



Evaluation of Serum Alkaline Phosphatase and Acid Phosphatase in Relation to Abo Blood Groups and Genotypes of Male Blood Donors in University College Hospital Ibadan, Oyo State

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Abstract

Background: Blood consists of plasma, erythrocytes, leucocytes and platelets. The relationship between serum levels of alkaline phosphatase (ALP), total acid phosphatase (TAP), tartrate inhibited acid phosphatase (TIAP) activities and blood groups and genotypes were studied.

Methods: By standard blood grouping and electrophoretic techniques, ABO blood groups and genotypes were determined in 113 apparently healthy male blood donors who reported at University College Hospital, Ibadan (UCH). By the use of the standard methods the activities of alkaline phosphatase, total acid phosphatase, tartrate inhibited acid phosphatase were measured respectively. Standard routine tests were also used for blood grouping and blood genotypes. Participants gave their written informed consent.

Results: The % distributions of ABO blood groups were: 21.2, 28.3, 8.8 and 41.6 for blood groups A, B, AB and O respectively. The % distributions of genotypes were: 70.8, 22.1 and 7.1 for genotypes AA, AS and AC respectively. The mean serum activity of alkaline phosphatase (K.A units/100ml) significantly increases in blood group O>B>A>AB as thus: 12.72 ± 3.24 , 11.05 ± 3.15 , 9.41 ± 3.46 , 9.21 ± 2.47 ($f= 7.33$; 0.000) respectively. The mean serum activity of total acid phosphatase (K.A units/100ml) significantly increases in blood group O>AB>B>A as thus: 4.69 ± 1.39 , 4.64 ± 1.02 , 4.16 ± 0.91 , 3.88 ± 1.15 ($f= 2.96$; 0.036 ($P<0.05$)) respectively. The mean serum activity of tartrate inhibited acid phosphatase was the same in the different blood groups studied ($f= 0.81$; 0.49 ($P>0.05$)) respectively. The mean serum activities of alkaline phosphatase, total acid phosphatase and tartrate inhibited acid phosphatase were the same in the different blood genotypes studied ($f= 1.80$; 0.17 ($P>0.05$)) respectively. There was a positive correlation between blood groups vs ALP, ALP vs TAP, ALP vs TIAP, TAP vs TIAP and a negative correlation between genotype AC and total acid phosphatase in blood donors.

Conclusion: Blood group O and genotype AA has the highest prevalence rate of 41.6 % and 70.8% respectively. Blood group O has the highest levels of both serum activities of ALP and total acid phosphatase respectively while blood group A has the least levels of both serum activities of ALP and total acid phosphatase respectively.

Keywords: Blood groups, genotypes, ALP, TAP, TIAP.

Introduction

Alkaline phosphatase (ALP)^[1] is a homodimeric protein enzyme with 86 kilodaltons. Each monomer of the enzyme contains five cysteine residues, two zinc atoms, and one magnesium atom crucial to its catalytic function. The mechanism of action of alkaline phosphatase involves the coordination of the substrate amid the Zn ions in the active sites, although the Mg site does not appear to be close enough to directly partake in the hydrolysis mechanism, however, it may contribute to the shape of the electrostatic potential around the active center^[2]. Alkaline phosphatase has a Km of 8.4×10^{-4} ^[3] and it is optimally active at alkaline pH environments^[3,4]. A phosphatase is an enzyme that uses water to cleave a phosphoric acid monoester from a substrate into a phosphate ion and an alcohol^[7]. In humans, alkaline phosphatase is present in all tissues throughout the entire body, as an isoforms, mainly in the liver, bile duct, kidney, bone, intestinal mucosa and placenta. Intestinal alkaline phosphatase is secreted by enterocytes, and seems to play a pivotal role in inflammation^[5], intestinal homeostasis and protection. It seems to regulate lipid absorption^[6] and bicarbonate secretion^[7] in the duodenal mucosa, which regulates the surface pH.

In the serum, two types of alkaline phosphatase isozymes predominate: skeletal and liver. During childhood the majority of alkaline phosphatase is of skeletal origin^[8].

Factors such as age, gender, blood type affect the levels of ALP in the blood. Abnormal levels of alkaline phosphatase in the blood could indicate issues relating to the liver, gall bladder or bones. Kidney tumors, infections and malnutrition have also shown abnormal level of alkaline phosphatase in blood^[9].

