

WhatsApp [LIVE] Tanya Jawab Ramadh... (89 unread) - asliswirda@yahoo.com

mail.yahoo.com/d/folders/52/messages/44187guc...\_referrer=sig=AQAA...

HOME MAIL NEWS FINANCE SPORT LIFESTYLE ENTERTAINMENT WEATHER MORE...

yahoo!mail Find messages, documents, photos or people Advanced Aslis Wirda Home

Compose

Back Archive Move Delete Spam

[Auto-Reply] Manuscript Dr. Aslis - BIMJ Yahoo/2017 Jur... ☆

**bimj\_editors@bimjonline.com** <bimj\_editors@bimjonline.com> Fri, 12 May 2017 at 10:00 am ☆  
To: Aslis Wirda Hayati

Thank you for submitting your manuscripts to bimjonline. We will endeavour to reply to your email within 48hrs.

Sincerely  
bimj editors

**Aslis Wirda Hayati** <asliswirda@yahoo.com> Fri, 12 May 2017 at 10:02 am ☆  
To: Haiza Ahmad, Alkausyari Aziz Ari, Widia Ningsih

Show original message

24°C Berawan 07:37 05/04/2023

Adobe Students save over 60% on Adobe Creative Cloud. Take your career to new heights. Buy now

AND LYON MARKET EDUCATION

1  
2 **Pyridinium Crosslinks (*Pyd*) is associated with**  
3 **stunting in neonates**

4  
5 **Aslis Wirda Hayati<sup>1\*</sup>, Alkausyari Aziz<sup>1</sup>, Siti Rohaiza Ahmad<sup>2</sup>**  
6 **and Sri Widya Ningseh<sup>3</sup>**

7  
8 <sup>1</sup>*Nutrition Department of Riau Health Polytechnic, Ministry of Health Republic Indonesia*

9 <sup>2</sup>*PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam*

10 <sup>3</sup>*Pharmacy Department of Medan Health Polytechnic, Ministry of Health Republic*

---

11  
12  
13  
14 **ABSTRACT**

15  
**Aims:** The aim of the research is to evaluate the reliability of bone resorption biomarkers called Pyridinium Crosslinks (*Pyd*) in the urine of the neonates as an evaluation to bone growth of the neonate (based on their length).

**Study design:** A cross-sectional study.

**Place and Duration of Study:** Place of the study was Andini Hospital (Pekanbaru, Indonesia). Study duration was between, August - September 2014.

**Methodology:** Subjects of study were 35 healthy neonates. Subjects were recruited at the first 3 days of life. Body length gauges, digital weighting scale, family socioeconomic questionnaires and *Pyd* kit were used to collect the data. Differences in the mean of the research variables were tested using an Independent t-test.

**Results:** Results showed that there were significant differences between z-score height for age (HAZ) and *Pyd* in the urine of stunted versus normal neonates. The contents of *Pyd* urine stunted neonates (body length <48 cm) was 982.9±61.6 and normal neonates (body length ≥48 cm) was 594.1±266.1 nmol/mmol Cr ( $p<0.01$ ). Neonates classified as stunted if their length <48 cm and the contents of *Pyd*>859,7 nmol/mmol Cr.

**Conclusion:** Therefore, HAZ and *Pyd* in the urine can be potential early indicators to identify stunted and normal growth neonates.

16  
17 *Keywords: body length, neonates, pyridinium crosslinks, stunting, z-score height for age*

21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74  
75  
76  
77

## 1. INTRODUCTION

Stunting is a condition by which an individual failed to reach the linear growth potential which could be resulted from the conditions of their health and nutrition WHO (2010). Worldwide, childhood stunting declined from 39.7% in 1990 to 26.7% in 2010 (De Onis, 2011). This expected to incline towards 21.8%, or 142 million, in the year 2020 (De, Onis 2011). In Indonesia, stunting is one of the major nutrition problems in Indonesia, with the prevalence of 35.6% (Minstry of Health of Republic Indonesia 2010). Stunting is resulted from poor standard of living, exposure to adverse conditions such as diseases and poor eating habits and practices (WHO 2010). The most common cause of stunting in the developing countries includes: impaired absorption of nutrients due to intestinal infections or parasites; or combinations of these problems (Prendergast, 2014). Stunting may also lead to several long-term effects when they reach adulthood period, which includes poor cognitive development, poor in academic, poor productivity towards the economy and negative impact on the reproductive health (Dewey, 2011) (Prendergast, 2014).

Improved early nutrition and care can compensate in part for stunted in utero. Stunting that began at a very early infancy age, leads to a more severe impairment to their cognitive development in later life (Prendergast, 2014). Therefore, an early determination of stunting among neonates is very important in order to support for proper feeding support and the gain of better optimal health (Kumar, 2006). The use of classical anthropometric measurements for body length measurements is widely accepted since many decades ago (Group, 1986). However, the use of such measurements to determine stunting has drawbacks such as possible human error or issues with the devices. The use of biomarkers in radiology is being debated to measure the infant's bone density to monitor the growth of the baby; however this method is deemed unsuitable as it involves unnecessary invasive procedures for the babies. Therefore, the use of other readily obtained biological fluids or wastes such as urine can be used as a possible early indicator to stunting among neonates.

In this research, the use of urine as a possible early indicator to stunting is tested. The condition of the urine is associated with health condition of the kidney. Stunted babies as a result from malnutrition, has shown to have disruption to their metabolic process and increased risk of renal impairment (Kartawinata, Hilmanto & Nataprawira 2012). In order to find out whether the babies are having these issues, the creatinine level can be measured from their urine. Creatinine is derived from the metabolism of proteins, either from food or from muscle. Human bone is formed through the process of pairing between bone resorption process (release of a cell or tissue with a gradual preparation of the compounds into smaller and dispersed in circulation) by osteoclasts and bone formation by osteoblast. This process happens normally in bone and skeletal growth. As many as 90% of the organic matrix of bone is made of Collagen Type I is a helical protein is stabilized by cross-linking between terminals N and C terminals on the basis of the formation of bone tissue. During maturation of collagen, Pyd formed by hydroxy Lysine or Lysine residues at the end of the C- and N- terminal telopeptide of collagen molecules and is released during the resorption of the matrix and is excreted through the urine. Pyd is expected to be specific and sensitive biomarker of bone resorption and are able to evaluate bone metabolism or disorder in neonates.

Based on the biological processes, Pyd seems to be of potential use to evaluate bone metabolism among neonates, which indirectly explains the possibility of stunting condition. Therefore, the main objective of the study is to test the possibility of Pyd level in urine as an early indicator to stunted growth.

## 2. MATERIAL AND METHODS

The study design was cross-sectional and was conducted between January to December 2014. Subjects of the study were 35 healthy neonates born at the Andini's Mothers and Children Hospital at Tuanku Tambusai street 55, Pekanbaru (middle class hospital and population strategic location in Pekanbaru) between August 28<sup>th</sup> until September 30<sup>th</sup> 2014. Subjects were recruited at around 1-3 days of neonates life. Inclusion criteria were normal gestation (36 to 40 weeks), spontaneous and caesarean delivery. The study complies with the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects and was approved by the Institutional Review Board of the Faculty of Medicine, University of Riau, Ministry of Education and

78 Culture of Republic Indonesia. Parents of all subjects were given a written informed consent and  
79 signed them upon agreement to join the study.

80 Family socioeconomic questionnaires (e.g. name, gender, age, race, and height parent), body length  
81 gauges (BUTERFLY), digital weighting scale for baby (BABY SCALE TANITA), MicroVue™ PYD EIA  
82 kit, USA (Quidel Corporation, San Diego, CA 92121, USA, Cat: 8010, Lot: 015210, ED: 2015-07 and  
83 Spectrophotometer Microplate Reader 680 (Bio-Rad Laboratories, Inc., Hercules, CA 94547, USA),  
84 Creatinine measurements were performed with the use of Jaffe method and Spectrophotometer  
85 ADVIA 1800: ADVIA, Germany, baby urine bags (PEDIATRIC URINE COLLECTOR, Japan).

86 The 24-hours neonates' urine was collected using paediatrics urine bags by trained nurses, aliquoted  
87 to 6 mL. Mothers were briefed about the study, one day prior to neonates urine collection. Baby urine  
88 that has been collected was stored in the refrigerator at a temperature of -20°C at Pekanbaru Prodia  
89 Clinical Laboratory and then was sent to Prodia Center in Jakarta for analysis.

90 Statistical analysis and results are reported based on the data. Statistical outliers, defined as outside  
91 the 95% confidence limits of the normal probability plots, two subjects were removed before the  
92 analysis. In all statistical tests performed, the null hypothesis (no effect) was rejected at the 0.01 level  
93 of probability. Differences in mean body weight, head circumference, concentrations of Pyd urine,  
94 age, WAZ, HAZ, basal metabolisme indeks -BMI- for age (BAZ), mother's height, mother's BMI,  
95 mother's weight before pregnancy, mother's prenatal weight, mother's pregnancy age, the number of  
96 children in family were evaluated by using a Independent t-test. All analyses were performed by using  
97 SPSS version 20 (IBM SPSS Statistics 20).

98

### 99 3. RESULTS AND DISCUSSION

100

101 All neonates were healthy and have received full enteral feeding (formula and/or breast feeding).  
102 There are 74.3% of the subjects involved in the study were male (Table 1). The reason for the high  
103 percentage is because it was found to be much easier to collect urine from male than female  
104 neonates. Most of the mothers stayed at Pekanbaru, except for two of them. This is because their  
105 other family members also stayed at the same area and would like to be close to them when their  
106 baby born. Apart from that, at Pekanbaru, the mothers thought that they can get a proper medical  
107 attention when needed compared to outside the city. Most of the mothers were Malay. 50% of the  
108 study subjects mother were full housewife. Some of the mothers also working as officers within the  
109 government and private sector. Overall, the education level among all the subjects mothers were at  
110 the Diploma Level and above.

111 Table 1 Familial socioeconomic status of the subjects

Variabels	Criteria	Value*
Sex	Male	74.3 (26)
	Female	25.7 (9)
Residence	Pekanbaru	91.4 (32)
	Others	5.7 (2)
Ethnic group	Malay	97.1 (34)
	Chinese	2.9 (1)

\* Tel.: +62818106440; fax: (0761) 20656.  
E-mail address: asliswirda@yahoo.com

Mother's job	Teacher	8.6 (3)
	Housewife	51.4 (18)
	Employed	31.4 (11)
	Entrepreneur	8.6 (3)
Mother's education	Elementary school	5.7 (2)
	Junior high school	2.9 (1)
	Senior high school	22.9 (8)
	Diploma 3	20.0 (7)
	Diploma 4	2.9 (1)
	Scholar	37.1 (13)

---

112 \*% (n)

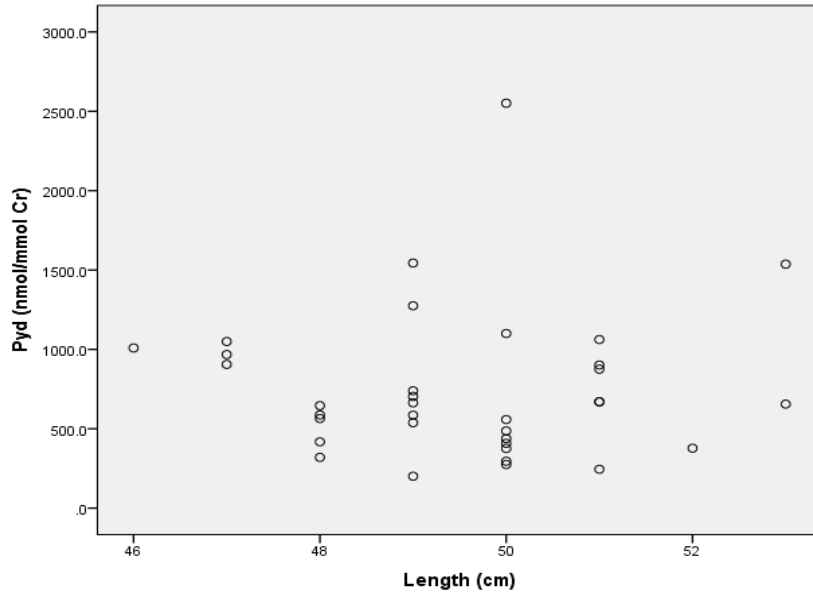
113 The proportion of stunted neonates in the study was 22.9%. It was a similar findings as  
 114 reported by Atmarita research is 20.2% (Atmarita 2014). Pyd excretion from neonates is  
 115 found to be more than that elementary school children and adult. Pyd excretion from  
 116 neonates is about 201.2 – 1275.1 nmol/mmol Cr. Pyd excretion from elementary  
 117 school children is about 50--500 nmol/mmol Cr (Beardsworth, Eyre & Dickson 1990).  
 118 Pyd excretion on children 3-5 year was 238.3±22.7 pmol/mumol Cr (male) and  
 119 261.8±14.2 pmol/mumol Cr (female) (Fujimoto et al. 1995). Pyd excretion from  
 120 elementary school children is more than adult (Husain et al. 1999). Pyd excretion from  
 121 malnourished children is less than recovery children, and there were positive correlation  
 122 Pyd excretion and linier growth (Robins SP (1994). Pyd excretion is specific to become  
 123 children bone resorption (Fujimoto et al. 1995).

124 Table 2. Characteristics of the stunted and normal subject

Variables	Stunted	Normal
Length (cm)	46.8±0.5 (46:47) <sup>a</sup>	49.9±1.4 (48.0:53.0) <sup>b</sup>
Weight (g)	2846±360 (2400:3480)	3215±404 (2380:4080)
Head circumference (cm)	33.3±1.0 (31.0:34.5)	33.6±1.2 (31.0:35.0)
Pyd (nmol/mmol Cr)	982,9±61.6 (967,8:1049.6) <sup>a</sup>	594.1±266,1 (564,4:2550.8) <sup>b</sup>
Age (days)	1±1 (1:1)	1±1 (1:3)
WAZ	-1.03±0.82 (-2.15:0.33)	-0.21±0.87 (-2.15:1.74)
HAZ	-1.26±0.27 (-1.67:-1.00) <sup>a</sup>	0.23±0.66 (-0.56:1.97) <sup>b</sup>
BAZ	-0.69±1.29 (-2.27:1.66)	-0.60±1.11 (-3.21:1.34)
Mother's BMI (kg/m <sup>2</sup> )	21.5±4.3 (18.0:30.5)	22.0±2.9 (16.9:26.7)
Mother's height (cm)	156±4 (150:165)	161±7 (150:185)
Mother's weight before pregnancy (kg)	53±13 (42:83)	57±8 (42:70)
Mother's prenatal weight (kg)	66±15 (53:101)	69±11 (50:86)
Pregnancy age (weeks)	38±1 (37:39)	38±3 (35:49)
Number of children (person)	2±1 (1:4)	2±1 (1:4)

Independent t test: <sup>ab</sup> $\rho < 0.01$

126 There was significant difference between HAZ and Pyd in urine of stunted and normal  
 127 neonates. HAZ of neonates are expected to be optimally. There is significant  
 128 difference between Pyd urine of stunted and normal neonates as indicated by body  
 129 length. The contents of Pyd urine stunted neonates (body length <48 cm) was  
 130  $982.9 \pm 61.6$  but normal neonates (body length  $\geq 48$  cm) was  $594.1 \pm 266.1$  nmol/mmol Cr  
 131 ( $p < 0.01$ ). The Pyd urine neonates had quadratic relationship with HAZ of infants ('U-  
 132 shaped' scatter plot). The Pyd urine was negatively associated with body length in  
 133 stunted neonates and can be used as biomarkers of linear growth. Neonates classified  
 134 as stunted if their length <48 cm and the contents of Pyd >859.7 nmol/mmol Cr. Further  
 135 study required for infants aged 6 to 12 months and larger number of sample.  
 136



137

138 Figure 1 Association of urine Pyd and body length in neonates

139

140

141 Therefore, the study has shown that the concentration of Pyd in the urine among  
 142 neonates with the of <48cm is higher compared to those within the >48cm.

