

Alkaline phosphatase (ALPP) in placental extract and in the serum of black mixed parturients from Bahia, Brazil

Maria Christina Bahiana Olympio da Silva, Eliane S. Azevêdo, Maria das Graças de Freitas Sousa[†],
Rosemary Duarte Sales de Carvalho and Maria Rita Passos Bueno

ABSTRACT

This paper analyses the levels of heat-stable placenta alkaline phosphatase (ALPP) in maternal serum and placental tissue extracts in 325 parturients of a Brazilian black mixed population. Based on a skewed to the right and departed from normal distribution of ALPP activity in placental extracts two groups were defined: group I (312 patients with values lower than 280 mg phenol/g/15') and group II (42 patients with values > 280). The cut-off point between groups is suggested by the histogram of enzyme levels. The levels of ALPP activity were correlated with a number of fetal and maternal parameters. The results of the separated analysis of each group added evidence favoring group independence. The main characteristics differing between the groups were: positive correlation of enzyme levels with birthweight, fetal maturity and phenotypes in group I and heavier babies and excess of primiparity in group II. A biological explanation of these results is not possible at this time because the function of ALPP is unknown.

INTRODUCTION

Human placental alkaline phosphatase (ALPP) is an enzyme of interest to geneticists, biochemists and obstetricians. ALPP-1, the heat-stable alkaline phosphatase, is encoded by a gene mapped to the long arm of chromosome 2 (Kam *et al.*, 1985), is synthesized in the syncytiotrophoblastic microvillous membrane (Abu-Hasan and Sutcliffe, 1984) and seems to be easily released into the maternal blood stream (Kanzaki *et al.*, 1977). Measurable levels of ALPP appear in the mother's serum by the end of the first trimester, and increase, exponentially, up to the end of gestation (Fishman *et al.*, 1972; Okpere *et al.*, 1986). In spite of a tentative

correlation between maternal serum levels and clinical or obstetric characteristics of mother and newborn, ALPP function remains unknown (Kapoor and Melita, 1973; Jones and Fox, 1976; Yamaguchi and Shimozato, 1978; Okpere *et al.*, 1986).

SUBJECTS AND METHODS

ALPP was dosed in both placental tissue and maternal serum. In addition, maternal and fetal attributes such as age, ethnicity, smoking habits, gestation age, reproductive history, sex, birthweight, fetal maturity index (Capurro *et al.*, 1978), placental weight and ALPP phenotypes were studied in relation to ALPP levels in both sera and placentas.

Laboratório de Genética Médica, Hospital Universitário Prof. Edgard Santos, Universidade Federal da Bahia, Rua Augusto Viana s/n Canel, 40110-160 Salvador, BA, Brasil. Send correspondence to M.C.B.O.S.

[†]Deceased.

The data were collected from the delivery room of the two largest public maternity hospitals (Hospital Central Roberto Santos, 85% of the sample and Maternidade Tsylla Balbino, 15%) of Salvador, Bahia, Brazil. Information on reproductive history, gestation age, maternal age and smoking habits were obtained by interviewing the parturients. Black admixture of mother and newborn were subjectively estimated following criteria previously described (Krieger *et al.*, 1965; Azevêdo, 1980b), and largely tested in the triracially mixed population of Brazil (Tavares-Neto and Azevêdo, 1977; Azevêdo, 1980a; Azevêdo *et al.*, 1981, 1982, 1986).

Placenta and newborn were weighted upon delivery. Samples of placental tissue were obtained, refrigerated and frozen for transportation to the laboratory, on the same day. The mother's venous blood was collected without anticoagulant, immediately after delivery.

Fragments of approximately 2 g of tissue were cut from the still-frozen placenta sample and used for preparing the lipid-free aqueous enzyme extract (Harris and Hopkinson, 1976). Starch gel electrophoresis was carried out at pH = 8.6 and 6.0. The ALPP phenotypes were classified by combining the results from both zymograms (Robson and Harris, 1965). Enzyme levels in the parturient sera were assayed following the method of Fishman *et al.* (1972), using a thermo-regulated Gilford II spectrophotometer. The enzyme activity in the placenta was similarly assayed by Fishman's method, using a 1:500 dilution, and the results expressed as milligrams phenol per gram per 15 minutes.

