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Original Research Article

Reference limits of serum total and bone alkaline phosphatases in apparently healthy Sudanese children aged 5-16 years

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Abstract

Alkaline phosphatase (ALP) activity is present in most organs of the body and is especially associated with membranes and cell surfaces located in the small intestines, bone osteoblasts, liver and placenta. Elevations in serum ALP activity commonly originate from the liver and bone. Consequently, serum ALP measurements are of particular interest in the investigation of hepatobiliary diseases and bone disease associated with increased osteoblastic activity such as in growing children due to rapid growth of bone. An elevated ALP almost always requires other tests to determine the origin of the enzyme. One of these tests is measurement of ALP isoenzymes using electrophoresis or immunoassays. The aim of this study is to estimate the reference limits of the total and bone ALP in growing children according to age and sex, and determine the age at which these enzymes reach their maximum peaks in both sexes as well as determining the percentage of bone ALP from total ALP according to age and sex. This study is a descriptive cross-sectional study done in Khartoum State in which 120 children aged 5-16 years from both sexes were included in this study. All children were weighed and height was determined for each child and compared with the growth charts for age and sex to ensure normal growth. Total ALP and bone isoenzyme were measured for each child then the normal limits were set according to age and sex. Total and bone ALP reference limits were recorded as the 5th and 95th percentiles for each age between 5-16 years and sex. Those levels were found to be higher than those obtained in other studies in other countries and that levels were higher in boys than girls. The peaked levels of total ALP and bone isoenzyme were found to be at the age of 14 and 13-14 years age group in boys and at the age of 10 and 9-10 years age group in girls. The percentage of bone isoenzyme from the total ALP was 76%-97% in boys and 71%-95% in girls. There was no relationship between the level of bone ALP and the height of children studied. Total ALP and bone isoenzyme have different levels at different ages and between sexes in growing children and by setting the normal limits, physiological and pathological elevations could be distinguished accordingly.

Key words; Isoenzyme, Liver, Osteoblastic, Phosphatase

INTRODUCTION

Alkaline phosphatases (ALP) are a group of cell membrane metalloenzymes and belong to a group of enzymes that catalyze the hydrolysis of various phosphomonoesters at an alkaline pH. Consequently,

ALP is a non-specific enzyme capable of reacting with many different substrates. Specifically, ALP functions to liberate inorganic phosphate from organic phosphate esters with the concomitant production of an alcohol. (Bishop L et al., 2005)

Sources of Alkaline 2hosphatase

ALP is expressed mainly in bone, liver, intestines, proximal convoluted tubules of the kidney, and in the placenta. ALP released from these tissues constitutes the total amount measured in the blood. Total ALP activity changes with age and bone fraction, varying from 77% to 89% in children and from 58% to 67% in adults. (Serap T et al., 2011) In contrast to ALP isoenzymes, total serum ALP is widely used in routine biochemical tests and can be performed in almost all laboratories. Traditionally, total serum ALP activity has been used as a biochemical marker for bone formation to assess osteoblastic activity in primary hyperparathyroidism, rickets, osteomalacia and Paget's disease. Since ALP is a marker for osteoblastic activity, growing children have higher levels than fully grown individuals. Highest levels of ALP are detected during the rapid growth phases of childhood such as infancy and puberty and it is known that reference values of serum ALP are highly dependent on age and sex in childhood. (Serap T et al., 2011)

Causes of elevated ALP activity

Normal physiological elevation

Increased values occur in the first 3 months of life and at puberty, normal values in women are reached by 18 years, in men by 20, between age of 20 and 50 men are 20% higher than women, after menopause women are higher. Increased values occur in puberty, pregnancy (placental isoenzyme), and postmenopause. (Carin E et al., 2001)

Pathological elevation

In serum ALP activity commonly originates from the liver and bone. Consequently, serum ALP measurements are of particular interest in the investigation of hepatobiliary disease and bone disease associated with increased osteoblastic activity. (Burtis A et al 2004) Causes other than bone or liver can be due to gastrointestinal inflammation. Some Systemic infections (sepsis), certain viral infections, sarcoidosis, certain cancers such as Hodgkin's lymphoma, hypernephroma, gynaecological malignancies, and neoplastic production of ectopic ALP. Acute tissue damage in the heart or lungs (myocardial or pulmonary infarctions), Polycythemia vera and myelofibrosis. (Gordan T. 1993) The intestinal isoenzyme may be increased in patients with cirrhosis and in individuals who are blood group O or B secretors. The placental (carcinoplacental antigen) and Regan isoenzyme can be elevated in cancer patients. (Jassam N, 2009)

