 hours by phone. Caregiver interviews were conducted with both parents, and others entrusted with the task of caring for these patients. Disadvantages of this method is recall accuracy is highly dependent on the memory of respondents, or need to employ personnel who are trained and skilled in the use of assistive devices household size and precision tools are used according to the customs of the people as well as the flat slope syndrome has the tendency for respondents who are underweight to report over estimate and for respondents who are obese tend to report fewer [6]. Validation studies on dietary assessment methods show no tendency for greater energy reported with increasing age of the subject. Accurate reporting is also affected by the increasing diversity of the types of food consumed. Over reporting recall method common in children with a weight for height Z-score was lower than that reported by Olinto *et al.* [7]. Commercial grains mix formula in this study fortified with enzyme amylase, lipase and protease as well as micronutrients. The addition of amylase and micronutrient fortification were able to improve the quality of food and prevent micronutrient deficiencies. Amylase enzyme breaks down starch into sugars (maltose, maltodextrin and dextrin),

make food sweeter and tastes better than without the addition of amylase. It also increases the caloric density [2,3].

Weight gain in healthy children aged 12-24 months is about 7-10 grams/day [8], while in malnourished children is about 50 g/kg/week [9]. The observations were carried out for 6 weeks (42 days) expected gain weight of the subjects is about 300-420 grams. In this study the expected body weight gain were achieved and statistically significant ($p<0.05$) in both group. However, differences in weight gain between two groups was not significant statistically, which is similar to the study conducted by [3] at the 6 months baby fed with complementary food. [10] examines the effectiveness of the base material substitution of F100 with soybean flour (modification) in the diet therapy of malnutrition children for 7 days, and concluded that weight gain before and after the intervention were significant in both modified group (substituting soybean flour) or a group of F-100 as control [2] using rice flour fortified with amylase, the average weight gain for 6 weeks in malnourished children were 500±340 grams in fortified group, and 400±470 grams in the group without the addition of amylase. The increase in MGMWF group has a wider range (10-1110 g), because this formula was designed for children with severe malnutrition with the expected increase of weight gain was 1 kg for 6 weeks. Patients with cow's milk allergy who were malnourished in this study is higher than the cross-sectional study conducted by [11], reported 15.1% of children with underweight, 8.7% wasted and 23.9% were stunted. Other study reported that 23% of children allergic to cow's milk with underweight, 7.7% of children with wasted and 11.5% of patients were stunted [12]. Nutritional status is an important variable in this study, since most of the subjects are children with soy formula comsumption due to cow´s milk allergies. The subjects had nutritional disturbance because of elimination diet is not accompanied by proper nutrition care. Stunted can be caused by endocrine disorder or not, including the condition of malnutrition, but stunted condition is not performed in the evaluation of the cause, whether familial, constitutional delay, or hormonal disorders in this study [13] assess pancreatic exocrine function (serum amylase and lipase) in Protein Energy Malnutrition (PEM) patients and concluded that the levels of serum amylase and lipase significantly lower in all subgroups of PEM compared with controls, and significantly increased after administration of nutritional therapy.

There is no difference between CGMF group and MGMWF group in the of serum amylase and lipase levels. Fortification of enzymes in CGMF group will affect the absorption of macronutrients that weight gain is expected to be greater than in the provision of non-fortified formula. The research hypothesis is not proved that there was no difference in weight gain between GGMF and MGMWF group due to the amount of calories and macronutrient content is similar in both types of formulas and no difference in the number of calories of food intake in both groups. Pancreatic exocrine glands synthesize digestive enzymes essential for digestion and absorption of carbohydrates, fats, and proteins. Although low levels of cell regeneration in the pancreas, the organ is very adaptable to the changes of macronutrient intake and affect the synthesis of digestive enzymes [14]. Maintenance of pancreatic mass and digestive enzyme depends on the adequacy of nutrients in the digestive tract, suggests that diet plays a central role in this regulatory process. It is shown in this study the presence of elevated levels of pancreatic enzymes amylase and lipase after the administration of formula that satisfies 50% of daily calories during the 6 weeks of observation [15] proves that the synthesis of pancreatic digestive enzymes changes in accordance with specific changes in macronutrient intake where the consumption of a diet high in protein, fat, or carbohydrates will increase the enzyme protease, lipase and amylase. So the pancreatic exocrine gland is able to recognize a particular macronutrient and regulate the synthesis of digestive enzymes. The introduction of nutrients at least partially mediated through the signal coming from the gut. However, whether there is a direct role of nutrients in acinar