143

144

145 **4. CONCLUSION**

146

147 Therefore, based on this study, it has been shown that, Pyd in the urine is very specific  
 148 as a bone resorption indicator.

149

150

151 **ACKNOWLEDGEMENTS**

152

153 Thank you very much for the financial support from the Riau Health Polytechnic, Prodia  
 154 Laboratory, and Andini Hospital.

155

156

157 **COMPETING INTERESTS**

158

159 Authors have declared that no competing interests exist.

160

161 **AUTHORS' CONTRIBUTIONS**

162

163 AWH designed the study, performed the statistical analysis, wrote the protocol, and

164 wrote the first draft of the manuscript. AA and SWN managed the analyses of the study.

165 SRA managed the literature searches and contributed the write up of the manuscript.

166 All authors read and approved the final manuscript.

167

168 **CONSENT**

169

170 All authors declare that written informed consent was obtained from the patient (or

171 other approved parties) for publication of this case report and accompanying images. A

172 copy of the written consent is available for review by the Editorial office/Chief

173 Editor/Editorial Board members of this journal.

174

175 **ETHICAL APPROVAL (WHERE EVER APPLICABLE)**

176

177 All authors hereby declare that all experiments have been examined and approved by

178 the appropriate ethics committee and have therefore been performed in accordance

179 with the ethical standards laid down in the 1964 Declaration of Helsinki.

180

181 **REFERENCES**

182

183 **[1]**



Pydurine as indicator to linear growth neonates

1

1

**Pyridinium Crosslinks (Pyd) in the urine as  
a potential indicator to linear growth among neonates**

2

3

4 Dr. Aslis Wirda Hayati

5 B.Sc (Community Nutrition), M.Sc (Community Nutrition), PhD (Human Nutrition)

6 Nutrition Department of Riau Health Polytechnic, Ministry of Health Republic Indonesia

7 Jl. Melur 103 Pekanbaru, Indonesia 28122

8 Telepon (0761) 36581

9 Facsimili (0761) 20656,

10 Handphone +62818106440

11 Email: asliswirda@yahoo.com

12

13 Dr. Siti Rohaiza Ahmad

14 B.Sc (Nutrition), M.Sc. (Biotech), PhD (Public Health)

15 PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam

16

17 Alkausyari Aziz, M.Kes

18 B.Sc (Public Health), M.Sc (Public Health)

19 Nutrition Department of Riau Health Polytechnic, Ministry of Health Republic Indonesia

20

21 Sri Widia Ningsih, M.Si

22 B.Sc (Chemistry), M.Sc (Chemistry)

23 Farmacy Department of Medan Health Polytechnic, Ministry of Health Republic Indonesia

24

**Pyridinium Crosslinks (Pyd) in the urine as  
a potential indicator to linear growth among neonates**

25  
26  
27

**28 ABSTRACT**

29 The aim of the research is to assess the reliability of bone resorption biomarkers called  
30 Pyridinium Crosslinks (Pyd) in the urine of the neonates as an evaluation to bone growth of  
31 the neonate (based on their length). The study design was cross-sectional. Subjects of study  
32 were 35 healthy neonates born at the Andini Hospital (Pekanbaru, Indonesia) between August  
33 to September 2014. Subjects were recruited at around the first 3 days of life. Body length  
34 gauges, digital weighting scale, family socioeconomic questionnaires and Pyd kit were used  
35 to collect the data. Differences in the mean of the research variables were tested using an  
36 Independent t-test. Results showed that there were significant differences between z-score  
37 height for age (HAZ) and Pyd in the urine of stunted versus normal neonates. The contents of  
38 Pyd urine stunted neonates (body length <48 cm) was  $982.9 \pm 61.6$  but normal neonates (body  
39 length  $\geq 48$  cm) was  $594.1 \pm 266.1$  nmol/mmol Cr ( $p < 0.01$ ). Neonates classified as stunted if  
40 their length <48 cm and the contents of Pyd >859,7 nmol/mmol Cr. Therefore, HAZ and Pyd  
41 in the urine can be a potential early indicators to identify stunted and normal growth neonates.

42

43 *Keywords: length, neonates, pyridinium crosslinks, stunted, z-score height for age,*

44

45

46 **INTRODUCTION**

47 Stunted growth reflects failure to reach linear growth potential as a result of  
48 suboptimal health and/or nutritional conditions WHO (2010). Stunting is one of the major  
49 nutrition problems in Indonesia, with the prevalence of 35.6% (Minstry of Health of Republic  
50 Indonesia 2010). The global prevalence of stunting in the developing countries, among  
51 children under-five years on average is about 33%. Stunting is associated with poor socio-  
52 economic conditions, increased risk of frequent and early exposure to adverse conditions such  
53 as illness and/or inappropriate feeding practices (WHO 2010). The most common cause of  
54 stunting in the developing countries includes: impaired absorption of nutrients due to  
55 intestinal infections or parasites; or combinations of these problems (WHO 2001).

56 Stunting in infant is often associated with poor cognitive and motor development.  
57 Improved early nutrition and care can compensate in part for stunted in utero. In addition to  
58 the adverse effects of early stunting (in utero and in early infancy) on brain development and  
59 their stimulation by their environment. Stunting that began at a very early infancy age, leads  
60 to a more severe impairment to their cognitive development in later life (WHO 2001).

61 No particular method has been reported as an indicator of stunting among neonates.  
62 Anthropometric measurements (body length) to determine stunting is found to be less  
63 accurate because of many possible errors such as human error or issues with the devices used  
64 for measuring. The use of biomarkers in radiology is being debated to measure the infant's  
65 bone density to monitor the growth of the baby, however this method is deemed unsuitable as  
66 it involves unnecessary invasive procedures for the babies.

67 The condition of the urine is associated with health condition of the kidney. Babies  
68 who suffered stunting as a result from malnutrition, has shown to have disruption to their  
69 metabolic process and increasing the risk of renal impairment (Kartawinata, Hilmanto &  
70 Nataprawira 2012). In order to find out whether the babies are having these issues, the

71 creatinine level can be measured from their urine. Creatinine is derived from the metabolism  
72 of proteins, either from food or from muscle. Human bone is formed through the process of  
73 pairing between resorption process (release of a cell or tissue with a gradual preparation of the  
74 compounds into smaller and dispersed in circulation) bone by osteoclasts and bone formation  
75 followed by osteoblast. This process happens normally in bone growth and skeletal. As many  
76 as 90% of the organic matrix of bone is made of Collagen Type I is a helical protein is  
77 stabilized by cross-linking between terminals N and C terminals on the basis of the formation  
78 of bone tissue. During maturation of collagen, Pyd formed by hydroxy Lysine or Lysine  
79 residues at the end of the C- and N- terminal telopeptide of collagen molecules and is released  
80 during the resorption of the matrix and is excreted through the urine. Pyd is expected to be  
81 specific and sensitive biomarker of bone resorption and are able to evaluate bone metabolism  
82 or disorder in neonates.

83

#### 84 **MATERIALS AND METHODS**

85 The study design was cross-sectional and was conducted between January to  
86 December 2014. Subjects of the study were 35 healthy neonates born at the Andini's Mothers  
87 and Children Hospital at Tuanku Tambusai street 55, Pekanbaru (middle class hospital and  
88 population strategic location in Pekanbaru) between August 28<sup>th</sup> until September 30<sup>th</sup> 2014.  
89 Subjects were recruited at around 1-3 days of neonates life. Inclusion criteria were normal  
90 gestation (36 to 40 weeks), spontaneous and caesarean delivery. The study complies with the  
91 World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research  
92 Involving Human Subjects and was approved by the Institutional Review Board of the  
93 Faculty of Medicine, University of Riau, Ministry of Education and Culture of Republic  
94 Indonesia. Parents of all subjects were given a written informed consent.

95 Family socioeconomic questionnaires (e.g. name, gender, age, race, and height parent),

Pydurine as indicator to linear growth neonates

5

96 body length gauges (BUTERFLY), digital weighting scale for baby (BABY SCALE  
97 TANITA), MicroVue™ PYD EIA kit, USA (Quidel Corporation, San Diego, CA 92121,  
98 USA, Cat: 8010, Lot: 015210, ED: 2015-07 and Spectrophotometer Microplate Reader 680  
99 (Bio-Rad Laboratories, Inc., Hercules, CA 94547, USA), Creatinine measurements were  
100 performed with the use of Jaffe method and Spectrophotometer ADVIA 1800: ADVIA,  
101 Germany, baby urine bags (PEDIATRIC URINE COLLECTOR, Japan).

102         The 24-hours neonates' urine was collected using paediatrics urine bags by trained  
103 nurses, aliquoted to 6 mL. Mothers were briefed about the study, one day prior to neonates  
104 urine collection. Baby urine that has been collected was stored in the refrigerator at a  
105 temperature of -20°C at Pekanbaru Prodia Clinical Laboratory and then was sent to Prodia  
106 Center in Jakarta for analysis.

107         Statistical analysis and results are reported based on the data. Statistical outliers,  
108 defined as outside the 95% confidence limits of the normal probability plots, two subjects  
109 were removed before the analysis. In all statistical tests performed, the null hypothesis (no  
110 effect) was rejected at the 0.01 level of probability. Differences in mean body weight, head  
111 circumference, concentrations of Pyd urine, age, WAZ, HAZ, basal metabolisme indeks -  
112 BMI- for age (BAZ), mother's height, mother's BMI, mother's weight before pregnancy,  
113 mother's prenatal weight, mother's pregnancy age, the number of children in family were  
114 evaluated by using an Independent t-test. All analyses were performed by using SPSS version  
115 20 (IBM SPSS Statistics 20).

116

## 117 **RESULTS**

118         All neonates were healthy and has received full enteral feeding (formula and/or breast  
119 feeding). There are 74.3% of the subjects involved in the study were male (Table 1). The  
120 reason for the high percentage is because it was found to be much easier to collect urine from

121 male than female neonates. Most of the mothers stayed at Pekanbaru, except for two of them.  
122 This is because their other family members also stayed at the same area and would like to be  
123 close to them when their baby born. Apart from that, at Pekanbaru, the mothers thought that  
124 they can get a proper medical attention when needed compared to outside the city. Most of  
125 the mothers were Malay. There are 50% of the study subjects mother were full housewife.  
126 Some of the mothers also working as officers within the government and private sector.  
127 Overall, the education level among all the subjects mothers were at the Diploma Level and  
128 above.

129

## 130 **DISCUSSION**

131 The proportion of stunted neonates in the study was 22.9%. It was a similar findings  
132 as reported by Atmarita research is 20.2% (Atmarita 2014).

133 Pyd excretion from neonates is found to be more than that elementary school children  
134 and adult. Pyd excretion from neonates is about 201.2 – 1275.1 nmol/mmol Cr. Pyd  
135 excretion from elementary school children is about 50--500 nmol/mmol Cr (Beardsworth,  
136 Eyre & Dickson 1990). Pyd excretion on children 3-5 year was 238.3±22.7 pmol/mumol Cr  
137 (male) and 261.8±14.2 pmol/mumol Cr (female) (Fujimoto *et al.* 1995). Pyd excretion from  
138 elementary school children is more than adult (Husain *et al.* 1999). Pyd excretion from  
139 malnourished children is less than recovery children, and there were positive correlation Pyd  
140 excretion and linier growth (Robins SP (1994). Pyd excretion is specific to become children  
141 bone resorption (Fujimoto *et al.* 1995).

142 There was significant difference between HAZ and Pyd in urine of stunted and normal  
143 neonates. HAZ of neonates are expected to be optimally. There is significant difference  
144 between Pyd urine of stunted and normal neonates as indicated by body length. The contents  
145 of Pyd urine stunted neonates (body length <48 cm) was 982.9±61.6 but normal neonates

Pydurine as indicator to linear growth neonates

7

146 (body length  $\geq 48$  cm) was  $594.1 \pm 266.1$  nmol/mmol Cr ( $p < 0.01$ ). The Pyd urine neonates had  
147 quadratic relationship with HAZ of infants ('U-shaped' scatter plot). The Pyd urine was  
148 negatively associated with body length in stunted neonates and can be used as biomarkers of  
149 linear growth. Neonates classified as stunted if their length  $< 48$  cm and the contents of  
150 Pyd  $> 859.7$  nmol/mmol Cr. Further study required for infants aged 6 to 12 months and larger  
151 number of sample.

152

### 153 **ACKNOWLEDGMENTS**

154 Thank you very much for the financial support from the Riau Health Polytechnic,  
155 Prodia Laboratory, and Andini Hospital.

156

### 157 **AUTHOR DISCLOSURES**

158 No author has a conflict of interest.

159

### 160 **REFERENCES**

161 Atmarita. The Future Figures of Indonesian Human Resources. Ministry of Health of  
162 Republic Indonesia, Jakarta; 2014.

163 Beardsworth LJ, Eyre DR & Dickson IR. Changes with age in the urinary excretion of lysyl-  
164 and hydroxylysylpyridinoline, two new markers of bone collagen turnover. *J. Bone*  
165 *Miner.* 1990; 5: 671.

166 Fujimoto S, Kubo T, Tanaka H, Miura M & Seino Y. Urinary Pyridinoline and  
167 Deoxypyridinoline in Healthy Children and in Children with Growth Hormone  
168 Deficiency. *J Clin Endocrinol Metab.* 1995; 80:1922–8.

- 169 Husain SM, Mughal Z, Williams G, Ward K, Smith CS, Dutton J & Fraser WD. Urinary  
170 Excretion of Pyridinium crosslinks in Healthy 4–10 Year Olds. *Arch Dis Child*.  
171 1999; 80:370–373.
- 172 Kartawinata Y, Hilmanto D & Nataprawira HM. Serum Levelsof Creatininedan Cystatin-C  
173 on Group of Children with Undernutrition and Normal Status. Department of  
174 Pediatrics, Faculty of Medicine, University of Padjadjaran/Hasan Sadikin Hospital,  
175 Bandung. *J Indon Med Assoc*. 2012; 62:46-51.
- 176 Ministry of Health of Republic Indonesia. National Health Survey. MOH, Jakarta; 2010.
- 177 Robins SP. Biochemical markers for assessing skeletal growth. *European Journal of Clinical*  
178 *Nutrition*. 1994; 48:S199-S209.
- 179 WHO. Improving Child Growth. WHO, Geneva; 2001.
- 180 WHO. Child Growth Indicators and Their Interpretation. WHO, Geneva; 2010.