Causes of decreased ALP activity

Hypophosphatasia, children with achondroplasia and cretinism. (Maldonado O, 1998). Hypothyroidism.Vitamin C deficiency (scurvy) Folic acid deficiency, vitamin B12 insufficiency, and pernicious anaemia, excess vitamin D intake. Zinc deficiency, malnutrition with low protein assimilation, insufficient parathyroid gland function. (Gordan T. 1993) Aplastic anaemia and chronic granulocytic leukaemia cause low ALP activity. (Maldonado O, 1998)

Objectives

The main objectives of this study are to determine the reference limits of the total alkaline phosphatase (ALP) and its bone isoenzyme according to age and sex and to measure the percentage of bone ALP from total ALP in apparently healthy Sudanese children aged 5-16 years, with determining the age of peaked level and to associate between the level with the height of the children.

METHODS

This is a descriptive, cross-sectional and community based study, conducted in kindergarten, primary, and secondary schools in Omdurman province in Khartoum State. 120 Sudanese children aged 5-16 years, 60 girls and 60 boys, were selected.

Blood samples were collected from apparently healthy Sudanese children attending their school day at the time of sampling were selected. This was done after ensuring normal weight and height for age and sex (within the 5^{th} and 95^{th} percentiles) and excluding physical complaints or known diseases (as claimed by the children).

Written consents were filled and signed by the parents of the children selected; this consent letter was attached to the Ministry of Education letters to the selected schools.

Data was collected using designed questionnaire including the required information and clinical evaluation. Measurement of weight was done for each of the children in this study using digital scale and recorded in kg. Height was also recorded for each child chosen using a measuring tape which was fixed to the wall, and then was recorded in cm.