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cells via the bloodstream remains unclear. Direct role of glucose in the acinar cells with a significant increase in pancreatic amylase shown in intraperitoneal glucose infusion or continuous parenteral in mice [14]. This research used a commercial formula fortified with enzyme amylase, lipase and protease derived from fortified grains (plant-based enzyme). Enzyme supplementation is generally used in cases of pancreatic insufficiency, with the symptoms include abdominal pain, maldigesti, steatorrea and weight loss due to malabsorption. Other circumstances that needs enzyme supplementation in the management of lactose intolerance. It is estimated that 75% of people in the world, even 90% in Asia and Africa suffer hypolactasia or decrease of lactase activity, particularly in adults. Symptoms associated with lactose intolerance are diarrhea, bloating improved by enzyme supplementation [16]. We conclude that commercial grain mix formula is equivalent to modified grain mix WHO formula F-100 so that it can be used as an alternative formula in the nutritional management of cow´s milk allergy in children aged 1-3 years old to ensure optimal growth.

Conclusion

We conclude that commercial grain mix formula is equivalent to modified grain mix WHO formula F-100 so that it can be used as an alternative formula in the nutritional management of cow´s milk allergy in children aged 1-3 years old to ensure optimal growth.

What is known about this topic

- *The special diets for children with cow´s milk generally allergy using modified grain mix WHO formula F-100, but no supplementation were added;*
- *The commercial modified grain mix formulas were supplemented with lactase and amylase beside other nutrients with exact nutrient contents as needed during childhood growth and development.*

What this study adds

- *The new formula as addition foods/beverage for children with cow´s milk allergy aged more than 5 years old supplemented enzymes (lactase and amylase) and other micronutrients to ensure optimal nutrient intake during childhood;*
- *There is no significant different in body weight change in children with cow´s milk allergy consuming modified grain mix WHO formula F-100 and commercial modified grain mix formulas;*
- *No adverse reaction in subjects consuming commercial modified grain mix formulas.*

Competing interests

The authors declare no competing interests.

Authors' contributions

All authors participated in Conception and design analysis, collection of data, contributed to the data and analysis tool, performed the analysis, drafting and writing the article. They all approved the final version of the article.

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Tables

Table 1: subject characteristics

Table 2: the Z-score subjects before administration of CGMF and MGMWF

Table 3: number and percentage of children with malnutrition (Z-score <-2 SD) before administration commercial and modified formula

Table 4: body weight of patients before and after administration of commercial and modified formula

Table 5: difference in levels of serum amylase between commercial formula group and modified formula group

Table 6: difference in levels of serum lipase between commercial formula group and modified formula group

