Serum Zinc Level in Children with β-Thalassemia Major

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ABSTRACT

Background: Beta thalassemia is one of the most common inherited single gene disorders result from absent or reduced β-chain production. Zinc as a second trace element of human body plays an important role in numerous functions. Thalassemic patients are at risk of zinc deficiency due to various causes including the use of iron chelating agents oral or injectable.
Objectives: To determine the level of serum zinc in a group of β-thalassemia major patients and its relationship to some variables.
Methods: A cross-sectional study was extended from 1st of April 2016 to 31st of August 2016 carried out in Al-Karama Teaching Hospital, Center of Inherited Blood Diseases in Baghdad. The control group were 62 apparently healthy (32 males, 30 females) within the age group of (2-16 years) with normal weight, height, body mass index for their ages. Serum zinc was measured by using colorimetric test kit measured by atomic absorption spectrophotometer UV/VIS.
Results: Seventy-three patients with β-thalassemia major (42 males 57.5% and 31 females 42.5%) with age range (2-16 years). Thirty eight patients (52.1%) were found to have serum zinc level < 70 µg/dl (low zinc), thirty (41.1%) patients had normal s. zinc level (70-115µg/dl), and only 5 (6.8%) patients were found to have high serum zinc level (more than 115µg/dl). Forty-three (58.9%) patients used deferasirox had mean of s. zinc level (82.02±15.42) while 25 (34.2%) patients used desferrioxamin had mean s. zinc level (73.08±17.76). So, p value was significant (0.032) on comparing between patients using desferrioxamin and deferasirox. There were no significant correlations observed in thalassemic patients between serum zinc measurements and serum ferritin (P value = 0.337).
Conclusion: Majority of patients with β-thalassemia major had low serum zinc level. A prominent low serum zinc was detected in short stature thalassemic patients.
Keywords: **Thalassemia, Serum zinc.**
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The thalassemias are a heterogeneous group of inherited anemias caused by mutations affecting the synthesis of hemoglobin[1].

Beta thalassemia is a group of autosomal recessive hereditary disorders characterized by genetic mutations resulting in reduced synthesis of β globin chains[2]. In individuals with beta thalassemia, there is a complete absence of globin production (β -thalassemia major)[3].

This results in an imbalanced accumulation of globin chains and ineffective erythropoiesis with hemolysis[4].

It was first described by a Detroit pediatrician Thomas Cooley, in 1925[5]. Cooley recognized similarities in the appearance and clinical course of four children of Greek and Italian ancestry. These children exhibited severe anemia (hemoglobin concentration of 3 to 7 g/dL), massive hepatosplenomegaly, and severe growth retardation[1]. In addition, bone deformities, such as frontal bossing and maxillary prominence, gave the patients a characteristic faces. Deformities of the long bones of the legs were also commonly seen and thought to reflect severe osteoporosis[1]. Autopsy studies revealed extraordinary expansion of bone marrow at the expense of bony structures. Extramedullary hematopoiesis was often a

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striking feature and appeared as either isolated massive hepatosplenomegaly[1].

Children were also noted to have significant iron deposition in almost all organs, the significance of which was not initially appreciated, before regular blood transfusion regimens were used, these changes progressed rapidly and were invariably fatal during the first few years of life[1]. Recurrent blood transfusions in beta thalassemia major lead to accumulation of excess iron in the body tissues. This secondary iron overload is responsible for peroxidative damage within the erythrocytes leading to oxidative stress. This oxidative stress will cause growth failure as well as liver, cardiovascular, endocrine, and neurological complications in beta thalassemia major[6]. Ferritin is a protein that plays a key role in iron metabolism by binding and storing excess iron within cells, it is an intracellular hollow protein shell composed of 24 subunits surrounding an iron core that may contain as many as 4000-4500 iron atoms[7]. The concentration of serum ferritin is positively correlated with the size of the total body iron stores in the absence of inflammation and used for the assessment of iron stores[8,9].