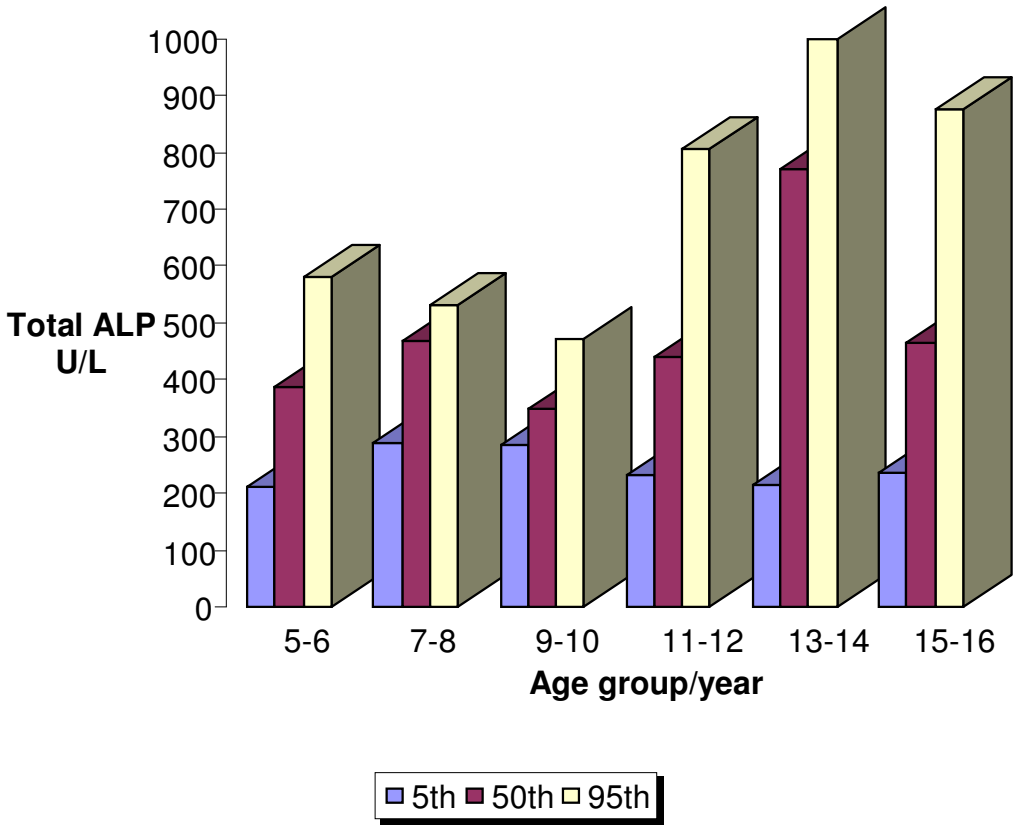

Figure 1. Reference Limits of Total ALP in Boys According to Age Groups

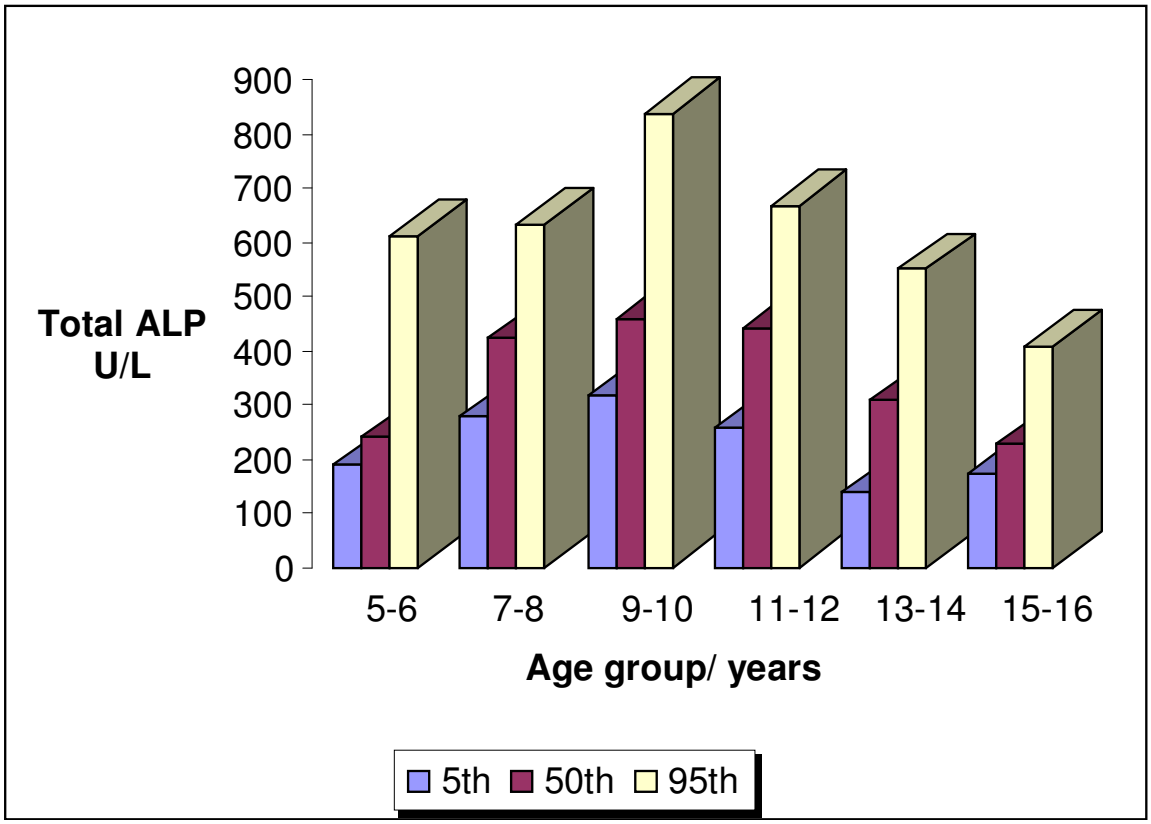

Figure 2. Reference Limits of Total ALP (U/L) in Girls According to Age Group

Table 1. Percentage of Bone ALP from Total ALP in Boys According to Age Group

Age Group	Bone ALP percent
5-6	84%
7-8	86%
9-10	87%
11-12	88%
13-14	86%
15-16	83%