181 Table 1. Familial socioeconomic status of the subjects

Variabels	Criteria	Value*
Sex	Male	74.3 (26)
	Female	25.7 (9)
Residence	Pekanbaru	91.4 (32)
	Others	5.7 (2)
Ethnic group	Malay	97.1 (34)
	Chinese	2.9 (1)
Mother's job	Teacher	8.6 (3)
	Housewife	51.4 (18)
	Employed	31.4 (11)
	Entrepreneur	8.6 (3)
Mother's education	Elementary school	5.7 (2)
	Junior high school	2.9 (1)
	Senior high school	22.9 (8)
	Diploma 3	20.0 (7)
	Diploma 4	2.9 (1)
	Scholar	37.1 (13)

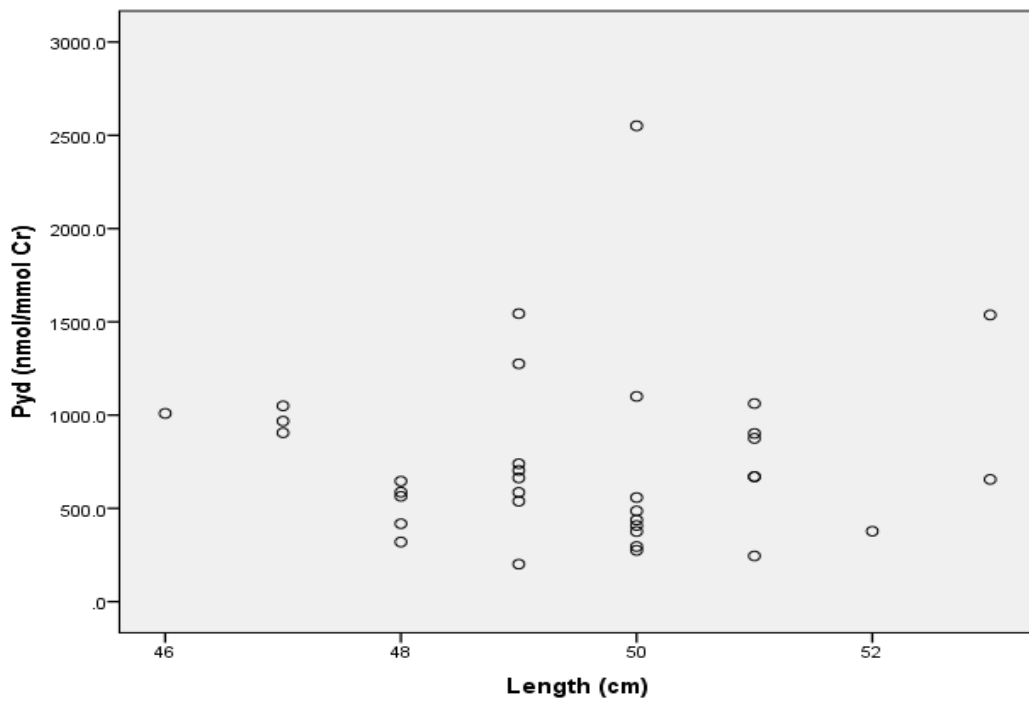
182 \*% (n)

183 Table 2. Characteristics of the stunted and normal subject

Variabels	Stunted	Normal
Length (cm)	46.8±0.5 (46:47) <sup>a</sup>	49.9±1.4 (48.0:53.0) <sup>b</sup>
Weight (g)	2846±360 (2400:3480)	3215±404 (2380:4080)
Head circumference (cm)	33.3±1.0 (31.0:34.5)	33.6±1.2 (31.0:35.0)
Pyd (nmol/mmol Cr)	982,9±61.6 (967,8:1049.6) <sup>a</sup>	594.1±266,1 (564,4:2550.8) <sup>b</sup>
Age (days)	1±1 (1:1)	1±1 (1:3)
WAZ	-1.03±0.82 (-2.15:0.33)	-0.21±0.87 (-2.15:1.74)
HAZ	-1.26±0.27 (-1.67:-1.00) <sup>a</sup>	0.23±0.66 (-0.56:1.97) <sup>b</sup>
BAZ	-0.69±1.29 (-2.27:1.66)	-0.60±1.11 (-3.21:1.34)
Mother's BMI (kg/m <sup>2</sup> )	21.5±4.3 (18.0:30.5)	22.0±2.9 (16.9:26.7)
Mother's height (cm)	156±4 (150:165)	161±7 (150:185)
Mother's weight before pregnancy (kg)	53±13 (42:83)	57±8 (42:70)
Mother's prenatal weight (kg)	66±15 (53:101)	69±11 (50:86)
Pregnancy age (weeks)	38±1 (37:39)	38±3 (35:49)
Number of children (person)	2±1 (1:4)	2±1 (1:4)

Independent t test: <sup>ab</sup> $p < 0.01$

185



186

187

Figure 1. Association of urine Pyd and body length in neonates

Chief Editor  
Dr Chong Vui Heng  
Brunei International Medical Journal  
Brunei Darussalam

10<sup>th</sup> May 2017

Dear Sir/Madam,

We wish to submit a new manuscript entitled “*Pyridinium Crosslinks (Pyd) in the urine as a potential indicator to linear growth among neonates*” for consideration by the Brunei International Medical Journal.

The work conducted and presented in this manuscript has not been published or submitted for publication in another journal. All authors named in the manuscript have made substantial contribution each to qualify for authorship according to BIMJ authorship criteria and have approved of the content of the manuscript. We have disclosed all financial support for our work and other potential conflicts of interests.

In this paper, we report potential biomarkers in the urine as early indicators to identify stunted and normal growth among neonates. This is significant because early diagnosis can support early intervention to stunting among neonates. This paper should be of interest to readers especially baby doctors.

Please address all correspondence concerning this manuscript to me at [asliswirda@yahoo.com](mailto:asliswirda@yahoo.com)

Thank you for your consideration of this manuscript.

Sincerely,



Dr. Aslis Wirda Hayati

B.Sc (Community Nutrition), M.Sc (Community Nutrition), PhD (Human Nutrition)  
Nutrition Department of Riau Health Polytechnic, Ministry of Health Republic Indonesia



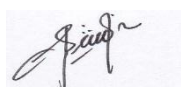
Dr. Siti Rohaiza Ahmad

B.Sc (Nutrition), M.Sc. (Biotech), PhD (Public Health)  
PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam



Alkausyari Aziz, M.Kes

B.Sc (Public Health), M.Sc (Public Health)  
Nutrition Department of Riau Health Polytechnic, Ministry of Health Republic Indonesia



Sri Widia Ningsih, M.Si

B.Sc (Chemistry), M.Sc (Chemistry)  
Pharmacy Department of Medan Health Polytechnic, Ministry of Health Republic Indonesia





# Brunei International Medical Journal

## COPYRIGHT ASSIGNMENT FORM

Manuscript No: ..... Category: .....

Manuscript Title: *Pyridinium crosslinks (Pxd) in the urine as a potential indicator to linear growth among neonates*

(Please fax completed copyright assignment form to BIMJ at +673-2242690: Attention to BIMJ, Clinical Research Unit, Medical Education Centre, RIPAS Hospital, or scan the completed form and email to [bimj\\_editors@bimjonline.com](mailto:bimj_editors@bimjonline.com))

Copyright Transfer Agreement: Each author must sign this form to certify that:

1. I/We hereby assign completely and absolutely to BIMJ with effect from the date of acceptance of the above titled manuscript for publication in BIMJ, all present and future copyrights to the manuscript. Such assignment of copyright shall include, without limitation to the foregoing, the exclusive right to do any and all acts in all countries in which the copyright (or analogous rights) in the manuscript subsists (or in the future subsists) together with all rights of action in respect of any past or existing infringement of such copyright;
2. The manuscript above is my/our original work without fabrication, fraud, or plagiarism and has not been published previously elsewhere (printed or electronic form in the internet/discussion groups/electronic bulletin boards) or has been submitted or under consideration for publication elsewhere.
3. That the manuscript contains no violation of any existing copyright or other third party right or any material of an obscene, libelous or otherwise unlawful nature, and that I/we will indemnify the Editors of BIMJ against all claims and expenses (including legal costs and expenses) arising from breach of this warranty and the other warranties on my/our behalf in this agreement.
4. That I/we have obtained permission for and acknowledged the original authors of the source of any illustrations, diagrams or other materials used in the manuscript of which I am/we are not the original copyright owner/s .
5. All authors warrant that they each meet the requirements for authorship enumerated in the Journal's Instructions for Authors and understand that if the paper or part of the paper is found to be faulty or fraudulent, each shares the responsibility.

I have read and understand the above conditions and provide the appropriate signatures and information below:

Name (in FULL): *Dr. Asli Winda Hayati, S.P., M.Si*  
(Corresponding or senior author/Copyright holder)

Signature: 

Date: *May 9<sup>th</sup> 2017*



Please tick if co-authors have agreed for corresponding author to sign on behalf of them.




# Brunei International Medical Journal


## COPYRIGHT ASSIGNMENT FORM

Co-Authors (Names in full with signatures and date). Attached an additional sheet if there is insufficient space below.

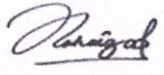
.....  
Full name, signatures and date

  
ALICIA SYARIF MBBS, SKM-MKES 12 May 2017  
Full name, signatures and date

.....  
Full name, signatures and date

Sri Widia Ningsih, M.Si, , 04th May 2017  
Full name, signatures and date

.....  
Full name, signatures and date

Siti Rohaiza Ahmad, 9.5.17   
Full name, signatures and date

.....  
Full name, signatures and date

.....  
Full name, signatures and date

.....  
Full name, signatures and date

.....  
Full name, signatures and date

Pydurine as indicator to linear growth neonates

1

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17

**Pyridinium Crosslinks (Pyd) in the urine as  
a potential indicator to linear growth among neonates**

**Reviewers comments:**

- This manuscript is poorly written with some degree of plagiarism where some of the text has been lifted completely from the reference.
- There are grammatical and spelling errors.
- There is no study aims or objective
- Half the results are missing from the main text.
- Discussion is rather short
- References are not in compliance with BIMJ requirements.
- No limitation paragraph
- No conclusion
- No description of how the cutoff for stunting was chosen. Was this based on WHO criteria? Were parental height taken into consideration?

**Decision: Reject.**



Pydurine as indicator to linear growth neonates

2

18  
19  
20

**Pyridinium Crosslinks (Pyd) in the urine as  
a potential indicator to linear growth among neonates**

21 **ABSTRACT**

22 The aim of the research is to assess the reliability of bone resorption biomarkers called  
23 Pyridinium Crosslinks (Pyd) in the urine of the neonates as an evaluation to bone growth of  
24 the neonate (based on their length). The study design was cross-sectional. Subjects of study  
25 were 35 healthy neonates born at the Andini Hospital (Pekanbaru, Indonesia) between August  
26 to September 2014. Subjects were recruited at around the first 3 days of life. Body length  
27 gauges, digital weighting scale, family socioeconomic questionnaires and Pyd kit were used  
28 to collect the data. Differences in the mean of the research variables were tested using an  
29 Independent t-test. Results showed that there were significant differences between z-score  
30 height for age (HAZ) and Pyd in the urine of stunted versus normal neonates. The contents of  
31 Pyd urine stunted neonates (body length <48 cm) was  $982.9 \pm 61.6$  but normal neonates (body  
32 length  $\geq 48$  cm) was  $594.1 \pm 266.1$  nmol/mmol Cr ( $p < 0.01$ ). Neonates classified as stunted if  
33 their length <48 cm and the contents of Pyd  $> 859,7$  nmol/mmol Cr. Therefore, HAZ and Pyd  
34 in the urine can be a potential early indicators to identify stunted and normal growth neonates.

35

36 *Keywords: length, neonates, pyridinium crosslinks, stunted, z-score height for age,*

37

38

Pydurine as indicator to linear growth neonates

3

## 39 INTRODUCTION

40 Stunted growth reflects failure to reach linear growth potential as a result of  
41 suboptimal health and/or nutritional conditions (WHO (2010)). Stunting is one of the major  
42 nutrition problems in Indonesia, with the prevalence of 35.6% (Minstry of Health of Republic  
43 Indonesia 2010). The global prevalence of stunting in the developing countries, among  
44 children under-five years on average is about 33%. Stunting is associated with poor socio-  
45 economic conditions, increased risk of frequent and early exposure to adverse conditions such  
46 as illness and/or inappropriate feeding practices (WHO 2010). The most common cause of  
47 stunting in the developing countries includes: impaired absorption of nutrients due to  
48 intestinal infections or parasites; or combinations of these problems (WHO 2001).

49 Stunting in infant is often associated with poor cognitive and motor development.  
50 Improved early nutrition and care can compensate in part for stunted in utero. In addition to  
51 the adverse effects of early stunting (in utero and in early infancy) on brain development and  
52 their stimulation by their environment. Stunting that began at a very early infancy age, leads  
53 to a more severe impairment to their cognitive development in later life (WHO 2001).

54 No particular method has been reported as an indicator of stunting among neonates.  
55 Anthropometric measurements (body length) to determine stunting is found to be less  
56 accurate because of many possible errors such as human error or issues with the devices used  
57 for measuring. The use of biomarkers in radiology is being debated to measure the infant's  
58 bone density to monitor the growth of the baby, however this method is deemed unsuitable as  
59 it involves unnecessary invasive procedures for the babies.

60 The condition of the urine is associated with health condition of the kidney. Babies  
61 who suffered stunting as a result from malnutrition, has shown to have disruption to their  
62 metabolic process and increasing the risk of renal impairment (Kartawinata, Hilmanto &  
63 Nataprawira 2012). In order to find out whether the babies are having these issues, the

Commented [BR1]: This sentence is lifted from the reference

Commented [BR2]: Please use vancouver style referencing as required by BIMJ.

Commented [BR3]: This statement is lifted off the reference.

Pydурine as indicator to linear growth neonates

4

64 creatinine level can be measured from their urine. Creatinine is derived from the metabolism  
65 of proteins, either from food or from muscle. Human bone is formed through the process of  
66 pairing between **resorption process** (release of a cell or tissue with a gradual preparation of the  
67 compounds into smaller and dispersed in circulation) **bone** by osteoclasts and bone formation  
68 **followed** by osteoblast. This process happens normally in **bone growth and skeletal**. As many  
69 as 90% of the organic matrix of bone is made of Collagen Type I is a helical protein is  
70 stabilized by cross-linking between terminals N and C terminals on the basis of the formation  
71 of bone tissue. During maturation of collagen, Pyd formed by hydroxy Lysine or Lysine  
72 residues at the end of the C- and N- terminal telopeptide of collagen molecules and is released  
73 during the resorption of the matrix and is excreted through the urine. Pyd is expected to be  
74 specific and sensitive biomarker of bone resorption and are able to evaluate bone metabolism  
75 or disorder in neonates. ]

Commented [BR4]: Bone resorption process

Commented [BR5]: Delete 'bone'

Commented [BR6]: Delete 'followed'

Commented [BR7]: Change to 'bone and skeletal growth'.

Commented [BR8]: Please provide an objective for this study.

76 [

## 77 MATERIALS AND METHODS

78 The study design was cross-sectional and was conducted between January to  
79 December 2014. Subjects of the study were 35 healthy neonates born at the Andini's Mothers  
80 and Children Hospital at Tuanku Tambusai street 55, Pekanbaru (middle class hospital and  
81 population strategic location in Pekanbaru) between August 28<sup>th</sup> until September 30<sup>th</sup> 2014.  
82 Subjects were recruited at around 1-3 days of neonates life. Inclusion criteria were normal  
83 gestation (36 to 40 weeks), spontaneous and caesarean delivery. The study complies with the  
84 World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research  
85 Involving Human Subjects and was approved by the Institutional Review Board of the  
86 Faculty of Medicine, University of Riau, Ministry of Education and Culture of Republic  
87 Indonesia. **Parents of all subjects were given a written informed consent.**

Commented [BR9]: This does not mean that the parents signed the consent. Author needs to be more precise in stating that parents of all subjects gave their written informed consent for their infants to be included in the study.