Zinc is an essential element which in thalassemia can be either removed by iron chelating drugs[10] as well as from inadequate dietary intake, poor absorption[11]. Zinc deficiency has been shown to affect growth and sexual maturation, and may also cause hair loss, diarrhea, skin disorders, and loss of appetite. In addition, it is also essential for the immune system, particularly for lymphocyte function, there have been few studies examining the effects of supplementation[12]. Zinc supplementation is usually delivered in the form of zinc sulphate, although other formulations are also available. The usual dose is 125 mg 1-3 times daily, although doses of 220 mg 3 times daily have been quoted for haemoglobin disorders. Caution is however needed for high doses, as toxicity can occur; including gastrointestinal irritation, as well as interactions with other minerals and drugs[10].

Methods

This a case-control study extending from 1st of April 2016 to 31st of August 2016 carried out at Al-Karama Teaching Hospital, Center of inherited blood diseases in Baghdad. The study population included 73 patients diagnosed with β-thalassemia major (42 males, 31 females) within the age group of 2-16 years, all of them on iron chelating therapy, the diagnosis of thalassemia major were based on the clinical and hematological with hemoglobin electrophoresis profiles. The control group include 62 apparently healthy subjects with normal weight, height, body mass index for their ages (32 males, 30 females) with age group of (2-16 years) were included in the study after taken their informed consents with fully explanation of the nature of this study.

Inclusion criteria: Confirmation of beta thalassemia major by hemoglobin electrophoresis. Included patients received chelating drug, deferoxamine subcutaneously in dose 25-45 mg/kg over 8-12 hours for at least five nights/week or oral deferasirox tablet (exjade) of 20-40mg/kg once daily dose/seven day a week, half hour before meal. They were 25 patients taken desferroxamine, 43 patients taken deferasirox and five patients taken combined therapy. The group of thalassemic patients on deferoxamine received folic acid tab and vitamin C.

Exclusion criteria: Patients with hepatitis C and/or B, chronic liver disease. Patients receiving minerals supplements including zinc. Patients with history of recent infection, fever, diarrhea. Patients with diabetes mellitus, celiac, hypothyroidism, growth hormone deficiency.

In this study, center for disease control and prevention growth charts were regarded as reference for evaluation of growth status[13], as these charts are recommended at inherited blood diseases center at AL-Karama Teaching Hospital for evaluation of growth status of each patient.

All data was collected by the same person. Secastadiometer was used for both height and weight measurement.

By using these charts, the measurements like height, weight can be compared with that of control of the same gender and age. Each chart has smoothed curves or lines that represent growth percentiles, these curves (percentiles) serves as a reference for comparison. The height or weight will be plotted on the grid and then compared to these percentiles[13].

Patients with height for age percentile below 5th were classified as having short stature, and those with weight for age percentile below 5th were classified as having underweight. BMI less than 5th percentile considered as underweight, 5th percentile to less than 85th percentile as healthy weight and 85th percentile to less than 95th percentile as overweight[13].

Zinc assay: Three milliliters of blood was drawn before transfusion in fasting state with plastic syringe from each thalassemic and control patient subject collected in biochemical tube (gel and plain) tube. Then centrifugation at 3000-4000 rpm for 5 minutes was done, separated sera were kept frozen at -20 °C. Haemolysed sera were excluded from the study.

Serum zinc assessment by using colorimetric test kit (ZINC –LTA s.r.l.-s.u.-via Milano) measured by atomic absorption spectrophotometer UV/VIS with thermostataion device model (PD – 303 – APEL Co.) of wavelength 578nm.

A cut-off value of 70 µg/dl - 115 µg/dl was used for serum zinc, samples below 70 g/dl was regarded as low (hypozincemia), while level > 115µg regarded as hyperzincemia. Serum ferritin, frequency of transfusions, and dose, type of iron chelating agent collected from patient's files.

Statistical package for social sciences version 22 (SSPS) was used for data analysis. Discrete variables presented as numbers and percentages and continuous variables presented as mean with standard deviation**.** Chi square test for independence used to test the significance of associations between discrete variables and for goodness of fit to test the significance of observed distribution. T test for two independent variables and ANOVA were used to test the significance of mean variation between independent samples. The level of significance was set at p value equals to 0.05 and below (≤ 0.05).

Results

Seventy-three patients with B thalassemia classified according to the age, age group, sex, weight percentile, height percentile, BMI percentile, type of iron chelating agent, splenectomy, serum zinc, and serum ferritin.