Table 2. Percentage of Bone ALP from Total ALP in Boys According to Age Group

Age Group	Bone ALP percent
5-6	84%
7-8	86%
9-10	90%
11-12	87%
13-14	88%
15-16	75%

Table 3. Reference Limits of Bon ALP (U/L) in Boys According to Age groups

AGE GROUP	5^{th}	50^{th}	95^{th}
5-6	199	330	440
7-8	285	376	460
9-10	238	310	441
11-12	228	384	695
13-14	112	742	877
15-16	126	404	803

Table 4. Reference Limits of Bon ALP (U/L) in Girls According to Age groups

Age Group	5^{th}	50^{th}	95^{th}
5-6	136	207	518
7-8	273	362	553
9-10	267	408	696
11-12	253	375	646
13-14	126	271	454
15-16	111	189	340

After comparing the recorded weight and height with the growth charts according to age and sex, those who were within the normal range (5^{th}-95^{th}) were included in the study.

Total ALP was measured using the MINDRAY BS-200 chemistry analyser then the results were recorded. ALP isoenzymes were measured using SAS-MX ALP isoenzymes gel electrophoresis kit from Helena-biosciences.

Data was analysed using Microsoft Excel (windows, 2003) and STATA 6.0 for windows 2003. Non-parametric methods were used as the data didn't show normal distribution. The reference limits were determined using rank method.

RESULTS

These children were divided equally into 12 sets according to age, each containing 10 children (8.33%) having the same age so they were studied together, and each age set was studied separately. The data was