Placental alkaline phosphatase is elevated in seminomas^[10] and active forms of rickets^[11], Biliary obstruction, Bone conditions, Osteoblastic bone tumors, Osteomalacia^[11], Osteoporosis, Hepatitis, Cirrhosis, Leukemia, Leukemoid reaction, Lymphoma, Paget's disease, Sarcoidosis,

Hyperthyroidism, Hyperparathyroidism, Myocardial infarction, Pregnancy^[12].

Acid phosphatase is a phosphatase, an enzyme, used to free attached phosphoryl groups from other molecules during digestion. Acid phosphatase acts to liberate phosphate under acidic conditions. It is found in the liver, spleen, bone marrow, and prostate gland. Abnormally high serum levels of acid phosphatase may indicate infection, injury, or cancer of the prostate^[13].

Acid phosphatase is stored in lysosomes and functions when these fuse with endosomes, which are acidified while they function; therefore, it has an acid pH optimum^[14]. Different forms of acid phosphatase are found in different organs, and their serum levels are used to evaluate the success of the surgical treatment of prostate cancer^[14].

Tartrate-resistant acid phosphatase (TRAP) is used as a biochemical marker of osteoclast function during the process of bone resorption^[15]. TRAP is also called acid phosphatase 5, tartrate resistant (ACP5)^[16]. TRAP is synthesized as latent proenzyme and activated by proteolytic cleavage and reduction^[17,18]. It is differentiated from other mammalian acid phosphatases by its resistance to inhibition by tartrate and molecular weight. Tartrate-labile phosphatase or prostatic acid phosphate is strongly inhibited by L (+) tartrate^[19].

The mechanism of phosphate ester hydrolysis by TRAP is through a nucleophilic attack mechanism,^[20] whereby, catalysis occurs with the binding of a phosphate-substrate to the Fe²⁺ in the active site of TRAP. Under normal circumstances, TRAP is highly expressed by osteoclasts, activated macrophages, neurons, and by the porcine endometrium during pregnancy^[15,21].

Blood contains antigens on red blood cells used in ABO blood group system that stratifies individuals into four groups of blood namely A, B, AB and O. Individual who lacks either antigen A belongs to O, B antigen to A blood group, individual who lacks both antigen A and B belongs to O group and individual who has both

antigen A and B belongs to AB group^[22,23]. Blood groups are inherited from both parents. The ABO blood group type is controlled by a single gene (ABO gene) with three types of alleles inferred from classical genetics: I , I^A and I^B . The I designation stands for Isoagglutinogen or antigen^[24,25].

Haemoglobin (Hb) molecule consists of four polypeptide chains-two identical alpha (α) chains and two identical beta (β) chains controlled by genes at separate loci. The great majority of people everywhere have one type of Hb known as normal adult HbA. The HbA is the predominant form (97%) and the chains consist of 141 amino acids, the β , delta (δ), gamma (γ) chains consist of 146 amino acids respectively. The remaining 3% is from foetal Haemoglobin^[26].

Haemoglobinopathies are inherited abnormalities of Hb structure known as Hb variant. The most common form of Hb variant known as sickle cell disease is HbSS^[27,28]. The heterozygous of HbS inherited from one parent has one gene for HbS and the other for HbA, having more than HbA and 20-40% of HbS. It is a benign condition, having rare complications but in anaerobic exertion, the red blood cells turn sickle-shaped, which can cause death during sporting activity^[39].

Materials and Methods

113 apparently healthy male blood donors between 30 and 55 years who reported at University College Hospital (UCH) Ibadan, Oyo State for blood donation between 1995 and 1996 were studied. The blood from each subject was collected from 8.00 am to 10.00 am every Wednesday and Friday. 2 mls of fasting blood samples were collected from the participants into EDTA sample for the determination of ABO blood grouping and genotyping. The remaining 3 ml was dispensed into plain tube for clotting and centrifugation for 5 minutes at 4,000 rates per minute and serum stored frozen for ALP, TAP and TIAP analyses. The method of Andre- Liandet *et al*,^[30] was used for the determination of blood group; the method of Kohn *et al*,^[31] was used for

genotype determination. The method of King and Armstrong^[32] was used for the determination of the activity of alkaline phosphatase, the method of King and Armstrong was adapted by Gutman and Gutman^[32,33] was used for the determination of the activity of total acid phosphatase and the method of King and Jegatheesan^[34] was used for the determination of tartrate inhibited acid phosphatase. Student t-test was used for statistical analysis with $p < 0.05$ as a cut-off point of significance. Correlation of parameters was determined using Pearson's r correlation coefficient.