88 Family socioeconomic questionnaires (e.g. name, gender, age, race, and height parent),

Pydurate as indicator to linear growth neonates

5

89 body length gauges (BUTERFLY), digital weighting scale for baby (BABY SCALE  
90 TANITA), MicroVue™ PYD EIA kit, USA (Quidel Corporation, San Diego, CA 92121,  
91 USA, Cat: 8010, Lot: 015210, ED: 2015-07 and Spectrophotometer Microplate Reader 680  
92 (Bio-Rad Laboratories, Inc., Hercules, CA 94547, USA), Creatinine measurements were  
93 performed with the use of Jaffe method and Spectrophotometer ADVIA 1800: ADVIA,  
94 Germany, baby urine bags (PEDIATRIC URINE COLLECTOR, Japan).

95 The 24-hours neonates' urine was collected using paediatrics urine bags by trained  
96 nurses, aliquoted to 6 mL. Mothers were briefed about the study, one day prior to neonates  
97 urine collection. Baby urine that has been collected was stored in the refrigerator at a  
98 temperature of -20°C at Pekanbaru Prodia Clinical Laboratory and then was sent to Prodia  
99 Center in Jakarta for analysis.

100 Statistical analysis and results are reported based on the data. Statistical outliers,  
101 defined as outside the 95% confidence limits of the normal probability plots, two subjects  
102 were removed before the analysis. In all statistical tests performed, the null hypothesis (no  
103 effect) was rejected at the 0.01 level of probability. Differences in mean body weight, head  
104 circumference, concentrations of Pyd urine, age, WAZ, HAZ, basal metabolism index -  
105 BMI- for age (BAZ), mother's height, mother's BMI, mother's weight before pregnancy,  
106 mother's prenatal weight, mother's pregnancy age, the number of children in family were  
107 evaluated by using an Independent t-test. All analyses were performed by using SPSS version  
108 20 (IBM SPSS Statistics 20). ]

109 [

## 110 RESULTS

111 All neonates were healthy and has received full enteral feeding (formula and/or breast  
112 feeding). There are 74.3% of the subjects involved in the study were male (Table 1). The  
113 reason for the high percentage is because it was found to be much easier to collect urine from

**Commented [BR10]:** This statement does not make any sense. In order to be certain of statistical outliers, Author will need to have carried out some form of analysis and yet author stated that the two subjects were removed before analysis.

**Commented [BR11]:** Author has not provided a paragraph on how the outcome was measured. How did the author decide on what is considered as stunting and what is normal?

**Commented [BR12]:** Past tense please. And this statement needs to be written better. The grammar and flow is in correct.

Pydurine as indicator to linear growth neonates

6

114 male than female neonates. Most of the mothers stayed at Pekanbaru, except for two of them.  
115 This is because their other family members also stayed at the same area and would like to be  
116 close to them when their baby born. Apart from that, at Pekanbaru, the mothers thought that  
117 they can get a proper medical attention when needed compared to outside the city. Most of  
118 the mothers were Malay. There are 50% of the study subjects mother were full housewife.  
119 Some of the mothers also working as officers within the government and private sector.  
120 Overall, the education level among all the subjects mothers were at the Diploma Level and  
121 above. ]

**Commented [BR13]:** Will this bias the results since a selective process for recruiting has been used.

122 [

**Commented [BR14]:** Where are the results of the study? There is no mention of table 2 results here.

## 123 DISCUSSION

124 The proportion of stunted neonates in the study was 22.9%. It was a similar findings  
125 as reported by Atmarita research is 20.2% (Atmarita 2014).

126 Pyd excretion from neonates is found to be more than that elementary school children  
127 and adult. Pyd excretion from neonates is about 201.2 – 1275.1 nmol/mmol Cr. Pyd  
128 excretion from elementary school children is about 50--500 nmol/mmol Cr (Beardsworth,  
129 Eyre & Dickson 1990). Pyd excretion on children 3-5 year was 238.3±22.7 pmol/mumol Cr  
130 (male) and 261.8±14.2 pmol/mumol Cr (female) (Fujimoto *et al.* 1995). Pyd excretion from  
131 elementary school children is more than adult (Husain *et al.* 1999). Pyd excretion from  
132 malnourished children is less than recovery children, and there were positive correlation Pyd  
133 excretion and linier growth (Robins SP (1994). Pyd excretion is specific to become children  
134 bone resorption (Fujimoto *et al.* 1995).

**Commented [BR15]:** This comparison is not entirely valid since the author is comparing with different geographical population which may have different diet, prevalence etc.

135 There was significant difference between HAZ and Pyd in urine of stunted and normal  
136 neonates. HAZ of neonates are expected to be optimally. There is significant difference  
137 between Pyd urine of stunted and normal neonates as indicated by body length. The contents  
138 of Pyd urine stunted neonates (body length <48 cm) was 982.9±61.6 but normal neonates

**Commented [BR16]:** On what basis is this reference used to define stunting? A neonate that is 49cm is normal and a neonate that is 47cm is considered stunted, with a difference of only 2cm. How does author define this? Was parents height taken into account? Short parents tend to have short offsprings.

Pyd urine as indicator to linear growth neonates

7

139 (body length  $\geq 48$  cm) was  $594.1 \pm 266.1$  nmol/mmol Cr ( $p < 0.01$ ). The Pyd urine neonates had  
140 quadratic relationship with HAZ of infants ('U-shaped' scatter plot). The Pyd urine was  
141 negatively associated with body length in stunted neonates and can be used as biomarkers of  
142 linear growth. Neonates classified as stunted if their length  $< 48$  cm and the contents of  
143 Pyd  $> 859.7$  nmol/mmol Cr. Further study required for infants aged 6 to 12 months and larger  
144 number of sample. ]

**Commented [BR17]:** Author needs to provide a limitation paragraph and a conclusion for the study.

145 [

#### 146 **ACKNOWLEDGMENTS**

147 Thank you very much for the financial support from the Riau Health Polytechnic,  
148 Prodia Laboratory, and Andini Hospital.

149

#### 150 **AUTHOR DISCLOSURES**

151 No author has a conflict of interest.

152

#### 153 **REFERENCES**

154 Atmarita. The Future Figures of Indonesian Human Resources. Ministry of Health of  
155 Republic Indonesia, Jakarta; 2014.

156 Beardsworth LJ, Eyre DR & Dickson IR. Changes with age in the urinary excretion of lysyl-  
157 and hydroxylysylpyridinoline, two new markers of bone collagen turnover. J. Bone  
158 Miner. 1990; 5: 671.

159 Fujimoto S, Kubo T, Tanaka H, Miura M & Seino Y. Urinary Pyridinoline and  
160 Deoxypyridinoline in Healthy Children and in Children with Growth Hormone  
161 Deficiency. J Clin Endocrinol Metab. 1995; 80:1922-8.

Pydumine as indicator to linear growth neonates

8

- 162 Husain SM, Mughal Z, Williams G, Ward K, Smith CS, Dutton J & Fraser WD. Urinary  
163 Excretion of Pyridinium crosslinks in Healthy 4–10 Year Olds. *Arch Dis Child*.  
164 1999; 80:370–373.
- 165 Kartawinata Y, Hilmanto D & Nataprawira HM. Serum Levelsof Creatininedan Cystatin-C  
166 on Group of Children with Undernutrition and Normal Status. Department of  
167 Pediatrics, Faculty of Medicine, University of Padjadjaran/Hasan Sadikin Hospital,  
168 Bandung. *J Indon Med Assoc*. 2012; 62:46-51.
- 169 Ministry of Health of Republic Indonesia. National Health Survey. MOH, Jakarta; 2010.
- 170 Robins SP. Biochemical markers for assessing skeletal growth. *European Journal of Clinical*  
171 *Nutrition*. 1994; 48:S199-S209.
- 172 WHO. Improving Child Growth. WHO, Geneva; 2001.
- 173 WHO. Child Growth Indicators and Their Interpretation. WHO, Geneva; 2010.

Pydurine as indicator to linear growth neonates  
9

174 Table 1. Familial socioeconomic status of the subjects

Variabels	Criteria	Value*
Sex	Male	74.3 (26)
	Female	25.7 (9)
Residence	Pekanbaru	91.4 (32)
	Others	5.7 (2)
Ethnic group	Malay	97.1 (34)
	Chinese	2.9 (1)
Mother's job	Teacher	8.6 (3)
	Housewife	51.4 (18)
	Employed	31.4 (11)
	Entrepreneur	8.6 (3)
Mother's education	Elementary school	5.7 (2)
	Junior high school	2.9 (1)
	Senior high school	22.9 (8)
	Diploma 3	20.0 (7)
	Diploma 4	2.9 (1)
	Scholar	37.1 (13)

175 \*% (n)



Pydurate as indicator to linear growth neonates  
10

176 Table 2. Characteristics of the stunted and normal subject

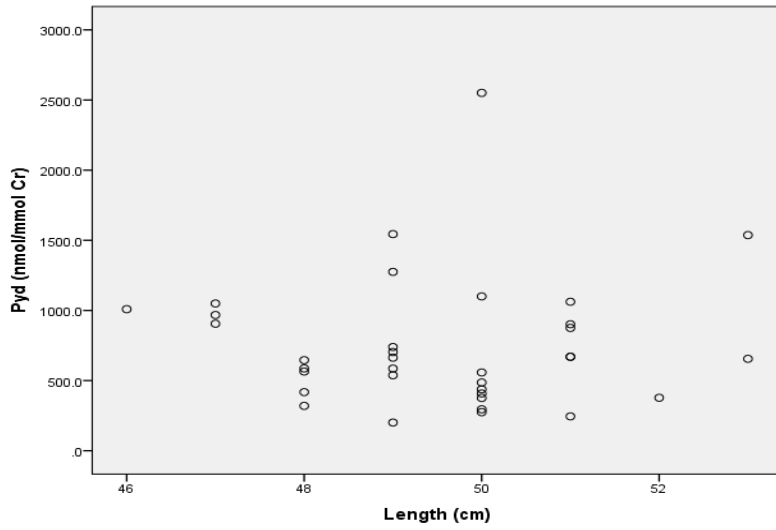
Variabels	Stunted	Normal
Length (cm)	46.8±0.5 (46:47) <sup>a</sup>	49.9±1.4 (48.0:53.0) <sup>b</sup>
Weight (g)	2846±360 (2400:3480)	3215±404 (2380:4080)
Head circumference (cm)	33.3±1.0 (31.0:34.5)	33.6±1.2 (31.0:35.0)
Pyd (nmol/mmol Cr)	982,9±61.6 (967,8:1049.6) <sup>a</sup>	594.1±266,1 (564,4:2550.8) <sup>b</sup>
Age (days)	1±1 (1:1)	1±1 (1:3)
WAZ	-1.03±0.82 (-2.15:0.33)	-0.21±0.87 (-2.15:1.74)
HAZ	-1.26±0.27 (-1.67:-1.00) <sup>a</sup>	0.23±0.66 (-0.56:1.97) <sup>b</sup>
BAZ	-0.69±1.29 (-2.27:1.66)	-0.60±1.11 (-3.21:1.34)
Mother's BMI (kg/m <sup>2</sup> )	21.5±4.3 (18.0:30.5)	22.0±2.9 (16.9:26.7)
Mother's height (cm)	156±4 (150:165)	161±7 (150:185)
Mother's weight before pregnancy (kg)	53±13 (42:83)	57±8 (42:70)
Mother's prenatal weight (kg)	66±15 (53:101)	69±11 (50:86)
Pregnancy age (weeks)	38±1 (37:39)	38±3 (35:49)
Number of children (person)	2±1 (1:4)	2±1 (1:4)

Independent t test: <sup>ab</sup> $p < 0.01$

Pydurate as indicator to linear growth neonates

11

178



179

180

Figure 1. Association of urine Pyd and body length in neonates

WhatsApp [LIVE] Tanya Jawab Ramadhan (89 unread) - asliswirda@yahoo.com

mail.yahoo.com/d/search/keyword=subject%253Aneonatus/messages/11780?guce\_referrer=aHR0cHM6Ly9sb2dpb5YWwhvby5j...

HOME MAIL NEWS FINANCE SPORT LIFESTYLE ENTERTAINMENT WEATHER MORE...

yahoo!mail Find messages, documents, photos or people Advanced Aslis Wirda Home

Compose Back Archive Move Delete Spam

Inbox 89 Unread Starred Drafts Sent Archive Spam Deleted Items ^ Less Views Show Folders Hide + New folder 2001 Sos 4 77 2017 Jurnal

Follow Up with reference to Pyridinium Crosslinks Pyd in the Urine Is Associated with Stunting in Neonates Yahoo/Aslis

Dr. Jixin Zhong <chiefauthor@medicalresearchjournal.org> To: Aslis Wirda Hayati Mon, 30 Sept 2019 at 6:50 pm

To,  
Dr. Aslis Wirda Hayati,  
Poltekkes Kemenkes Riau,  
Indonesia.

Dear Dr. Aslis Wirda Hayati,

I am writing this follow-up for the email I sent you few days ago. I think that you had a glimpse of our invitation email and regarded our. We will be glad to develop an academic relationship with you. I would like to answer any query that may have come up. Looking forward from you soon.

With Regards,

Adobe  
Students save up to 60% on Adobe Creative Cloud.  
Stand out from the crowd.  
Buy now

24°C Berawan 07:32 05/04/2023

WhatsApp [LIVE] Tanya Jawab Ramadhan (89 unread) - asliswirda@yahoo.com

mail.yahoo.com/d/search/keyword=subject%253Aneonatus/messages/11780?guce\_referrer=aHR0cHM6Ly9sb2dpb5YWwhvby5j...

HOME MAIL NEWS FINANCE SPORT LIFESTYLE ENTERTAINMENT WEATHER MORE...

yahoo!mail Find messages, documents, photos or people Advanced Aslis Wirda Home

Compose Back Archive Move Delete Spam

Inbox 89 Unread Starred Drafts Sent Archive Spam Deleted Items ^ Less Views Show Folders Hide + New folder 2001 Sos 4 77

Poltekkes Kemenkes Riau, Indonesia.

Dear Dr. Aslis Wirda Hayati,

I am writing this follow-up for the email I sent you few days ago. I think that you had a glimpse of our invitation email and regarded our. We will be glad to develop an academic relationship with you. I would like to answer any query that may have come up. Looking forward from you soon.

With Regards,  
Dr. Jixin Zhong  
Editor,  
Global Journal of Medical Research  
<https://globaljournals.org/journals/medical-research/b-pharma-drug-discovery-toxicology-medicine>

Not interested anymore? [Unsubscribe](#) to no longer receive emails from us.