Regarding the control group, s. zinc level mean ± SD was (90.6±17.1) µg/dl with range levels of (62.8–123.7µg/dl). Nine children were found to be with low s. zinc (14.5%), 46 children (74.2%) considered normal and 7 children (11.3%) with high serum zinc level, (Table 2).

There was no significant difference in mean age level between the two study groups.

The result of present study revealed that mean zinc level found to be significantly lower in thalassemic patient (79.25+17 µg/dl) than in non-thalassemic patient (90.58+17.09 µg/dl) (P <0.05, Table 3)

There was no significant association between age group and type of study group (P>0.05, Table 3).

This study observed a significant association between thalassemia and zinc level that low zinc levels are more likely to be observed in thalassemic patients (52.1%) than in non-thalassemic patients (14.5%) (P < 0.05, Table 3).

There were no significant correlations observed in thalassemic patients between serum zinc measurements and each of s. zinc, age, height, weight and BMI (P > 0.05, table 4-a),

There were no significant correlations observed in thalassemic patients between serum ferritin measurements and each of s.

zinc and BMI (P >0.05, table 4-b). In thalassemic patients, serum ferritin measurements significantly directly correlated with each of age, weight and height (P < 0.05, table 4-b). In non-thalassemic patients, there was no significant correlation between serum zinc measurements and age of patients (P >0.05, table 4-c).

In this study, the mean of serum zinc levels was significantly associated with age, weight, height, and splenectomy. There were 52 patients > 5 years of age had lower s. zinc level mean than the other age group (p value <0.001), (Table 5).

Regarding weight there were 17 patients (23.3%) underweight and 56 patients (76.7%) were appropriate weight for their age and sex, a significant relationship was observed between mean of s. zinc levels and patients with underweight (p value < 0.001), (Table 5).

Table 1: Characteristics of enrolled thalassemia major patients.

Variables	Category	N=73	100.0%
Age (y)	Mean ±SD	8.5±3.8	
	Min-Max	2-16	
Age Group	< 2 years	0	0.0%
	2-5 years	21	28.8%
	> 5 years	52	71.2%
Sex	Male	42	57.5%
	Female	31	42.5%
Weight Percentile	Low	17	23.3%
	Normal	56	76.7%
Height Percentile	Low	37	50.7%
	Normal	36	49.3%
BMI Percentile	Low	6	8.2%
	Normal	64	87.7%
	High	3	4.1%
Type of Iron Chelating Agent	Oral	43	58.9%
	DFO	25	34.2%
	Combined	5	6.8%
Splenectomy	Yes	9	12.3%
	No	64	87.7%
Frequency of blood transfusion	Every 1 wk	2	2.7%
	Every 2 wk	25	34.2%
	Every 3 wk	41	56.2%
	Every 4 wk	5	6.8%
Serum Zinc (µg/dl)	Mean ±SD	79.3±17.1	
	Min-Max	60.8-122.8	
Serum Zinc Level	Low	38	52.1%
	Normal	30	41.1%
	High	5	6.8%
Serum Ferritin (ng/ml)	Mean ±SD	2702.6±1040.2	
	Min-Max	891.0-4851.0	
Serum Ferritin Level	Low	2	2.7%
	Normal	19	26.0%
	High	52	71.2%

Table 2: Characteristics of comparison of healthy controls group.

Variables	Category	N=62	100.0%
Age (y)	Mean ±SD	9.1±3.9	
	Min-Max	2-16	
Sex	Male	32	51.6%
	Female	30	48.4%
Age Group	< 2 years	0	0.0%
	2-5 years	16	25.8%
	> 5 years	46	74.2%
Serum Zinc (µg/dl)	Mean ±SD	90.6±17.1	
	Min-Max	62.8-123.7	
Serum Zinc Level	Low	9	14.5%
	Normal	46	74.2%
	High	7	11.3%

Table 3: Comparing characteristics of the two study groups.