Quality control measures

Quality control sera were run along test in each batch of analysis these were compared with the reference values of the control sera.

Results

The % distributions of ABO blood groups were: 21.2, 28.3, 8.8 and 41.6 for blood groups A, B, AB and O respectively. The % distributions of genotypes were: 70.8, 22.1 and 7.1 for genotypes AA, AS and AC respectively.

The variance of analysis of variance showed that the mean serum activities of ALP and total acid phosphatase levels were significantly different amongst the blood groups studied ($f = 7.33, 0.00$; $f = 2.96, 0.04$) at $p < 0.05$ respectively. The mean serum activity of alkaline phosphatase (K.A units/100ml) significantly increases in blood group O>B>A>AB as thus: $12.72 \pm 3.24, 11.05 \pm 3.15, 9.41 \pm 3.46, 9.21 \pm 2.47$ respectively. Again, the mean serum activity of total acid phosphatase (K.A units/100ml) significantly increases in blood group O>AB>B>A as thus: $4.69 \pm 1.39, 4.64 \pm 1.02, 4.16 \pm 0.91, 3.88 \pm 1.15$ ($f = 2.96; 0.036$ ($P < 0.05$)) respectively.

The study revealed that the mean serum activities of ALP and total acid phosphatase levels were significantly higher in blood group O when compared with blood group A at $p < 0.05$ respectively. Also, the mean serum activity of ALP level was significantly higher in blood group O when compared with blood group B at $p < 0.05$. But the mean serum activity of tartrate inhibited

acid phosphatase was the same in the different blood groups studied ($f= 0.81; 0.49 (P>0.05)$) respectively.

The mean serum activities of alkaline phosphatase, total acid phosphatase and tartrate inhibited acid phosphatase were the same in the different blood genotypes studied ($f= 1.80; 0.17 (P>0.05)$) respectively.

There was a positive correlation between blood groups and alkaline phosphatase, alkaline phosphatase and total acid phosphatase, alkaline phosphatase, alkaline and tartrate inhibited acid phosphatase, total acid phosphatase and tartrate inhibited acid phosphatase and a negative correlation between genotype AC and total acid phosphatase in blood donors.

Table 1: Comparison of mean \pm SD serum activities of ALP, total and tartrate inhibited acid phosphatase in blood groups of blood donors

Blood group (n, %)	ALP (KA/100 ml)	TAP (KA/100 ml)	TIAP (KA/100 ml)
O (n=47) 42 %	12.72 \pm 3.24	4.69 \pm 1.39	1.29 \pm 0.41
B (n=32) 28 %	11.05 \pm 3.15	4.16 \pm 0.91	1.22 \pm 0.40
A (n=24) 21.2 %	9.41 \pm 3.46	3.88 \pm 1.15	1.18 \pm 0.37
AB (n=10) 8.8 %	9.21 \pm 2.47	4.64 \pm 1.02	1.38 \pm 0.40
f- (p)value	7.33 (0.000 < 0.05)	2.96 (0.000 < 0.05)	0.81 (0.000 > 0.05)
O v A	0.70 (0.00 < 0.05)	4.56 (0.02 < 0.05)	1.65 (0.28 > 0.05)
O v B	0.15 (0.03 < 0.05)	10.70 (0.65 > 0.05)	0.18 (0.48 > 0.05)
O v AB	2.58 (0.00 < 0.05)	3.13 (0.92 > 0.05)	0.14 (0.51 > 0.05)
A v B	1.27 (0.07 > 0.05)	0.15 (0.31 > 0.05)	0.69 (0.68 > 0.05)
A v AB	6.26 (0.08 > 0.05)	2.13 (0.41 > 0.05)	5.22 (0.55 > 0.05)
B v AB	0.29 (0.67 > 0.05)	0.22 (0.46 > 0.05)	0.43 (0.73 > 0.05)

Key: f- (p) value = mean \pm SD of parameter compared among blood group O, B, A, and AB (using ANOVA).

O v A= mean \pm SD of parameter compared between blood group O and A (using t-test).

O v B= mean \pm SD of parameter compared between blood group O and B (using t-test).

O v AB= mean \pm SD of parameter compared between blood group O and AB (using t-test).

A v B= mean \pm SD of parameter compared between blood group A and B (using t-test).

A v AB= mean \pm SD of parameter compared between blood group A and AB (using t-test).