SCOOT BECAUSE TRAVEL DESERVES BETTER flyscot.com

BILANG 'KE MANA SELANJUTNYA' DENGAN SCOOT!  
Sekali jalan ke Phuket  
Rp 1.014.000\*  
Pesan sekarang

Waiting for jll.fc.yahoo.com... 24°C Berawan 07:33 05/04/2023



**SDI Review Form 1.6**

Journal Name:	<a href="#">Asian Journal of Research in Medical and Pharmaceutical Sciences</a>
Manuscript Number:	Ms_AJRIMPS_48579
Title of the Manuscript:	Pyridinium Crosslinks (Pyd) In the Urine is Associated With Stunting In Neonates
Type of the Article	Original Research Article

**General guideline for Peer Review process:**

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

(<http://www.sciencedomain.org/page.php?id=sdi-general-editorial-policy#Peer-Review-Guideline>)

**PART 1: Review Comments**

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<b>Compulsory</b> REVISION comments	In this short communication, the authors document the relationship between a marker for bone density (expressed as fraction of creatinine in urine) to underdeveloped growth in newborns. Their findings are interesting, however seem preliminary and somewhat anecdotal. This is due largely to the small sample size, the skew towards male infants and the fact that mothers in this study who give birth to smaller babies are shorter and lighter individuals themselves. The study as is can therefore not be accepted for publication.	
<b>Minor</b> REVISION comments		
<b>Optional/General</b> comments		



SDI Review Form 1.6

**PART 2:**

	<b>Reviewer's comment</b>	<b>Author's comment</b> <i>(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)</i>
<b>Are there ethical issues in this manuscript?</b>	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	

Reviewer Details:

Name:	<b>Anonymous Reviewer, Reviewer preferred to be anonymous.</b>
Department, University & Country	



## **Pyridinium Crosslinks (*Pyd*) in the Urine is Associated With Stunting in Neonates**

**Aslis Wirda Hayati<sup>1\*</sup>, Alkausyari Aziz<sup>1</sup>, Siti Rohaiza Ahmad<sup>2</sup> and Sri Widya Ningseh<sup>3</sup>**

<sup>1</sup>*Nutrition Department of Riau Health Polytechnic, Ministry of Health Republic, Indonesia.*

<sup>2</sup>*PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam, Brunei.*

<sup>3</sup>*Pharmacy Department of Medan Health Polytechnic, Ministry of Health Republic, Indonesia.*

### **Authors' contributions: Please write this section**

*This work was carried out in collaboration among all authors. 'Author A' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Author B' and 'Author C' managed the analyses of the study. 'Author C' managed the literature searches. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/AJRIMPS/2019/XXXXX

Editor(s):

(1)

(2)

Reviewers:

(1)

(2)

(3)

(4)

Complete Peer review History:

**Original Research Article**

**Received ..... 20YY**  
**Accepted ..... 20YY**  
**Published ..... 20YY**

### **ABSTRACT**

**Aims:** The aim of the research is to evaluate the reliability of bone resorption biomarkers called Pyridinium Crosslinks (*Pyd*) in the urine of the neonates as an evaluation to bone growth of the neonate, as an indicator of stunting.

**Study design:** A cross-sectional study.

**Place and Duration of Study:** Andini Mothers and Children Hospital (*Pekanbaru*, Indonesia). Duration of the study was between, August until September 2014.

**Methodology:** Subjects of study were 35 healthy neonates. Subjects were recruited at the first 3 days of life. Body length gauges, digital weighting scale, family socioeconomic questionnaires and *Pyd* kit were used to collect the data. Differences in the mean of the research variables were tested using an Independent t-test.

\*Corresponding author: E-mail: [asliswirda@yahoo.com](mailto:asliswirda@yahoo.com);

**Results:** Results showed that there were significant differences ( $p < 0.01$ ) in terms of height for age and *Pyd* in the urine of stunted (body length  $< 48$  cm) versus normal (body length  $\geq 48$  cm) neonates. The contents of *Pyd* in the urine of stunted neonates were  $982.9 \pm 61.6$  and normal neonates was  $594.1 \pm 266.1$  nmol/mmol.

**Conclusion:** Therefore, there is a possible association between height for age and *Pyd* in the urine as a potential early indicators to identify stunted and normal neonates.

**Keywords:** *Body length; neonates; pyridinium crosslinks; stunting; height for age; normal neonates.*

## 1. INTRODUCTION

Stunting is a condition by which an individual failed to reach the linear growth potential which could be resulted from the conditions of their health and nutrition [1]. Worldwide, childhood stunting declined from 39.7% in 1990 to 26.7% in 2010 [2]. This expected to incline towards 21.8%, or 142 million, in the year 2020 [2]. In Indonesia, stunting is one of the major nutrition problems in Indonesia, with the prevalence of 35.6% [3]. Stunting is resulted from poor standard of living, exposure to adverse conditions such as diseases and poor eating habits and practices [1]. The most common cause of stunting in the developing countries includes: impaired absorption of nutrients due to intestinal infections or parasites; or combinations of these problems [4], [5]. Stunting may also lead to several long-term effects when they reach adulthood period, which includes poor cognitive development, poor in academic, poor productivity towards the economy and negative impact on the reproductive health [4].

Improved early nutrition and care can compensate in part for stunted in utero. Stunting that began at a very early infancy age, leads to a more severe impairment to their cognitive development in later life [4]. Therefore, an early determination of stunting among neonates is very important in order to support for proper feeding support and the gain of better optimal health [6]. The use of classical anthropometric measurements for body length measurements is widely accepted since many decades ago [7]. However, the use of such measurements to determine stunting has drawbacks such as possible human error or issues with the devices. The use of biomarkers in radiology is being debated to measure the infant's bone density to monitor the growth of the baby; however this method is deemed unsuitable as it involves unnecessary invasive procedures for the babies. Therefore, the use of other readily obtained biological fluids or wastes such as urine can be used as a possible early indicator to stunting among neonates.

In this research, the quantification of a bone resorption marker called *Pyd* in the urine as a possible early indicator to stunting is proposed [8]. The condition of the urine is associated with health condition of the kidney. Stunted babies (that has been exposed to malnutrition) has shown to have disruption to their metabolic processes and increased risk of renal impairment [9]. In order to find out whether the babies are having these issues, the creatinine level can be measured from their urine. Creatinine is derived from the metabolism of proteins, either from food or from muscle. Human bone is formed through the process of pairing between bone resorption process (release of a cell or tissue with a gradual preparation of the compounds into smaller and dispersed in circulation) by osteoclasts and bone formation by osteoblast. This process happens normally in bone and skeletal growth. As many as 90% of the organic matrix of bone is made of Collagen Type I is a helical protein is stabilized by cross-linking between terminals N and C terminals on the basis of the formation of bone tissue. During maturation of collagen, *Pyd* formed by hydroxy Lysine or Lysine residues at the end of the C- and N- terminal telopeptide of collagen molecules and is released during the resorption of the matrix and is excreted through the urine. *Pyd* is expected to be specific and sensitive biomarker of bone resorption and are able to evaluate bone metabolism or disorder in neonates.

Based on the biological processes, *Pyd* seems to be of potential use to evaluate bone metabolism among neonates, which may indirectly indicate the possibility of stunting condition. Therefore, the main objective of the study is to test the possibility of *Pyd* level in urine as an early indicator to stunting.

## 2. MATERIALS AND METHODS

The study design was cross-sectional and was conducted between January to December 2014. Subjects of the study were 35 healthy neonates born at the Andini's Mothers and Children Hospital at Tuanku Tambusai street 55,

Pekanbaru (middle class hospital and population strategic location in Pekanbaru) between the 28<sup>th</sup> of August 28<sup>th</sup> until 30<sup>th</sup> September 2014 (all babies born in a specific period of time that their mothers were willing to sign an informed consent). The minimum number of samples to compare two groups (stunted & normal) in this study is 30,29 neonates [10].

$$n = \frac{2\sigma^2(Z_{1-\alpha} + Z_{1-\beta})^2}{(\mu_1 - \mu_2)^2}$$

n = Sample size

$\sigma$  = Population standard deviation

$\mu_1$  = Test value of the population mean

$\mu_2$  = Anticipated population mean

The value of  $\alpha = 5\%$  (1.964) and  $\beta = 20\%$  (0.842) [11]. In order to obtain a number of samples that reflect population characteristics, statistical parameters (eg mean and standard deviation) from previous studies were used in this study. The research was "Food Supplementation with Encouragement to Feed It to Infants from 4 to 12 Months of Age" carried out by [12]. The study showed that  $\mu_1 - \mu_2 = 0.4$  cm (achievement of the subject body length increase), and standard deviation namely  $\sigma = 1.6$  cm. In anticipation of the drop out subjects, a number of neonates were added so that  $n = 35$  neonates. Subjects were recruited at around 1-3 days of neonatal life. Inclusion criteria were normal gestation (36 to 40 weeks), spontaneous and caesarean delivery. The study complies with the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects and was approved by the Institutional Review Board of the Faculty of Medicine, University of Riau, Ministry of Education and Culture of Republic Indonesia. Parents of all subjects were given a written informed consent and signed them upon agreement to join the study.

Family socioeconomic questionnaires (e.g. name, gender, age, race, and height parent), body length gauges (BUTERFLY), digital weighting scale for baby (BABY SCALE TANITA), MicroVue™ PYD EIA kit, USA (Quidel Corporation, San Diego, CA 92121, USA, Cat: 8010, Lot: 015210, ED: 2015-07 and Spectrophotometer Microplate Reader 680 (Bio-Rad Laboratories, Inc., Hercules, CA 94547, USA), Creatinine measurements were performed with the use of Jaffe method and Spectrophotometer ADVIA 1800: ADVIA, Germany, baby urine bags (PEDIATRIC URINE COLLECTOR, Japan).

The 24-hours neonates' urine was collected using pediatrics urine bags by trained nurses, aliquoted to 6mL. Mothers were briefed about the study, one day prior to neonates urine collection. Baby urine that has been collected was stored in the refrigerator at a temperature of -20°C at the Pekanbaru Prodia Clinical Laboratory and then was sent to Prodia Center in Jakarta for analysis.

Statistical analysis and results are reported based on the data. Statistical outliers, defined as outside the 95% confidence limits of the normal probability plots, two subjects were removed before the analysis. In all statistical tests performed, the null hypothesis (no effect) was rejected at the 0.01 level of probability. Differences in mean body weight, head circumference, concentrations of Pyd urine, age, weight-for-age-z-score (WAZ), length/height-for-age (HAZ), basal metabolic index (BMI) for age (BAZ), mother's height, mother's BMI, mother's weight before pregnancy, mother's prenatal weight, mother's pregnancy age, the number of children in family were evaluated by using a Independent t-test. Stunted neonates referred to babies with body length of <48 cm and normal babies referred to those with body length  $\geq 48$  [13]. All analyses were performed by using SPSS version 20 (IBM SPSS Statistics 20).

### 3. RESULTS AND DISCUSSION

The proportion of stunted neonates in the study was 22.9%. That is similar with reported by [13] that the proportion of stunting neonates in Indonesia is 20.2%.

All neonates were healthy and have received full enteral feeding (formula and/or breast feeding). 74.3% and 25.7% of the subjects involved in the study were male and female respectively (Table 1). It was found to be much easier to collect urine from male than female neonates, which reflected on the higher percentage of the subjects. Most of the mothers stayed at Pekanbaru, except for two of them. This is because their other family members also stayed at the same area and would like to be close to them when their baby is born. Apart from that, living at Pekanbaru, the mothers thought that they can get a proper medical attention when needed compared to outside the city. Most of the mothers were Malay and a small percentage was Chinese. 50% of the subject's mother was full housewife. Some of the mothers were also working as officers of the government and private



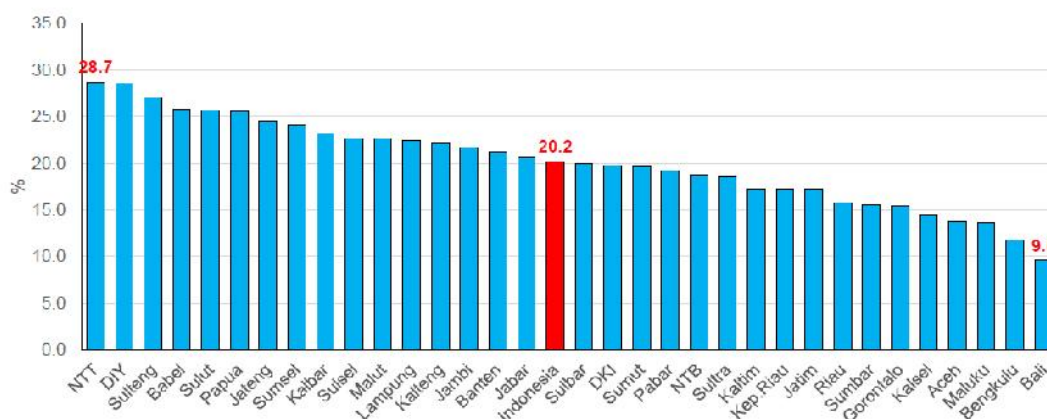


Fig. 1. The proportion of stunting neonates neonatal body length at birth < 48 cm) by province in Indonesia

Table 1. Growth problems

Z-score	Growth indicators			
	Length/height-for-age	Weight-for-age	Weight-for-length/height	BMI-for-age
Above 3	See note 1	See note 2	Obese	Obese
Above 2			Overweight	Overweight
Above 1			Possible risk of overweight (See note 3)	Possible risk of overweight (See note 3)
0 (median)				
Below -1				
Below -2	Stunted (See note 4)	Underweight	Wasted	Wasted
Below -3	Severely stunted (See note 4)	Severely underweight (See note 5)	Severely wasted	Severely wasted

Note 4: It is possible for a stunted or severely stunted child to become overweight [14]

sector. Overall, the education level among all the subjects mothers were from the Elementary to Scholar level.

Length for age males and female neonates in Indonesia is about same. [15] reported that length male neonatus is 49.9 cm and female neonatus is 49.1 cm. The growth of male and female neonates is almost the same in the early stages of life, differences will occur at the next stage of growth [16]. There were 42 neonates who were born during the urine collection period, as many as 5 neonates were not continued to be processed to the analysis stage of the urine Pyd content because the amount of urine collected was insufficient. The five neonates are female. Neonatal female urine collectors are relatively more difficult than men because female urine

sometimes spills out of a pediatric urine collector. An addition, there were 2 female neonates whose Pyd content was in the form of outliers so they were not included in the data analysis.

The proportion of stunted neonates in the study was 22.9%. It was a similar findings as reported by Atmarita research which was at the rate of 20.2% [13]. The characteristics of the stunted and normal subject were recorded as Table 2. The length of stunted and normal neonates was 46.8±0.5cm and 49.9±1.4 respectively. Their weight also differs, by which the normal neonates were much heavier compared to the stunted neonates (a difference of about 300-400g). The head circumference of stunted neonates and normal neonates were 33.3±1.0 and 33.6±1.2cm respectively. This study has shown that there

was a major difference (statistically significance) in terms of the level of *Pyd* in the urine between the stunted neonates and normal neonates. Among stunted neonates, the amount of *Pyd* in the urine was  $982.9 \pm 61.6$  nmol/mmol Cr, compared to only  $594.1 \pm 266.1$  among normal neonates. The HAZ was also found to be statistically significant between the stunted neonates and normal neonates.

Mothers of subjects with small BMI's did not give birth small subjects (independent t test). The mean BMI of mothers of stunting and normal subjects were 22.2 and 20.0, respectively. The mother of the subject with a normal BMI was 48.3% ( Table 4 ).

Classification of adult Asian body mass index (BMI) according to [14] is listed in Table 5.

The quantified amount of *Pyd* in the urine of the neonates had quadratic relationship with HAZ ('U-shaped' scatter plot) as shown in Fig. 1. The *Pyd* in the urine was negatively associated with body length in stunted neonates and can be used as biomarkers of linear growth. Neonates classified as stunted if their length <48 cm and the contents of  $Pyd > 859.7$  nmol/mmol Cr. Further study is recommended for infants aged 6 to 12 months to further confirm the hypothesis. In the previous studies, *Pyd* excretion were found to differ based on different age groups. *Pyd* excretion from elementary school children, for example, is about 50--500 nmol/mmol Cr [17]. *Pyd* excretion on children 3-5 year was  $238.3 \pm 22.7$  pmol/mumol Cr (male) and  $261.8 \pm 14.2$  pmol/mumol Cr (female) [18].