Variables	Thalassemic Group		Non-thalassemic Group		P value
	N=73	100.0%	N=62	100.0%	
Age (y); Mean ±SD	8.5±3.8		9.1±3.9		0.431(NS)
Age Group					0.701
• < 2 years	0	0.0%	0	0.0%	(NS)
• 2-5 years	21	28.8%	16	25.8%	
• > 5 years	52	71.2%	46	74.2%	
Sex					0.491
• Male	42	57.5%	32	51.6%	(NS)
• Female	31	42.5%	30	48.4%	
Serum Zinc (µg/dl);Mean ±SD	79.3±17.1		90.58±17.09		**<0.001**
Serum Zinc Level					**<0.001**
• Low	38	52.1%	9	14.5%	
• Normal	31	41.1%	46	74.2%	
• High	5	6.8%	7	11.3%	

NS: non-significant.

Table 4: Correlations between study variables.

a) Correlations of serum zinc measurements with studied variables in thalassemic group.

Variables	Pearson Correlation coefficient	P value
S. ferritin	-0.114	0.337
Age	-0.229	0.051
WT	-0.046	0.700
HT	-0.041	0.730
BMI	-0.093	0.436

Table 4: Correlations between study variables (*contd.*).

b) Correlations of serum ferritin measurements with studied variables in thalassemic group

Variables	Pearson Correlation coefficient	P value
S. zinc	-0.114	0.337
Age	**0.252**	**0.031**
Weight	**0.257**	**0.028**
Height	**0.236**	**0.045**
BMI	0.140	0.238

c) Correlations between serum zinc measurements and age in non-thalassemic group.

Variable	Pearson Correlation coefficient	P value
dwq Age	0.145	0.262

Table 5: Mean serum zinc and ferritin measurements in thalassemic patients according to study variables.

Variables	S. zinc (Mcg/dl)			S. ferritin (ng/ml)		
	N	Mean	SD	N	Mean	SD
Sex						
• Male	42	79.07	17.29	42	2798.69	879.42
• Female	31	79.48	17.12	31	2572.48	1228.56
P value		0.920			0.362	
Age Group						
• 2-5 years	21	81.10	15.62	21	2478.71	928.55
• > 5 years	52	78.50	17.75	52	2793.06	1077.21
P value		**<0.001**			0.586	
Weight Percentile						
• < 5th	17	67.47	7.60	17	2987.00	1067.73
• 5th – 95th	56	82.82	17.61	56	2616.30	1025.75
P value		**<0.001**			0.200	
Height Percentile						
• < 5th	37	71.19	9.91	37	2768.59	1130.92
• 5th – 95th	36	87.53	19.01	36	2634.83	949.16
P value		**<0.001**			0.586	
BMI Percentile						
• < 5th	6	66.83	6.55	6	3131.83	1061.81
• 5th – 95th	64	80.73	17.59	64	2666.67	1031.86
• > 95th	3	72.33	8.39	3	2611.33	1409.57
P value		0.126			0.577	
Iron Chelating Agent						
• Oral	43	82.02	15.42	43	2582.12	1023.68
• DFO	25	73.08	17.76	25	3042.64	1023.33
• Combined	5	86.20	22.23	5	2039.00	871.70
P value		0.072			0.069	
Splenectomy						
• Yes	9	67.89	7.99	9	3398.33	886.84
• No	64	80.84	17.47	64	2604.80	1028.49
P value		**0.032**			**0.031**	
Frequency of Blood Transfusion						
• Every 1 wk	2	76.00	19.80	2	3340.00	1186.53
• Every 2 wk	25	78.60	17.64	25	2808.64	1070.23
• Every 3 wk	41	79.41	17.44	41	2643.12	1058.63
• Every 4 wk	5	82.40	15.44	5	2405.60	790.15
P value		0.965			0.679	

Discussion

In the current study, the status of serum zinc was investigated in patients with β-thalassemia major and its correlation to different variables. Zinc is a key constituent, which is essential for maintenance of cellular homeostasis. The main reason for its mandatory requirement is the fact that hundred of metallo-enzymes require zinc as a cofactor to be functionally effective. Zinc insufficiency is considered as an important factor for impaired linear growth, delayed sexual maturity, immune dysfunction and endocrinopathies in thalassemic patients[14,15].