Statistical tests were performed with a micro-computer using the SPSS/PC+ system.

RESULTS

Assessment of Black ancestries in the sample - Out of 360 newborns 20% were light, 60% medium and 20% dark. Seventeen percent of the mothers were black, 20% dark mulatto, 28% medium mulatto, 25% light mulatto and 10% white.

Quantitative studies - Mean activity \pm standard deviations of ALPP in the 325 parturients were as follows: for serum, 34.97 ± 19.65 U/L; for placenta, 176.31 ± 89.20 mg phenol/g/15'. The distribution of activity in the serum did not fit a normal distribution (Kolmogorov-Smirnov Goodness of fit test, $z = 2.88$; $p < 0.0001$) nor did it in the placenta ($z = 1.97$; $p < 0.001$). In fact, the histogram of placental ALPP, shown in Figure 1 is skewed to the right suggesting bimodality with a suspected antimode at 280 mg phenol/g/15'.

Graphic evidence of bimodality is corroborated by the following findings: a) ALPP mean level to the left of the antimode (280.00 mg phenol/g/15') differs from ALPP mean levels to the right of it ($t = 22.85$; $p < 0.001$); b) up to 280.00 mg phenol/g/15' of placental ALPP there is a positive and significant correlation between enzyme levels in the placenta and in the serum ($r = 0.36$; $p < 0.001$); c) for placental levels higher than 280.00 mg phenol/g/15' there is an inversion in the correlation sign ($r = -0.12$) without reaching significance ($p > 0.46$). These findings led us to carry out analyses to explore the presumptive bimodality in the distribution of ALPP levels in placenta. Thus, two groups of parturients were considered: group I, having ALPP levels in placenta up to 280.00 mg phenol/g/15'; group II, having placental ALPP levels higher than 280.00 mg phenol/g/15'.

Group I - Three hundred and fifteen parturients had placental ALPP levels up to 280 mg phenol/g/15'. ALPP activity, in this group, followed a normal distribution ($z = 1.22$; $p > 0.05$ with a mean of 148.59 and a standard deviation equal to 56.65 mg phenol/g/15'. There was a significant and positive association between ALPP levels in the placenta and birthweight ($t = 2.55$; $p < 0.05$), electrophoretic type (greater in the heterozygotes) ($t = 2.23$; $p < 0.05$) and Capurro index of newborn maturity (Capurro *et al.*, 1978) ($t = 2.15$; $p < 0.05$). However, for placental weight the association was also significant but negative, i.e. the higher the ALPP levels the lower the placental weight ($t = 2.15$; $p < 0.05$). Finally, in group I, there was no association between ALPP and sex, smoking habits, ethnicity of mother, ethnicity of the newborn, maternal age or type of family name.

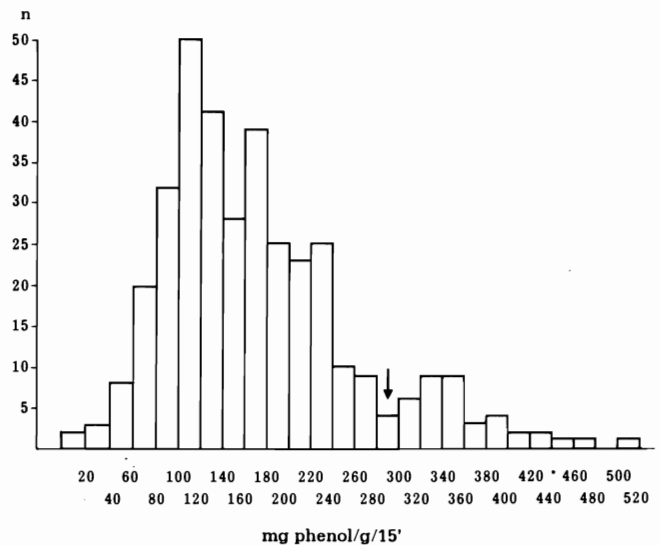


Figure 1 - The distribution of ALPP activity in placental extract of black mixed parturients from Bahia, Brazil. The arrow points to the antimode of a presumptive bimodal distribution.