**Table 2 .Familial socioeconomic status of the subjects**

Variables	Criteria	Value*
<b>Sex</b>	Male	74.3 (26)
	Female	25.7 (9)
<b>Residence</b>	<i>Pekanbaru</i>	91.4 (32)
	Others	5.7 (2)
<b>Ethnic group</b>	Malay	97.1 (34)
	Chinese	2.9 (1)
<b>Mother's job</b>	Teacher	8.6 (3)
	Housewife	51.4 (18)
	Employed	31.4 (11)
<b>Mother's education</b>	Entrepreneur	8.6 (3)
	Elementary school	5.7 (2)
	Junior high school	2.9 (1)
	Senior high school	22.9 (8)
	Diploma 3	20.0 (7)
	Diploma 4	2.9 (1)
	Scholar	37.1 (13)

\*% (n)



**Fig. 2. Pediatric urine collector**

**Table 3. Characteristics of the stunted and normal subject**

Variables	Stunted	Normal
Length (cm)	46.8±0.5 (46:47) <sup>a</sup>	49.9±1.4 (48.0:53.0) <sup>b</sup>
Weight (g)	2846±360 (2400:3480)	3215±404 (2380:4080)
Head circumference (cm)	33.3±1.0 (31.0:34.5)	33.6±1.2 (31.0:35.0)
Pyd (nmol/mmol Cr)	982,9±61.6 (967,8:1049.6) <sup>a</sup>	594.1±266,1 (564,4:2550.8) <sup>b</sup>
Age (days)	1±1 (1:1)	1±1 (1:3)
WAZ	-1.03±0.82 (-2.15:0.33)	-0.21±0.87 (-2.15:1.74)
HAZ	-1.26±0.27 (-1.67:-1.00) <sup>a</sup>	0.23±0.66 (-0.56:1.97) <sup>b</sup>
BAZ	-0.69±1.29 (-2.27:1.66)	-0.60±1.11 (-3.21:1.34)
Mother's BMI (kg/m <sup>2</sup> )	21.5±4.3 (18.0:30.5)	22.0±2.9 (16.9:26.7)
Mother's height (cm)	156±4 (150:165)	161±7 (150:185)
Mother's weight before pregnancy (kg)	53±13 (42:83)	57±8 (42:70)
Mother's prenatal weight (kg)	66±15 (53:101)	69±11 (50:86)
Pregnancy age (weeks)	38±1 (37:39)	38±3 (35:49)
Number of children (person)	2±1 (1:4)	2±1 (1:4)

Independent t test: <sup>ab</sup>p<0.01

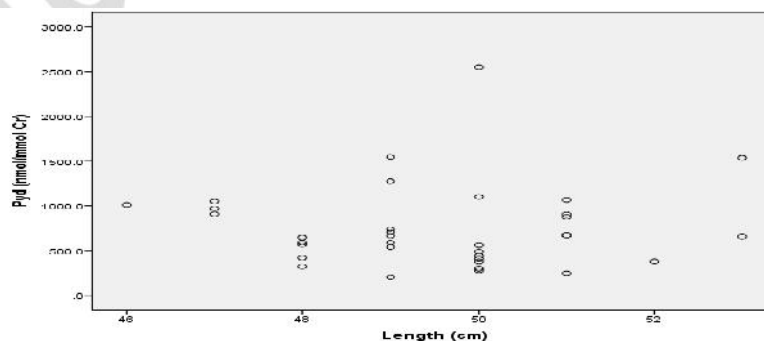
**Table 4. Nutritional status of subject mother**

Category	Amount	
	N	%
Underweight	5	17.2
Normal	14	48.3
Overweight:	4	13.8
At Risk	5	17.2
Obese I	0	0.0
Obese II	1	3.4
Total	29	100.0

**Table 5. Classification of adult Asian body mass index (BMI)**

Category	BMI (kg/m <sup>2</sup> )	Risk of co-morbidities
Underweight	< 18.5 kg/m <sup>2</sup>	Low (but the risk of other clinical problems increases)
Batas Normal	18.5 - 22.9 kg/m <sup>2</sup>	Mean
Overweight:	≥ 23	
At Risk	23.0 – 24.9 kg/m <sup>2</sup>	Increase
Obese I	25.0 - 29.9kg/m <sup>2</sup>	Moderate
Obese II	≥ 30.0 kg/m <sup>2</sup>	Dangerous

Source: [14]



**Fig. 3 Association of Pyd in the urine and body length in neonates**

#### 4. CONCLUSION

Pyd was significantly higher in the urine from stunted neonates than non-stunted neonates. Urine Pyd may become a candidate of a marker of stunted neonates. Further study on a large population is necessary.

#### CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

#### ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. World Health Organization, "Child growth indicators and their interpretation," *Global Database on Child Growth and Malnutrition*, 2018.
2. M. de Onis and F. Branca, "Childhood stunting: A global perspective," *Maternal and Child Nutrition*. 2016.
3. C. B. of S. (BPS) et al., "Indonesia Demographic and Health Survey," 2016.
4. A. J. Prendergast and J. H. Humphrey, "The stunting syndrome in developing countries," *Paediatr. Int. Child Health*, 2014.
5. P. A.J. et al., "Stunting is characterized by chronic inflammation in zimbabwean infants," *PLoS One*, 2014.
6. D. Kumar, N. K. Goel, P. C. Mittal, and P. Misra, "Influence of infant-feeding practices on nutritional status of under-five children," *Indian J. Pediatr.*, 2006.
7. "Use and interpretation of anthropometric indicators of nutritional status," *Bull. World Health Organ.*, 1986.
8. J. Marowska, M. Kobylińska, J. Lukaszewicz, A. Talajko, B. Rymkiewicz-Kluczyńska, and R. S. Lorenc, "Pyridinium crosslinks of collagen as a marker of bone resorption rates in children and adolescents: Normal values and clinical application," *Bone*, 1996.
9. H. N. Y Kartawinata, D Hilmanto, "Serum Creatinine and Cystatin C Level in Children with Undernutrition and Normal Nutritional Status," *J. Indones. Med. Assoc.*, vol. 62, no. 12, pp. 471–475, 2012.
10. S. K. Lwanga and S. Lameshow, *Sample Size Determination Health Study*. Geneva. World Health Organization. 1996.
11. R. E. Walpole, *Introduction of Statistic (3rd Ed)*. 1988.
12. N. Bhandari, R. Bahl, B. Nayyar, P. Khokhar, J. E. Rohde, and M.K. Bhan, "Food Supplementation with Encouragement to Feed It to Infants from 4 to 12 Months of Age Has a Small Impact on Weight Gain," *Am. J. Nutr.*, vol. 1, no. 1946–1961, 2001.
13. Atmarita, "The Future Figures of Indonesian Human Resources," 2014.
14. World Health Organization, *Improving Child Growth*. 2001.
15. Ministry of Health Republic Indonesia, *National Health Survey*. 2010.
16. "Keputusan Menteri Kesehatan Republik Indonesia Nomor 1995/Menkes/SK/XII/2010 tentang Standar Antropometri Penilaian Status Gizi Anak." [Online]. Available: <https://www.slideshare.net/vindhyatripta/buku-antropometrianakduniasehatnet>.
17. L. J. Beardsworth, D. R. Eyre, and I. R. Dickson, "Changes with age in the urinary excretion of lysyl- and hydroxylysylpyridinoline, two new markers of bone collagen turnover," *J. Bone Miner. Res.*, 1990.
18. H. Fujimoto, S; Kubo, T; Tanaka, M. Miura, and Y. Seino, "Urinary pyridinoline and deoxypyridinoline in healthy children and in children with growth hormone deficiency," *J. Clin. Endocrinol. Metab.*, vol. 80, no. 6, pp. 1922–1928, 1995.

**Group Statistics**

	Categorical of length	N	Mean	Std. Deviation	Std. Error Mean
Pyd (nmol/mmol Cr)	<48 cm	4	982.925	61.6458	30.8229
	>= 48 cm	28	594.118	266.1589	50.2993

**Independent Samples Test**

	Levene's Test of Equality of Variances	t-Test of Equality of Means								
		f	Sig.	t	df	Std. Error Difference	Mean Difference	95% Confidence Interval of the Difference Lower	Upper	
Pyd (nmol/mmol Cr)	Equal variances assumed	3.376	.079	2.872	30	363.807	383.807	137.368	112.347	652.267
	Equal variances not assumed			6.561	22.973	300	363.807	55.4921	296.6235	590.4978

© 2019 Hayati et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

WhatsApp | [LIVE] Tabligh Akbar Kaji | [InaB] #1644 Editor Decis | Yahoo Mail | (89 unread) - asliswirda

mail.yahoo.com/d/search/keyword=Asian%2520Journal%2520of%2520Research%2520in%2520Medical%2520and%2520Pharmaceuti...

HOME MAIL NEWS FINANCE SPORT LIFESTYLE ENTERTAINMENT WEATHER MORE...

yahoo/mail Find messages, documents, photos or people Advanced Aslis Wirda Home

Compose Back Archive Move Delete Spam

Inbox 88 Unread Starred Drafts Sent Archive Spam Deleted Items ^ Less Views Show Folders Hide + New folder

Aslis Wirda Hayati <asliswirda@yahoo.com>  
To: P Mondal  
Cc: Haiza Ahmad  
Tue, 4 June 2019 at 12:07 pm

Dear Ms. Ruma Bag  
**SCIENCEDOMAIN international**

I sent a manuscript that I fixed as you suggested.

Thank you  
With Best Regards  
Aslis Wirda Hayati

Show original message

Galley proof\_201...docx 14 Galley proof\_2...pdf Show all

25°C Berawan 08:43 05/04/2023

WhatsApp | [LIVE] Tabligh Akbar Kaji | [InaB] #1644 Editor Decis | Yahoo Mail | (89 unread) - asliswirda

mail.yahoo.com/d/search/keyword=Asian%2520Journal%2520of%2520Research%2520in%2520Medical%2520and%2520Pharmaceuti...

HOME MAIL NEWS FINANCE SPORT LIFESTYLE ENTERTAINMENT WEATHER MORE...

yahoo/mail Find messages, documents, photos or people Advanced Aslis Wirda Home

Compose Back Archive Move Delete Spam

Inbox 88 Unread Starred Drafts Sent Archive Spam Deleted Items ^ Less Views Show Folders Hide + New folder

Galley pro... .docx 582.7kB

Aslis Wirda Hayati <asliswirda@yahoo.com>  
To: P Mondal  
Tue, 4 June 2019 at 5:27 pm

Dear Ms. Ruma Bag  
**SCIENCEDOMAIN international**

I am sorry. May I change the affiliations as attached?

Galley proof\_201...docx 14 Galley proof\_2...pdf Show all

25°C Badai sore hari 08:44 05/04/2023



WhatsApp | [LIVE] Tabligh Akbar Kaji | [InaB] #1644 Editor Decis | Yahoo Mail | (89 unread) - asliswirda@... | +

mail.yahoo.com/d/search/keyword=Asian%2520Journal%2520of%2520Research%2520in%2520Medical%2520and%2520Pharmaceuti...

HOME MAIL NEWS FINANCE SPORT LIFESTYLE ENTERTAINMENT WEATHER MORE...

yahoo/mail Find messages, documents, photos or people Advanced

Aslis Wirda Home

Compose Back Archive Move Delete Spam

Galley Proof for manuscript number 2019/AJRIMPS/48579 4 Yahoo/Sent

P Mondal <editor.sciencedomain5@gmail.com>  
To: asliswirda@yahoo.com  
Cc: Siti Rohaiza Binti Ahmad

Dear Dr. Aslis Wirda Hayati,

Please find attached the pdf and MS word version of the Galley Proof of your accepted manuscript.

Please go through the manuscript very carefully. At galley proof stage no change is allowed. Authors are strongly advised not to do any change in the editor-approved version of the manuscript. Only minor spelling/style/punctuation/grammar changes are allowed at this stage. Kindly note that type setting of manuscript is already done. Any major change requires additional approval from reviewers and editors. **Additional 125 USD payment is required for processing of such changes after type-setting.** No charge is required if galley proof editor requests author to change some part of the manuscript. If any author is not doing any change (except spelling/grammatical correction and changes requested by galley proof editor) then he/she should not be worried about this charge.

**Important note 1:** Kindly send us the Authors' contributions section according to our template format. Authors may use the following wordings for this section:

"This work was carried out in collaboration between all authors. Author A (insert author name here) designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors B (insert author name here) and C (insert author name here) managed the analyses of the study. Author D (insert author name here) managed the literature searches. All

26°C Berawan 08:41 05/04/2023

WhatsApp | [LIVE] Tabligh Akbar Kaji | [InaB] #1644 Editor Decis | Yahoo Mail | (89 unread) - asliswirda@... | +

mail.yahoo.com/d/search/keyword=Asian%2520Journal%2520of%2520Research%2520in%2520Medical%2520and%2520Pharmaceuti...

HOME MAIL NEWS FINANCE SPORT LIFESTYLE ENTERTAINMENT WEATHER MORE...

yahoo/mail Find messages, documents, photos or people Advanced

Aslis Wirda Home

Compose Back Archive Move Delete Spam

"This work was carried out in collaboration between all authors. Author A (insert author name here) designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors B (insert author name here) and C (insert author name here) managed the analyses of the study. Author D (insert author name here) managed the literature searches. All authors read and approved the final manuscript."

**Important note 2:** Kindly note acknowledgement section is not mandatory part of a paper. If you think the acknowledgement section is necessary for your paper, kindly add 2-3 lines under acknowledgement section. After final publication no changes could be done.

Kindly send us the corrected Galley Proof within 2 days.

Thank you for your interest in this journal.

With Best Regards  
Ms. Ruma Bag  
**SCIENCEDOMAIN international**  
[www.sciencedomain.org](http://www.sciencedomain.org)

Reg. Offices:  
India: SCIENCEDOMAIN international, Guest House Road, Street no - 1/6, Hooghly, West Bengal, India. Firm Registration Number: L77527, Tele: +91 8617752708 Email: [contact@sciencedomain.org](mailto:contact@sciencedomain.org)  
UK: SCIENCEDOMAIN international, Third Floor, [207 Regent Street, London, W1B 3HH, UK](http://www.sciencedomain.org)  
Fax: +44 20-3031-1429 Email: [contact@sciencedomain.org](mailto:contact@sciencedomain.org)

Disclaimer: This email and any files transmitted with it are confidential and intended solely for the use of the individual or entity to whom they are addressed. If verification is required please request a

26°C Berawan 08:41 05/04/2023



WhatsApp x [LIVE] Tabligh Akbar Kaji: x [InaB] #1644 Editor Decis x Yahoo Mail x (89 unread) - aslswirda@ x +

mail.yahoo.com/d/search/keyword=Asian%2520Journal%2520of%2520Research%2520in%2520Medical%2520and%2520Pharmaceuti...