The present study showed agreement with many regional and international studies, where a decrease in serum zinc level have been reported in thalassemic patients compared with the matched healthy control. In Iraq many studies showed similar observations. Abdulkhader et al, observed in 50 thalassemic patients compared to 50 persons as control that the level of serum zinc was significantly lower in thalassemics[16], and the same results were observed by Zahra MA[17], Elham AM[18], Khalid et al[19], Jasim MH et al[20], and Nameer MW et al [21]. Also, Erdoğan E et al[10], Arcasoy A et al[22], Nasr MR et al[23] and Ghone RA et al[24] showed in their studies that serum zinc level was low in thalassemics compared to control similar to the current study. All these results indicates that most of patients with β-thalassemia major had hypozincemia, and this may be related to multifactorial causes such as nutritional as low intake of enriched zinc foods due to anorexia and fear of increasing iron, hyperzincuria induced by hemolysis and by iron chelating drugs, impaired zinc absorption, and hepatic dysfunction. In contrast to these results, Mohammed El Missiry et al revealed that among 30 thalassemic patients and 30 siblings, there were no significant differences in zinc levels between the two groups, suggesting that zinc level was not influenced by thalassemia or its treatment, the author also assumed that serum zinc levels are possibly more influenced by familial factors rather than by thalassemia per se or its treatment[25]. Interestingly, Mansi K et al[26] and Mehdizadeh M et al[26] found that the mean serum zinc levels of thalassemics was significantly higher than that in the control group, this suggest that regular blood transfusion somehow prevents zinc deficiency, and it may explain the normal and even higher serum zinc values in patients compared with their control group.

In the current study, the mean serum zinc level was significantly associated with age, as patients more than 5 years of age had lower serum zinc level. In contrast to other studies[27,28] were no significant correlation between serum zinc and ages of studied groups, this might be due to long duration of exposure to chelating agents, chronic hemolysis and poor nutrition.

Thirty seven (50.7%) of thalassemia patients in this study were considered as short stature, which was agree to the result observed by Zahraa MA as 80% of studied patient were short stature[17]. Also, Hamidah A et al[29] and Shamshirsaz AA et al[2] reported that short stature seemed to be more prevalent among thalassemia patients. In contrast, Mehdizadeh M et al showed that only 23.4% of thalassemics in his study were short stature[26].

Interestingly, this study showed 26 (70.27%) of short stature patients had hypozincemia with significant correlation between serum zinc and short stature (p value <0.014).

In conclusions; patients with β-thalassemia major who had low serum zinc level constitute 52.1%. Low serum zinc was detected in short stature and underweight thalassemia patients. There were more hypozincemia observed in thalassemic patient on desferrioxamine (parenteral DFO) compared to others on DFX and combined therapy. There were no significant correlation between serum zinc concentrations and serum ferritin.

References

1- Cunningham MJ, Sankaran VG, Nathan DG, et al. The thalassemias. In: Orkin SH, Nathan DG, Ginsburg D, Ginsburg (editors). Nathan and