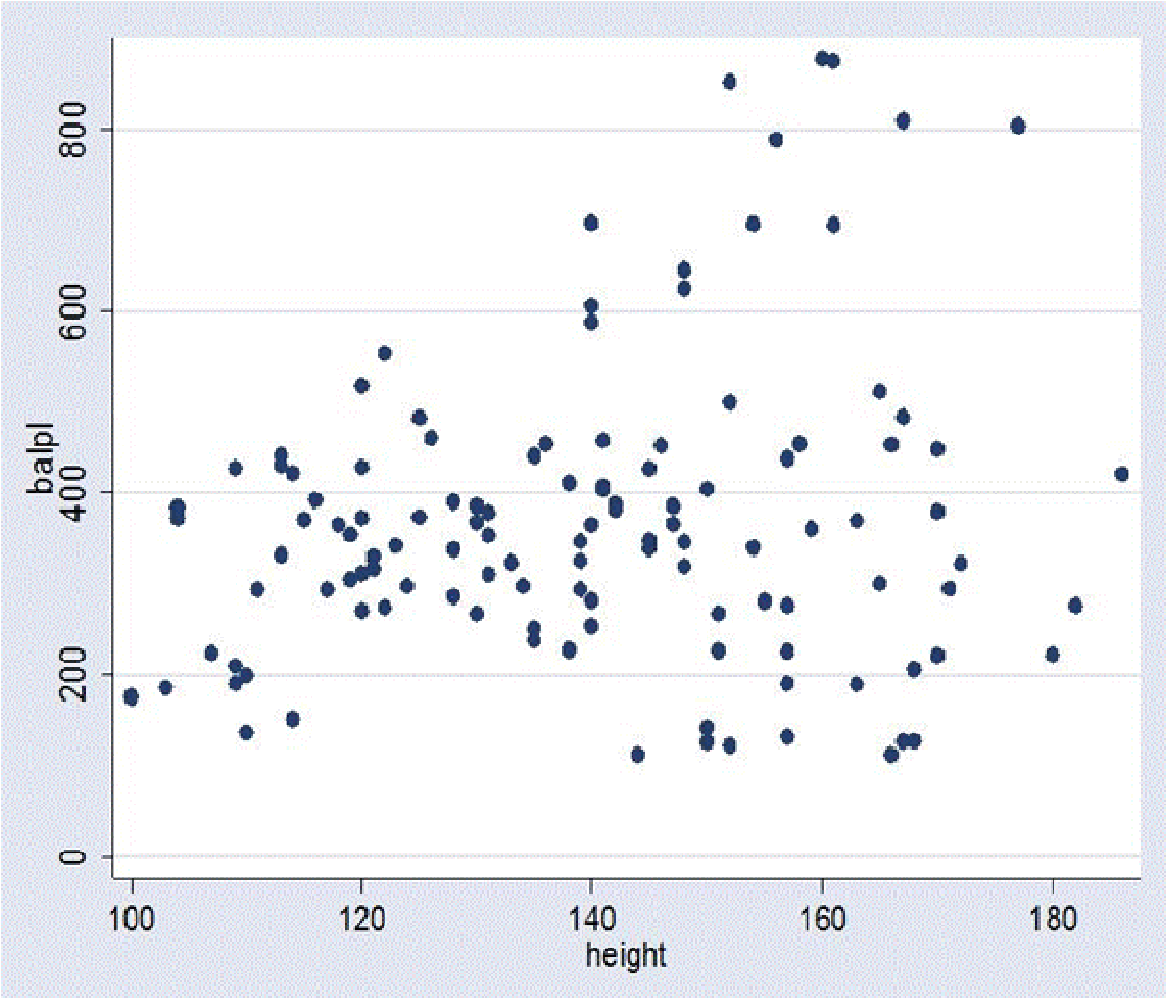

Figure 3. Pearson Correlation Coefficient between Bone ALP Level (U/L) and Height (cm) of Children

divided equally into 6 age groups each containing 20 children (16.67%), each age group includes 2 ages.

According to the sex, boys and girls were studied separately; so for each age, 5 boys (8.33%) were studied together. The same was done for the girls, for each age group, 10 boys (16.67%) were included in each group; the same was done to the girls.

The result showed; total ALP were grouped according to age and sex, and then the 5^{th} and 95^{th} percentiles (reference limits) of the total ALP for each age group according to sex seen in figure (1 and 2). The peaked level of total ALP for boys was at the age of 14 and at 13-14 age groups while for the girls the peaked total ALP level was at the age of 10 and at 9-10 age group.

The percentage of the bone ALP from the total ALP was found to range from 76% to 97% in boys table (1), and 71% to 95% in girls in table (2). The mean was 88% in boys and 83% in girls.

Bone ALP was calculated, then the 5^{th} and 95^{th} (reference limits) were determined for each age group according to sex illustrated in tables (3 and 4). The Pearson r value was 0.178 for the bone ALP and the height showed significant relationship between the level of bone ALP and the height of the children. The p value was 0.052.

DISCUSSION

Concerning this present study, the total ALP was measured, and then according to age and sex the reference limits were recorded as the 5^{th} and 95^{th} percentile for age and sex. Generally, the levels of total ALP obtained by this study were obviously higher than those obtained by other studies. This can be explained by the different environmental, racial, nutritional and physical structure between the different populations around the world. Exposure to the sun for longer time of the day can elevate the ALP too. It was also evident from the results of this study that there are different levels of total ALP

according to age and sex, and that those levels were found to be higher in boys than in girls.

A study done in France titled "Total ,Bone and Liver Alkaline Phosphatase in Plasma: Biological variations and Reference limits" studied factors affecting biological variation in total ALP in a population of apparently healthy subjects four years old or older. The main factors that modify ALP activity are age, sex, hormonal status and morphometric parameters (height, weight). The bone isoenzyme is mainly responsible for the variation associated with age, sex, and puberty and to some extent with the menopause. Activity of the liver isoenzyme was also altered at the menopause and by certain drugs. These data allow proposing reference limits for total plasma, bone, and liver ALP according to age and sex. (Schiele F et al., 1983)

Serum bone alkaline phosphatase (B-ALP) has been considered to be a good marker for bone formation. Recently, a specific immunoradiometric assay for serum B-ALP has been developed. Using this system, study was done measured the serum levels of B-ALP in normal children males and females, (age 0–18 yr). The study found that following in normal children, serum B-ALP levels were high in infants and gradually declined and increased again during puberty. During the pubertal period, the highest serum B-ALP values were observed at midpuberty. (Hitoshi T., 2013)

Those studies done demonstrated that there are sustained high values in the pre-pubertal girls and boys (due mainly to the elevation of bone ALP), and that at puberty and post- puberty the bone ALP showed a gradual decline in boys and girls. (Hitoshi T., 2013) And because the pre-pubertal age is different in girls from that of boys, the peaked levels of bone ALP according to age is different between boys and girls. (Hitoshi T., 2013) All of the findings found by this study were agreed with those obtained by other studies.