HOME MAIL NEWS FINANCE SPORT LIFESTYLE ENTERTAINMENT WEATHER MORE...

yahoo!mail Find messages, documents, photos or people Advanced

Aslis Wirda Home

Compose Back Archive Move Delete Spam

Inbox 88  
Unread  
Starred  
Drafts  
Sent  
Archive  
Spam  
Deleted Items  
Less  
Views Show  
Folders Hide  
+ New folder  
2001 Sos 4 77  
2017 Jurnal

Reg. Offices:  
India: SCIENCEDOMAIN international, Guest House Road, Street no - 1/6, Hooghly, West Bengal, India, Corp. Firm Registration Number: L77527, Tele: +91 8617752708 Email: [contact@sciencedomain.org](mailto:contact@sciencedomain.org)  
UK: SCIENCEDOMAIN international, Third Floor, 207 Regent Street, London, W1B 3HH, UK  
Fax: +44 20-3031-1429 Email: [contact@sciencedomain.org](mailto:contact@sciencedomain.org)

Disclaimer This email and any files transmitted with it are confidential and intended solely for the use of the individual or entity to whom they are addressed. If verification is required please request a signed hard-copy/scanned version. Our company accepts no liability for the content of this email, unless that information is subsequently confirmed in writing.  
[Download all attachments as a zip file](#)

Galley proof...pdf 741.3kB Galley pro...docx 574.5kB

Aslis Wirda Hayati ----- Pesan yang Diteruskan ----- Dari: P Monclal <editor.sciencedomain5> Tue, 4 June 2019 at 11:17 am

26°C Berawan 08.41 05/04/2023



**SDI FINAL EVALUATION FORM 1.1**

**PART 1:**

Journal Name:	<a href="#">Asian Journal of Research in Medical and Pharmaceutical Sciences</a>
Manuscript Number:	Ms_AJRIMPS_48579
Title of the Manuscript:	<b>Pyridinium Crosslinks (Pyd) In the Urine is Associated With Stunting In Neonates</b>
Type of Article:	<b>Original Research Article</b>

**PART 2:**

FINAL EVALUATOR'S comments on revised paper (if any)	Authors' response to final evaluator's comments
<p>The authors in their response point out the use of appropriate mathematics in their analysis.</p> <p>However, the point is that the sample size and the skewing towards males as well as mothers with smaller BMIs.</p> <p>Therefore, the data as presented are preliminary and the sample size should be increased.</p>	<p><b>The sample size</b></p> <p>The number of samples is determined based on a formula that compares two groups in a research (stunted &amp; normal). Lwanga and Lameshow (1996) explain the minimum number of samples for hypothesis testing which compares the average of two population groups using the formula:</p> $n = \frac{2\sigma^2(Z_{1-\alpha} + Z_{1-\beta})^2}{(\mu_1 - \mu_2)^2}$ <p>n = Sample size  σ = Population standard deviation  μ<sub>1</sub> = Test value of the population average  μ<sub>2</sub> = Anticipated population mean</p> <p>α = 5% (1,964) and β = 20% (0842) are used in the formula above (Walpole 1988). To obtain a number of samples that reflect population characteristics, statistical parameters (eg mean and standard deviation) from previous studies were used in this study. The research was "Food Supplementation with Encouragement to Feed It to Infants from 4 to 12 Months of Age" carried out by Bhandari et al. (2001). The study showed that μ<sub>1</sub> - μ<sub>2</sub> = 0.4 cm (achievement of the subject body length increase), and standard deviation namely σ = 1.6 cm. Based on this information, the number of samples of this study is 30.29 subjects.</p> <p>Bhandari, N., R. Bahl, B. Nayyar, P. Khokhar, J. E. Rohde and M.K. Bhan. 2001. Food Supplementation with Encouragement to Feed It to Infants from 4 to 12 Months of Age Has a Small Impact on Weight Gain. Am. J. Nutrition 1:1946-1961.</p> <p>Lwanga SK and Lameshow S. 1996. Sample Size Determination Health Study. Geneva. World Health Organization.</p> <p>Walpole RE. 1988. Introduction of Statistic (3<sup>rd</sup> Ed). Sumantry B (translator). Jakarta: Gramedia.</p> <p><b>Skewing towards males is not problema in the early bird.</b></p>



**SDI FINAL EVALUATION FORM 1.1**

Length for age males and female neonates in Indonesia is about same. Ministry of Health Republic Indonesia (2010) reported that length male neonatus is 49.9 cm and female neonatus is 49.1 cm.

In the early stages of life the growth of male and female neonates is almost the same so that although relatively many male than female in this study have no effect on conclusions. The growth of male and female is different at the next stage of growth.

[\[https://www.slideshare.net/vindhyatripta/buku-antropometrianakduniasehatnet\]](https://www.slideshare.net/vindhyatripta/buku-antropometrianakduniasehatnet)  
Keputusan Menteri Kesehatan Republik Indonesia Nomor 1995/Menkes/SK/XII/2010 tentang Standar Antropometri Penilaian Status Gizi Anak.

There were 42 neonates born in the study observation period. Five neonates' Pvd were not analyzed, because of the urine amount stored was insufficient. Those five people are female. Taking the urine of a female neonate is relatively more difficult than a male because the urine of the female is sometimes out of the urine bag. In addition, there were 2 neonates whose Pvd content was an outlier (the Pvd value was too large compared to the other values) so it was not included in the data analysis, they were also female. Thus, the data analyzed statistically in this study were 35 neonates.



**SDI FINAL EVALUATION FORM 1.1**



Figure 1. Pediatric Urine Collector

**Mothers with smaller BMIs did not significantly give birth to stunting babies based on the results of the Independent t test.**

Mothers who have a small BMI giving birth to a relatively small child are not significant in this study after being tested by independent t test.

The average BMI of stunting normal neonates mothers is 22.2, but the BMI of normal neonates mothers is 20.0.

Neonates mothers come from middle and upper economic groups. Neonates mothers pay attention to ideal body weight and regulate food intake.

48.3% of mothers have normal nutritional status according to body mass index (BMI).

Table 1. Mother's Nutritional Status

Category	Amount	
	N	%
<i>Underweight</i>	5	17.2
Normal Limit	14	48.3
<i>Overweight:</i>	4	13.8
<i>At Risk</i>	5	17.2
<i>Obese I</i>	0	0.0
<i>Obese II</i>	1	3.4
Total	29	100.0

Classification of body weight based on body mass index (BMI) or basal metabolism index (BMI) in Asian Adult population according to WHO 2001 is listed in Table 2.



**SDI FINAL EVALUATION FORM 1.1**

Table 2. The proposed Body Weight Classification based on BMI in Adult Asian Population (IOTF, WHO 2001)

Category	BMI (kg/m <sup>2</sup> )	Risk of co-morbidities
Underweight	< 18.5 kg/m <sup>2</sup>	Low (but the risk of other clinical problems increases)
Normal Limit	18.5 - 22.9 kg/m <sup>2</sup>	Average
Overweight:	≥ 23	
At Risk	23.0 – 24.9 kg/m <sup>2</sup>	Increase
Obese I	25.0 - 29.9kg/m <sup>2</sup>	Medium
Obese II	≥ 30.0 kg/m <sup>2</sup>	Dangerous



**SDI Journal Reprint Order Form 1.1**

Journal Name:	<a href="#">Asian Journal of Research in Medical and Pharmaceutical Sciences</a>	
Title of the Article:	<a href="#">Pyridinium Crosslinks (Pyd) In the Urine is Associated With Stunting In Neonates</a>	
Specify the number of Copies of journal required (Please select from these numbers: 1, 2, 3, 4, 5...)	1	
Name of the customer	Dr. Aslis Wirda Hayati	
Email ID of the customer	asliswirda@yahoo.com;	
Delivery Address (Delivery will be made to ONLY ONE Address)	Building number	103
	Street number	Jalan Melur
	Any nearby landmark	Poltekkes Kemenkes Riau
	City	Pekanbaru
	District	Kota Pekanbaru
	State or Province	Riau
	Country	Indonesia
Postal Code	28122	
Phone number of Customer	+62818106440	
Any other instruction / comment	-	

Please see more information in this link: <http://www.sciencedomain.org/page.php?id=article-reprints>



## **Pyridinium Crosslinks (*Pyd*) in the Urine is Associated with Stunting in Neonates**

**Aslis Wirda Hayati<sup>1\*</sup>, Alkausyari Aziz<sup>1</sup>, Siti Rohaiza Ahmad<sup>2</sup> and Sri Widia Ningsih<sup>3</sup>**

<sup>1</sup>*Department of Nutrition, Poltekkes Kemenkes Riau, Indonesia.*

<sup>2</sup>*PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam, Brunei.*

<sup>3</sup>*Department of Pharmacy, Poltekkes Kemenkes Medan, Indonesia.*

### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author AWH designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AA and SWN managed the analyses of the study. Author SRA managed the literature searches. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/AJRIMPS/2019/v7i130113

#### Editor(s):

(1) Dr. John Yahya I. Elshimali, Professor, Department of Pathology and Oncology, UCLA School of Medicine, Charles R. Drew University of Medicine and Science, California, USA.

(2) Dr. Lokendra Bahadur Sapkota, Assistant Professor, Department of Biochemistry, Chitwan Medical College, Bharatpur, Nepal.

#### Reviewers:

(1) Giuseppe Gregori, Italy.

(2) Erik De Leeuw, UMB School of Medicine, Maryland, USA.

(3) Shigeki Matsubara, Jichi Medical University, Japan.

Complete Peer review History: <http://www.sdiarticle3.com/review-history/48579>

**Received 15 March 2019**

**Accepted 31 May 2019**

**Published 07 June 2019**

**Original Research Article**

### **ABSTRACT**

**Aims:** The aim of the research is to evaluate the reliability of bone resorption biomarkers called Pyridinium Crosslinks (*Pyd*) in the urine of the neonates as an evaluation to bone growth of the neonate, as an indicator of stunting.

**Study Design:** A cross-sectional study.

**Place and Duration of Study:** Andini Mothers and Children Hospital (*Pekanbaru*, Indonesia). Duration of the study was between, August until September 2014.

**Methodology:** Subjects of study were 35 healthy neonates. Subjects were recruited at the first 3 days of life. Body length gauges, digital weighting scale, family socioeconomic questionnaires and *Pyd* kit were used to collect the data. Differences in the mean of the research variables were tested using an Independent t-test.

\*Corresponding author: E-mail: [asliswirda@yahoo.com](mailto:asliswirda@yahoo.com);

**Results:** Results showed that there were significant differences ( $p < 0.01$ ) in terms of height for age and *Pyd* in the urine of stunted (body length  $< 48$  cm) versus normal (body length  $\geq 48$  cm) neonates. The contents of *Pyd* in the urine of stunted neonates were  $982.9 \pm 61.6$  and normal neonates was  $594.1 \pm 266.1$  nmol/mmol.

**Conclusion:** Therefore, there is a possible association between height for age and *Pyd* in the urine as a potential early indicators to identify stunted and normal neonates.

**Keywords:** Body length; neonates; pyridinium crosslinks; stunting; height for age; normal neonates.

## 1. INTRODUCTION

Stunting is a condition by which an individual failed to reach the linear growth potential which could be resulted from the conditions of their health and nutrition [1]. Worldwide, childhood stunting declined from 39.7% in 1990 to 26.7% in 2010 [2]. This expected to incline towards 21.8%, or 142 million, in the year 2020 [2]. In Indonesia, stunting is one of the major nutrition problems in Indonesia, with the prevalence of 35.6% [3]. Stunting is resulted from poor standard of living, exposure to adverse conditions such as diseases and poor eating habits and practices [1]. The most common cause of stunting in the developing countries includes: Impaired absorption of nutrients due to intestinal infections or parasites; or combinations of these problems [4,5]. Stunting may also lead to several long-term effects when they reach adulthood period, which includes poor cognitive development, poor in academic, poor productivity towards the economy and negative impact on the reproductive health [4].

Improved early nutrition and care can compensate in part for stunted in utero. Stunting that began at a very early infancy age, leads to a more severe impairment to their cognitive development in later life [4]. Therefore, an early determination of stunting among neonates is very important in order to support for proper feeding support and the gain of better optimal health [6]. The use of classical anthropometric measurements for body length measurements is widely accepted since many decades ago [7]. However, the use of such measurements to determine stunting has drawbacks such as possible human error or issues with the devices. The use of biomarkers in radiology is being debated to measure the infant's bone density to monitor the growth of the baby; however this method is deemed unsuitable as it involves unnecessary invasive procedures for the babies. Therefore, the use of other readily obtained biological fluids or wastes such as urine can be used as a possible early indicator to stunting among neonates.

In this research, the quantification of a bone resorption marker called *Pyd* in the urine as a possible early indicator to stunting is proposed [8]. The condition of the urine is associated with health condition of the kidney. Stunted babies (that has been exposed to malnutrition) has shown to have disruption to their metabolic processes and increased risk of renal impairment [9]. In order to find out whether the babies are having these issues, the creatinine level can be measured from their urine. Creatinine is derived from the metabolism of proteins, either from food or from muscle. Human bone is formed through the process of pairing between bone resorption process (release of a cell or tissue with a gradual preparation of the compounds into smaller and dispersed in circulation) by osteoclasts and bone formation by osteoblast. This process happens normally in bone and skeletal growth. As many as 90% of the organic matrix of bone is made of Collagen Type I is a helical protein is stabilized by cross-linking between terminals N and C terminals on the basis of the formation of bone tissue. During maturation of collagen, *Pyd* formed by hydroxy Lysine or Lysine residues at the end of the C- and N- terminal telopeptide of collagen molecules and is released during the resorption of the matrix and is excreted through the urine. *Pyd* is expected to be specific and sensitive biomarker of bone resorption and are able to evaluate bone metabolism or disorder in neonates.

Based on the biological processes, *Pyd* seems to be of potential use to evaluate bone metabolism among neonates, which may indirectly indicate the possibility of stunting condition. Therefore, the main objective of the study is to test the possibility of *Pyd* level in urine as an early indicator to stunting.

## 2. MATERIALS AND METHODS

The study design was cross-sectional and was conducted between January to December 2014. Subjects of the study were 35 healthy neonates born at the Andini's Mothers and Children Hospital at Tuanku Tambusai street 55,



Pekanbaru (middle class hospital and population strategic location in Pekanbaru) between the 28<sup>th</sup> of August until 30<sup>th</sup> September 2014 (all babies born in a specific period of time that their mothers were willing to sign an informed consent). The minimum number of samples to compare two groups (stunted & normal) in this study is 30,29 neonates [10].

$$n = \frac{2\sigma^2(Z_{1-\alpha} + Z_{1-\beta})^2}{(\mu_1 - \mu_2)^2}$$

n = Sample size

$\sigma$  = Population standard deviation

$\mu_1$  = Test value of the population mean

$\mu_2$  = Anticipated population mean

The value of  $\alpha = 5\%$  (1.964) and  $\beta = 20\%$  (0.842) [11]. In order to obtain a number of samples that reflect population characteristics, statistical parameters (eg mean and standard deviation) from previous studies were used in this study. The research was "Food Supplementation with Encouragement to Feed It to Infants from 4 to 12 Months of Age" carried out by [12]. The study showed that  $\mu_1 - \mu_2 = 0.4$  cm (achievement of the subject body length increase), and standard deviation namely  $\sigma = 1.6$  cm. In anticipation of the drop out subjects, a number of neonates were added so that  $n = 35$  neonates. Subjects were recruited at around 1-3 days of neonatal life. Inclusion criteria were normal gestation (36 to 40 weeks), spontaneous and caesarean delivery. The study complies with the World Medical Association Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects and was approved by the Institutional Review Board of the Faculty of Medicine, University of Riau, Ministry of Education and Culture of Republic Indonesia. Parents of all subjects were given a written informed consent and signed them upon agreement to join the study.