Oski's Hematology of Infancy and Childhood. 7th ed. Philadelphia: Elsevier Health Sciences; 2015. P.1015-105.
2- Shamshirsaz AA, Bekheirnia MR, Kamgar M, et al. Metabolic and endocrinologic complications in beta-thalassemia major: A multicenter study in Tehran. BMC Endocrine Disorders 2003 Aug 12;3: 1.
3- DeBaun MR, Frei-Jones MJ, Vichinsky EP. Thalassemia syndromes, hemoglobinopathies. In: Kliegman RM, Stanton BMD, St. Geme J (editors). Nelson Textbook of Pediatrics. 20th, Philadelphia, PA, United States: Elsevier - Health Sciences Division; 2015. P. 2349-52.
4- Rund D, Rachmilewitz E. B-thalassemia. New England Journal of Medicine 2005;353(11):1135-46.
5- Hastings CA, Torkildson JC, Agrawal AK. Handbook of Pediatric Hematology and Oncology: Children's Hospital and Research Center Oakland. 2nd ed. Malden, MA, United States: Wiley, John & Sons; 2012. P. 36-43.
6- Khan FU, Khan MH, Tariq A et al. Frequency of complications in Beta thalassemia major. Biomedical 2007;23(6): 31–33.
7- Sanda K, Hnin O P, Myat T, et al. Iron status in type 2 diabetes patients with and without metabolic syndrome. Journal of Physiological and Biomedical Sciences 2015;28(2):29–33.
8- Domellöf M, Hernell O. Iron-deficiency anemia during the first two years of life. Scandinavian Journal of Nutrition 2002;46(1):20–30.
9- WHO. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011 WHO/NMH/NHD/MNM/11.2). http://www.who.int/vmnis/indicators/serumferritin
10- Erdoğan E, Canatan D, Örmeci AR, et al. The effects of chelators on zinc levels in patients with thalassemia major. Journal of Trace Elements in Medicine and Biology 2013;27(2):109–11.
11- Fung EB, Xu Y, Trachtenberg F, Odame I, et al. Inadequate dietary intake in patients with thalassemia. Journal of the Academy of Nutrition and Dietetics 2012;112(7):980–90.
12- Tienboon P. Effect of nutrition support on immunity in paediatric patients with beta-thalassaemia major. Asia Pac J Clin Nutr 2003;12:61-5.
13- Center for Disease Control and Prevention (CDC). National Center for Health Statistics (NCHS). National Health and Nutrition Examination Survey Data. Hyattsville MD: United States. Department of Health and Human Services, Center for Disease Control and Prevention, 2008. Vital and Health Statistics Series 11, Number 246. 2000 CDC Growth Charts for the United States: Methods and Development
14- Ackland ML, Michalczyk A. Zinc deficiency and its inherited disorders -a review. Genes Nutr 2006; 1(1):41-9.
15- Consolini R, Calleri A, Legitimo A et al. Immunological evaluation of patients with beta-thalassemia major. Acta Haematol 2001;105(1):7-12.
16- Abdulkhader AA, Ban AM, Alla NM. The effects of chelating therapy on the levels of serum ferritin, zinc, copper & its relation with malondialdehyde in patients with B-thalassemia major. Iraqi Journal of Community Medicine 2010;23(3):147–52.
17- Zahraa MA Naji. Serum trace elements (zinc, copper and magnesium) in Iraqi patients with thalassemia major receiving desferrioxamine and its relation with growth state. Iraqi JMS 2012;10(4).
18- Elham AM. Relationship between oxidative stress and antioxidant status in beta thalassemia major patients. Acta Chim Pharm Indica 2014;4(3):137–45.
19- Khaleed J K, Abeer AA, Maysem MA, et al. Biomarkers and trace elements in beta thalassemia major. Iraqi Journal of Cancer and Medical Genetics 2013;6(1):81-6.
20- Jasim M H, Shaimaa MK. Serum zinc level in children with β- thalassemia major on iron chelator. Journal of the Arab Board of Health Specializations 2014;15(1).
21- Nameer MW, Lamia Al-Naama, Meaad KH. Trace elements in patients with β-thalassaemia major. HAEMA 2003;6(3):376–83.
22- Arcasoy A, Canatan D, Sinav B, et al. Serum zinc levels and zinc binding capacity in thalassemia. Journal of Trace Elements in Medicine and Biology 2001;15(2-3):85–7.
23- Nasr MR, Ali S, Shaker M, et al. Antioxidant micronutrients in children with thalassaemia in Egypt. East Mediterr Health J 2002; 8(4-5):490-5.
24- Ghone RA, Kumbar KM, Suryakar AN, et al. Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. Indian J Clin Biochem 2008; 23(4): 337-40.
25- Mohammed El Missiry, Hamed HM, Khalid S, et al. Assessment of serum zinc levels of patients with thalassemia compared to their siblings. Anemia 2014;2014:1-6.
26- Mehdizadeh M, Zamani G, Tabatabaee. S. Zinc status in patients with major β- thalassemia. Pediatric Hematology and Oncology 2008;25(1):49–54.
27- Abolfazl M, Parviz A, Ali-Asghar P, et al. Zinc and copper status in children with beta-thalassemia major. Iran J Pediatr 2010;20(3):297–302.
28- Mashhadi MA, Sepehri Z, Heidari Z, et al. The prevalence of zinc deficiency in patients with thalassemia in south east of Iran, Sistan and Baluchistan province. Iranian Red Crescent Medical Journal 2014;16(8).
29- Hamidah A, Rahmah R, Azmi T, et al. Short stature and truncal shortening in transfusion dependent thalassemia patients: Results from a thalassemia center in Malaysia. The Southeast Asian Journal of Tropical Medicine and Public Health 2001;32(3).

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