B v AB= mean \pm SD of parameter compared between blood group B and AB (using t-test).

Table 2: Comparison of mean \pm SD serum activities of ALP, total and tartrate inhibited acid phosphatase in genotypes of blood donors

Blood group (n, %)	ALP (KA/100 ml)	TAP (KA/100 ml)	TIAP (KA/100 ml)
AA (n=80) 70.8 %	11.58 \pm 3.56	4.44 \pm 1.18	1.27 \pm 0.41
AS (n=25) 22.1 %	10.09 \pm 3.39	4.23 \pm 1.42	1.23 \pm 0.40
AC (n=8) 7.1 %	11.33 \pm 2.04	4.08 \pm 1.01	1.18 \pm 0.31
f- (p)value	1.80 (0.17 > 0.05)	0.51 (0.60 > 0.05)	0.21 (0.81 > 0.05)
AA v AS	0.30 (0.07 > 0.05)	0.22 (0.46 > 0.05)	0.43 (0.73 > 0.05)
AA v AC	6.26 (0.84 > 0.05)	2.13 (0.41 > 0.05)	5.22 (0.55 > 0.05)
AS v AC	4.30 (0.34 > 0.05)	1.28 (0.78 > 0.05)	2.27 (0.72 > 0.05)

Key: f- (p) value = mean \pm SD of parameter compared among blood group O, B, A, and AB (using ANOVA).

O v A= mean \pm SD of parameter compared between blood group O and A (using t-test).

O v B= mean \pm SD of parameter compared between blood group O and B (using t-test)

O v AB= mean \pm SD of parameter compared between blood group O and AB (using t-test).

Table 3: Levels of association between parameters studied in the blood donors

Parameters	Pearson r correlation coefficient	f- value	p-value
Blood group v ALP	0.29	0.03	< 0.05
ALP v TAP	0.31	0.01	< 0.05
ALP v TIAP	0.22	0.02	< 0.05
TAP v TIAP	0.24	0.01	< 0.05
Genotype AC v TAP	-0.77	0.03	< 0.05

Discussion

The study revealed that the % distributions of ABO blood groups were 21.2% , 28.3 % , 8.8 % and 41.6% for blood groups A, B, AB and O respectively. The prevalence rate of the blood groups of adult male blood donors studied in University teaching hospital, Ibadan were in this order (O>B>A>AB). Reports have it that the distribution of A, B, O and AB blood groups varies across the world according to population [35]. Again, blood groups are inherited from both parents [36,37].

The % distributions of genotypes were: 70.8 % , 22.1% and 7.1% for genotypes AA, AS and AC respectively. Nnaji *et al* [38] observed that three quarters of the premarital couples had genotype AA, one quarter had sickle cell trait and a very low percentage (0.9 %) had HbSS. Also, Taiwo *et al*, [39] reported that 73.1 % of Yoruba subjects had genotype AA and 24.5 % had sickle cell trait.

The present study showed that the serum activities of alkaline phosphatase and total acid phosphatase were significantly different in the blood groups of adult male blood donors studied. The activity of alkaline phosphatase was significantly higher in blood group O, followed by Blood group A, then by blood group B and least in blood group A. Researchers have observed that a high-fat meal is more likely to increase ALP activity than a low-fat meal ingestion at the early morning with the patient in a fasted state in blood group B or O secretors [40].

In this study, the activity of total acid phosphatase was significantly different amongst the blood groups studied in the adult male blood donors. The activity of total acid phosphatase was significantly higher in blood group O, followed by Blood group AB, then by blood group B and least in blood group A.

There was no significant difference observed in the mean serum activities of alkaline phosphatase, total acid phosphatase and tartrate inhibited acid phosphatase studied amongst the different blood genotypes of the blood donors.

The study showed a positive correlation between blood groups and alkaline phosphatase, alkaline phosphatase and total acid phosphatase, alkaline phosphatase, alkaline and tartrate inhibited acid phosphatase, total acid phosphatase and tartrate inhibited acid phosphatase and a negative correlation between genotype AC and total acid phosphatase in blood donors.

Conclusion

Blood group O and genotype AA has the highest prevalence rate of 41.6 % and 70.8% respectively. Blood group O has the highest levels of both serum activities of ALP and total acid phosphatase respectively while blood group A has the least levels of both serum activities of ALP and total acid phosphatase respectively. These findings may imply that blood groups have impact on the activities of serum levels of alkaline phosphatase and alkaline phosphatase respectively.

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