Family socioeconomic questionnaires (e.g. name, gender, age, race, and height parent), body length gauges (BUTERFLY), digital weighting scale for baby (BABY SCALE TANITA), MicroVue™ PYD EIA kit, USA (Quidel Corporation, San Diego, CA 92121, USA, Cat: 8010, Lot: 015210, ED: 2015-07 and Spectrophotometer Microplate Reader 680 (Bio-Rad Laboratories, Inc., Hercules, CA 94547, USA), Creatinine measurements were performed with the use of Jaffe method and Spectrophotometer ADVIA 1800: ADVIA, Germany, baby urine bags (PEDIATRIC URINE COLLECTOR, Japan).

The 24-hours neonates' urine was collected using pediatrics urine bags by trained nurses, aliquoted to 6 mL. Mothers were briefed about the study, one day prior to neonates urine collection. Baby urine that has been collected was stored in the refrigerator at a temperature of -20°C at the Pekanbaru Prodia Clinical Laboratory and then was sent to Prodia Center in Jakarta for analysis.

Statistical analysis and results are reported based on the data. Statistical outliers, defined as outside the 95% confidence limits of the normal probability plots, two subjects were removed before the analysis. In all statistical tests performed, the null hypothesis (no effect) was rejected at the 0.01 level of probability. Differences in mean body weight, head circumference, concentrations of *Pyd* urine, age, weight-for-age-z-score (WAZ), length/height-for-age (HAZ), basal metabolic index (BMI) for age (BAZ), mother's height, mother's BMI, mother's weight before pregnancy, mother's prenatal weight, mother's pregnancy age, the number of children in family were evaluated by using a Independent t-test. Stunted neonates referred to babies with body length of <48 cm and normal babies referred to those with body length  $\geq 48$  [13]. All analyses were performed by using SPSS version 20 (IBM SPSS Statistics 20).

### 3. RESULTS AND DISCUSSION

The proportion of stunted neonates in the study was 22.9%. That is similar with reported by [13] that the proportion of stunting neonates in Indonesia is 20.2%.

All neonates were healthy and have received full enteral feeding (formula and/or breast feeding). 74.3% and 25.7% of the subjects involved in the study were male and female respectively (Table 1). It was found to be much easier to collect urine from male than female neonates, which reflected on the higher percentage of the subjects. Most of the mothers stayed at *Pekanbaru*, except for two of them. This is because their other family members also stayed at the same area and would like to be close to them when their baby is born. Apart from that, living at *Pekanbaru*, the mothers thought that they can get a proper medical attention when needed compared to outside the city. Most of the mothers were Malay and a small percentage was Chinese. 50% of the subject's mother was full housewife. Some of the mothers were also working as officers of the government and private

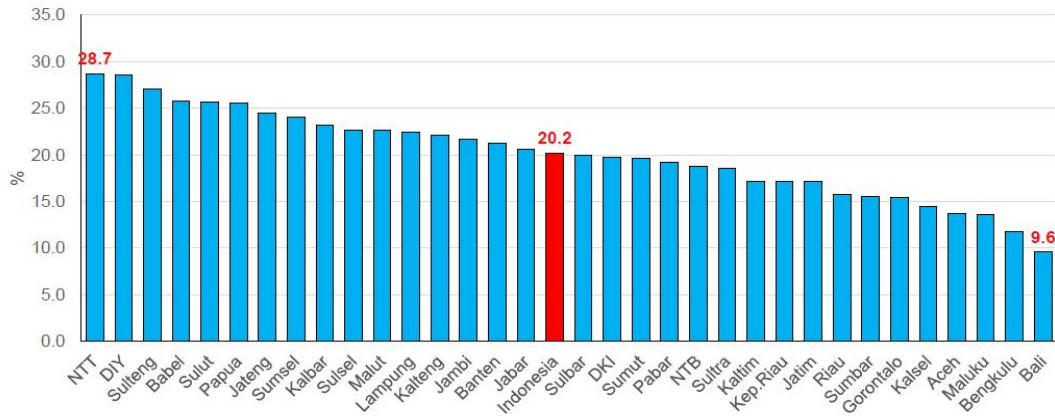


Fig. 1. The proportion of stunting neonates neonatal body length at birth < 48 cm) by province in Indonesia

Table 1. Growth problems

Z-score	Growth indicators			
	Length/height-for-age	Weight-for-age	Weight-for-length/height	BMI-for-age
Above 3	See note 1	See note 2	Obese	Obese
Above 2			Overweight	Overweight
Above 1			Possible risk of overweight (See note 3)	Possible risk of overweight (See note 3)
0 (median)				
Below -1				
Below -2	Stunted (See note 4)	Underweight	Wasted	Wasted
Below -3	Severely stunted (See note 4)	Severely underweight (See note 5)	Severely wasted	Severely wasted

Note 4: It is possible for a stunted or severely stunted child to become overweight [14]

sector. Overall, the education level among all the subjects mothers were from the Elementary to Scholar level.

Length for age males and female neonates in Indonesia is about same. [15] reported that length male neonatus is 49.9 cm and female neonatus is 49.1 cm. The growth of male and female neonates is almost the same in the early stages of life, differences will occur at the next stage of growth [16]. There were 42 neonates who were born during the urine collection period, as many as 5 neonates were not continued to be processed to the analysis stage of the urine Pyd content because the amount of urine collected was insufficient. The five neonates are female.

Neonatal female urine collectors are relatively more difficult than men because female urine sometimes spills out of a pediatric urine collector. An addition, there were 2 female neonates whose Pyd content was in the form of outliers so they were not included in the data analysis.

The proportion of stunted neonates in the study was 22.9%. It was a similar findings as reported by Atmarita research which was at the rate of 20.2% [13]. The characteristics of the stunted and normal subject were recorded as Table 2. The length of stunted and normal neonates was 46.8±0.5 cm and 49.9±1.4 respectively. Their weight also differs, by which the normal neonates were much heavier compared to the stunted

neonates (a difference of about 300-400 g). The head circumference of stunted neonates and normal neonates were  $33.3 \pm 1.0$  and  $33.6 \pm 1.2$  cm respectively. This study has shown that there was a major difference (statistically significance) in terms of the level of *Pyd* in the urine between the stunted neonates and normal neonates. Among stunted neonates, the amount of *Pyd* in the urine was  $982.9 \pm 61.6$  nmol/mmol Cr, compared to only  $594.1 \pm 266.1$  among normal neonates. The HAZ was also found to be statistically significant between the stunted neonates and normal neonates.

Mothers of subjects with small BMI's did not give birth small subjects (independent t test). The mean BMI of mothers of stunting and normal subjects were 22.2 and 20.0, respectively. The mother of the subject with a normal BMI was 48.3% (Table 4).

Classification of adult Asian body mass index (BMI) according to [14] is listed in Table 5.

The quantified amount of *Pyd* in the urine of the neonates had quadratic relationship with HAZ ('U-shaped' scatter plot) as shown in Fig. 1. The *Pyd* in the urine was negatively associated with body length in stunted neonates and can be used as biomarkers of linear growth. Neonates classified as stunted if their length <48 cm and the contents of *Pyd* >859.7 nmol/mmol Cr. Further study is recommended for infants aged 6 to 12 months to further confirm the hypothesis. In the previous studies, *Pyd* excretion were found to differ based on different age groups. *Pyd* excretion from elementary school children, for example, is about 50--500 nmol/mmol Cr [17]. *Pyd* excretion on children 3-5 year was  $238.3 \pm 22.7$  pmol/mumol Cr (male) and  $261.8 \pm 14.2$  pmol/mumol Cr (female) [18].

**Table 2 .Familial socioeconomic status of the subjects**

Variables	Criteria	Value*
<b>Sex</b>	Male	74.3 (26)
	Female	25.7 (9)
<b>Residence</b>	Pekanbaru	91.4 (32)
	Others	5.7 (2)
<b>Ethnic group</b>	Malay	97.1 (34)
	Chinese	2.9 (1)
<b>Mother's job</b>	Teacher	8.6 (3)
	Housewife	51.4 (18)
	Employed	31.4 (11)
<b>Mother's education</b>	Entrepreneur	8.6 (3)
	Elementary school	5.7 (2)
	Junior high school	2.9 (1)
	Senior high school	22.9 (8)
	Diploma 3	20.0 (7)
	Diploma 4	2.9 (1)
	Scholar	37.1 (13)

\*% (n)



**Fig. 2. Pediatric urine collector**

**Table 3. Characteristics of the stunted and normal subject**

Variables	Stunted	Normal
Length (cm)	46.8±0.5 (46:47) <sup>a</sup>	49.9±1.4 (48.0:53.0) <sup>b</sup>
Weight (g)	2846±360 (2400:3480)	3215±404 (2380:4080)
Head circumference (cm)	33.3±1.0 (31.0:34.5)	33.6±1.2 (31.0:35.0)
Pyd (nmol/mmol Cr)	982,9±61.6 (967,8:1049.6) <sup>a</sup>	594.1±266,1 (564,4:2550.8) <sup>b</sup>
Age (days)	1±1 (1:1)	1±1 (1:3)
WAZ	-1.03±0.82 (-2.15:0.33)	-0.21±0.87 (-2.15:1.74)
HAZ	-1.26±0.27 (-1.67:-1.00) <sup>a</sup>	0.23±0.66 (-0.56:1.97) <sup>b</sup>
BAZ	-0.69±1.29 (-2.27:1.66)	-0.60±1.11 (-3.21:1.34)
Mother's BMI (kg/m <sup>2</sup> )	21.5±4.3 (18.0:30.5)	22.0±2.9 (16.9:26.7)
Mother's height (cm)	156±4 (150:165)	161±7 (150:185)
Mother's weight before pregnancy (kg)	53±13 (42:83)	57±8 (42:70)
Mother's prenatal weight (kg)	66±15 (53:101)	69±11 (50:86)
Pregnancy age (weeks)	38±1 (37:39)	38±3 (35:49)
Number of children (person)	2±1 (1:4)	2±1 (1:4)

Independent t test: <sup>ab</sup>p<0.01

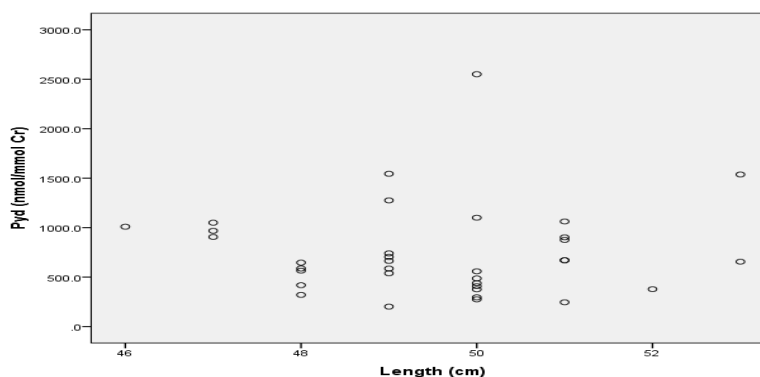
**Table 4. Nutritional status of subject mother**

Category	Amount	
	n	%
Underweight	5	17.2
Normal	14	48.3
Overweight:	4	13.8
At Risk	5	17.2
Obese I	0	0.0
Obese II	1	3.4
Total	29	100.0

**Table 5. Classification of adult Asian body mass index (BMI)**

Category	BMI (kg/m <sup>2</sup> )	Risk of co-morbidities
Underweight	< 18.5 kg/m <sup>2</sup>	Low (but the risk of other clinical problems increases)
Batas Normal	18.5 - 22.9 kg/m <sup>2</sup>	Mean
Overweight:	≥ 23	
At Risk	23.0 – 24.9 kg/m <sup>2</sup>	Increase
Obese I	25.0 - 29.9 kg/m <sup>2</sup>	Moderate
Obese II	> 30.0 kg/m <sup>2</sup>	Dangerous

Source: [14]



**Fig. 3. Association of Pyd in the urine and body length in neonates**

#### 4. CONCLUSION

Pyd was significantly higher in the urine from stunted neonates than non-stunted neonates. Urine Pyd may become a candidate of a marker of stunted neonates. Further study on a large population is necessary.

#### CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

#### ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. World Health Organization. Child growth indicators and their interpretation. Global Database on Child Growth and Malnutrition; 2018. .
2. M. de Onis, Branca F. Childhood stunting: A global perspective. Maternal and Child Nutrition; 2016.
3. C. B. of S. (BPS) et al. Indonesia demographic and health survey; 2016.
4. Prendergast AJ, Humphrey JH. The stunting syndrome in developing countries. Paediatr. Int. Child Health; 2014.
5. PAJ, et al. Stunting is characterized by chronic inflammation in Zimbabwean infants. PLoS One; 2014.
6. Kumar D, Goel NK, Mittal PC, Misra P. Influence of infant-feeding practices on nutritional status of under-five children. Indian J. Pediatr; 2006.
7. Use and interpretation of anthropometric indicators of nutritional status. Bull. World Health Organ; 1986.
8. Marowska J, Kobylińska M, Lukaszewicz J, Talajko A, Rymkiewicz-Kluczyńska B, Lorenc RS. Pyridinium crosslinks of collagen as a marker of bone resorption rates in children and adolescents: Normal values and clinical application. Bone; 1996.
9. Kartawinata HNY, Hilmanto D. Serum creatinine and cystatin C level in children with undernutrition and normal nutritional status. J. Indones. Med. Assoc. 2012; 62(12):471–475.
10. Lwanga SK, Lameshow S. Sample size determination health study. Geneva. World Health Organization; 1996.
11. Walpole RE. Introduction of Statistic (3rd Ed); 1988.
12. Bhandari N, Bahl R, Nayyar B, Khokhar P, Rohde JE, Bhan MK. Food supplementation with encouragement to feed it to infants from 4 to 12 months of age has a small impact on weight gain. Am. J. Nutr. 2001;1:1946–1961.
13. Atmarita. The future figures of Indonesian human resources; 2014.
14. World Health Organization. Improving child growth; 2001.
15. Ministry of Health Republic Indonesia, National Health Survey; 2010.
16. Keputusan Menteri Kesehatan Republik Indonesia Nomor 1995/Menkes/SK/XII/2010 tentang Standar Antropometri Penilaian Status Gizi Anak. Available: <https://www.slideshare.net/vindhayatRIPTA/buku-antropometrianakduniasehatnet>
17. Beardsworth LJ, Eyre DR, Dickson IR. Changes with age in the urinary excretion of lysyl- and hydroxylysylpyridinoline, two new markers of bone collagen turnover. J. Bone Miner. Res; 1990.
18. Fujimoto H, Kubo S, Tanaka T, Miura M, Seino Y. Urinary pyridinoline and deoxy-pyridinoline in healthy children and in children with growth hormone deficiency. J. Clin. Endocrinol. Metab. 1995;80(6):1922–1928.

**Group Statistics**

Categorical of length		N	Mean	Std. Deviation	Std. Error Mean
Pyd (nmol/mmol Cr)	<48 cm	4	982.925	61.6458	30.8229
	>= 48 cm	28	594.118	266.1589	50.2993

**Independent Samples Test**

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Pyd (nmol/mmol Cr)	Equal variances assumed	3.316	.079	2.872	30	.007	388.8071	135.3688	112.3471	665.2672
	Equal variances not assumed			6.591	22.513	.000	388.8071	58.9921	286.6265	510.9878

© 2019 Hayati et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Peer-review history:**

The peer review history for this paper can be accessed here:  
<http://www.sdiarticle3.com/review-history/48579>