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Table 1: subject characteristics			
Variable	**CGMF (n=17)**	**MGMWF (n=16)**	**P**
Sex [n(%)]			0.494**
Boys	10 (58.8)	7 (43.8)	
Girls	7 (41.2)	9 (56.3)	
Age, months [mean±SD]	22.8±7.89	21.2±6.64	0.525*
Father education [n(%)]			0.365**
Primary	0	0	
Secondary	1 (5.9)	3 (18.8)	
Tertiary Collage	10 (58.8)	10 (62.5)	
University	6 (35.3)	3 (18.8)	
Mother education [n(%)]			0.810**
Primary	1(5.9)	2 (12.5)	
Secondary	1(5.9)	2 (12.5)	
Tertiary Collage	10 (58.8)	9 (56.3)	
University	5 (29.4)	3 (18.8)	
Father employment [n(%)]			0.771**
Government employed	3 (17.6)	1 (6.3)	
Salaried	10 (53.8)	11 (52.4)	
Self employed	4 (23.5)	4 (25)	
Mother employment [n(%)]			0.240**
Government employed	2 (11.8)	0 (0)	
Salaried	4 (23.5)	7 (43.8)	
Self employed	0 (0)	1 (6.3)	
Housewife	11 (64.7)	8 (50)	
Parental income			0.764**
< Rp 1 milyon	1 (5.9)	1 (6)	
Rp 1 - 2 milyon	6 (35.3)	6 (37.5)	
Rp 2 - 3 milyon	6 (35.5)	3 (18.8)	
>Rp 3 milyon	4 (23.5)	6 (37.5)	
Breastfeeding history [n(%)]			0.678**
0-3 months	6 (35.3)	3 (18.8)	
3-6 months	3 (17.6)	4 (25.0)	
6-9 months	3 (17.6)	(12.5)	
9-12 months	3 (17.6)	2 (12.5)	
>12 months	2 (11.8)	5 (31.3)	
Caregiver [n%]			1.000**
Mother	11 (64.7)	9 (56.3)	
Family	3 (17.6)	7 (43.8)	
Others	3 (17.6)	0(0)	
Chi-square test, significant if p < 0,05, *Pearson Chi-Square**Fisher's exact test			

Table 2: the Z-score subjects before administration of CGMF and MGMWF			
Variable	CGMF [mean(SD)]	MGMWF [mean(SD)]	P
WAZ	-0.23±1.65	-0.99±1.82	0.219
HAZ	-0.41±1.43	-0.55±2.09	0.825
WHZ	-0.41±1.43	-0.55±2.09	0.825
HC	-0.63±0.89	-0.75±1.28	0.755
MUAC	-0.49±1.30	-1.77±1.60	0.018
Chi-square test (Pearson Chi-square) significant if p<0.05			

Table 3: number and percentage of children with malnutrition (Z-score <-2 SD) before administration commercial and modified formula			
Variable	**CGMF [n(%)]**	**MGMWF [mean(SD)]**	**P**
WAZ			1.000
-3< Z score<-2SD	5 (29.4)	0 (0)	
Z score<-3 SD	0	3 (18.8)	
HAZ			0.112
-3< Z score<-2SD	4 (23.5)	1 (6.3)	
Z score<-3SD	0 (0)	3 (18.8)	
WHZ			0.484
-3< Z score<-2SD	3 (17.6)	3 (18.8)	
Z score<-3SD	0	2 (12.5)	
Chi-square test (Pearson Chi-square) significant if p<0.05			

Table 4: body weight of patients before and after administration of commercial and modified formula			
Administration	**Body weight (mean±SD)**		**P**
	CGMF	**MGMWF**	
Before (kg)	11,65±2.93	10,18±2.46	0.30*
After (kg)	12.09±2.95	10.69±2.39	0.144**
Significant if p< 0.05, * independent sample t-test, ** Anacova test			

Table 5: difference in levels of serum amylase between commercial formula group and modified formula group			
Administration	**Amylase (mean±SD)**		**p**
	CGMF	**MGMWF**	
Before (U/l)	18.04±6.80	14.41±6.92	0.135*
After (U/l)	24.81±8.51	20.27±6.81	0.088**
p	< 0.001 ***	< 0.001***	
Significant if p< 0.05 *independent sample t-test, ** Anacova test, *** paired sample t-test			

Table 6: difference in levels of serum lipase between commercial formula group and modified formula group			
Administration	**Lipase (median[min-max])**		**p**
	CGMF	**MGMWF**	
Before (U/l)	28 (17.10-91.50)	26.25 (12.30-65.10)	0.358*
After (U/l)	32.65 (23.30-76.59)	32.3 (17-70)	0.460*
P	0.022**	0.001**	
Significant if p< 0.05,* Mann-Whitney test ** Wilcoxon sign rank			

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