It was evident by this study that the age at which the total ALP reaches its peak levels are different from boys to girls. The peaked level of the total ALP was found to be at the age of 14 years and age group of 13-14 years in boys (reaching 1000U/L). But this was different in the case of girls as the peaked level of total ALP was found to be at the age of 10 and age group 9-10 years. This difference can be explained by the physiologically different pre-pubertal age between girls and boys. Those findings were also stated by other studies; for example, in a study titled "Age and Sex Distribution of Alkaline Phosphatase Isoenzymes by Agarose Electrophoresis" done in Belgium in 1990, ALP isoenzymes were separated in sera of normal individuals (aged 4-65) by agarose electrophoresis. The predominant isoenzyme in children was of bone origin. The highest (median) bone ALP activity was reached at age 9-10 in girls and at age 13-14 in boys, followed by a gradual decline in girls and a steep decline in boys. (Vivian O et al., 1990).

During adulthood, activity of the bone fraction was constant and no significant differences were observed between sexes, neither for bone nor for liver ALP activity. The latter remained unchanged throughout life. Intestinal ALP was frequently present in some samples but no significant differences were observed between age groups and sexes for the intestinal isoenzymes. (Viviane O et al., 1990)

By stating the reference limits of the total ALP (like what had been done in this study), it can be possible to correlate the level of the total ALP to the age and sex in the growing children and to differentiate between physiological and pathological elevation accordingly. In the literature, it is stated that bone ALP normally forms about 50% of the total ALP in adults, but this percentage may reach 90% or more in growing children. (Serap T et al., 2011)

This was also demonstrated by the present study that revealed that the percentage of the bone ALP from total ALP is different according to age and sex of the children. Knowing the percentage of the bone ALP from total ALP, we can roughly estimate the level of the bone ALP and other isoenzymes; mainly liver ALP. In this study, the level of bone ALP was found to be different in different age groups and from sex to the other and also revealed that generally the levels were higher in boys. Compared to those of the girls, these finding were also seen in the total ALP in this study.

The level obtained in this study were higher than those obtained in other studies in different countries, this may be due to geographical, environmental, racial and physical structure variation between different populations around the world.

Concerning the peaked level of bone ALP, it was at the age of 14 years and age group of 13-14 years in boys, and in girls it was at the age of 10 years and age group of 9-10 years (as the total ALP). This difference is referred to the physiological difference in pre-pubertal and pubertal age between boys and girls. Those high levels then start to drop in the pubertal and post-pubertal periods in both boys and girls.

In another study titled:"Bone Alkaline Phosphatase Isoenzyme and Carboxy-Terminal Propeptide of Type-I Procollagen in Healthy Chinese Girls and Boys" fasting morning serum samples from 110 girls and 120 boys were taken from the urban Taipei area in 1997. Serum bone alkaline phosphatise (BAP) was measured with immunocatalytic kits. Both the values of BAP and Carboxy-Terminal Propeptide of Type-I Procollagen (PICP) showed mean values approximately fourfold higher than the upper reference limit for adults in the first 3 years of life in both genders. The BAP showed sustained high values in prepubertal girls and boys, however, BAP was higher during the first 3 years of life than the prepubertal values during the next 5 years. After puberty, BAP showed a gradual decrease in boys and girls. (Keh-Sung T et al., 1999).

As observed, the total and bone ALP go in one line

concerning the results obtained by this study because simply most (>80%) of the total ALP in the growing children is of bone origin. To compare the bone ALP level (U/L) with the height (cm) for presence of a relationship between them, Pearson correlation coefficient test was used. The result in this study showed positive relationship between bone ALP and the height. There may be other factors along with the height, age and sex that affect the levels of bone ALP in growing children.

CONCLUSION

This study concluded that serum total and bone ALP have different reference limits according to age and sex. The peaked level of total ALP and bone ALP were reached at the age of 14 years in boys and 10 years in girls and it is higher in boys than in girls.

Most of the total ALP is of bone origin in the growing children with different levels in according to sex. There is significant relationship between the level of bone ALP and the height of the children studied.

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