Independent of ALPP levels the general effect of smoking on newborn birthweight was once again confirmed: we had newborn birthweight and smoking information from 374 mothers, 247 being non-smokers. Birthweight figures were as follows: smokers = 2.984 ± 542 g; non-smokers 3.120 ± 566 ($t_{312} = 2.3$; $p < 0.05$).

Group II - Forty-three parturients had placental ALPP levels higher than 280 mg phenol/g/15'. The distribution of ALPP activity in this group also followed a normal distribution ($z = 1.03$; $p > 0.34$) with a mean of 357.25 and a standard deviation of 52.51 mg phenol/g/15'. There was no significant association between ALPP activity and birthweight, electrophoretic type, placental weight, newborn maturity index, sex, smoking, ethnicity, maternal age or family name ($F = 1.6$; $p = 0.36$).

Comparison of maternal and newborn attributes between groups I and II - The mean number of gestations was significantly higher in group I (mean equal to 3.4 gestations) than in group II (mean of 2.6 gestations) ($t = 2.57$; $p < 0.05$), with no difference for maternal age (group I: 24.2y; group II: 23.7y) ($t = 0.53$; $p = 0.60$) or for the mean number of abortions plus stillbirths (group I: 0.39; group II: 0.25) ($t = 1.54$; $p = 0.12$). These results speak in favor of parity as the main variable accounting for the difference between the mean number of gestations between groups I and II. In fact, there was a significant excess of primiparas in group II compared to group I (Table I). Since, in group I, birthweight was found to be significantly associated with placental ALPP activity, a joint analysis of parity and birthweight was carried out within each group (Table I). Birthweight was grouped as "small", when

equal to or less than 2500 g and "normal" when greater than 2500 g. The results showed near absence of "small" birthweight in group II. However, there was no effect of parity on birthweight (Table I).

Finally, there was no difference in the length of gestation for primiparas between the two groups (37.9 ± 3.43 weeks for group I and 38.1 ± 2.24 weeks for group II; $t = 0.2$; $p = 0.25$).

DISCUSSION

Two major challenges complicate the present discussion: a) except for the paper by Fishman *et al.* (1972) we know of no other investigation measuring ALPP levels in both human placental tissue and mother's serum. Thus, the overall correlation of findings becomes rather restricted; b) in spite of a considerably large number of papers on the serum levels of ALPP in relation to pregnancy outcome (Kappor and Melita, 1973; Marshall and Parisi, 1975; Jones and Fox, 1976; Oesterling *et al.*, 1977; McLaughlin *et al.*, 1983; Ashokraj *et al.*, 1985; Brock and Barron, 1988), and on ALPP biochemistry and molecular biology (Sakiyama *et al.*, 1980; Gogolin *et al.*, 1981; Orenberg *et al.*, 1981; Kam *et al.*, 1985; Millán, 1986; Henthorn *et al.*, 1987; Takami *et al.*, 1988) the function of this enzyme remains unknown.

Non-gaussian distribution of ALPP levels are found in the placental tissue. As a rule biological measures follow a normal (Gaussian) distribution (Swinscout, 1978). The departure from normality, in large samples, strongly suggests heterogeneity, i.e. the overlapping of two or more distributions. Thus, an effort must be made, in such situations, to untangle these distributions. The histogram shown in Figure 1 does not fit a normal curve, suggesting bimodality with an antimode at 280 mg phenol/g/15'. Thus, the selection of the 280 level as the cut-off point between the two presumptive distributions, named group I and II, was guided by the data itself. Also, from a biological point of view, the results from the separated analysis of each group, corroborate group independence.

Group I - The characteristics of group I, overall, are those previously described for serum ALPP: the higher the birthweight and/or the greater the fetal maturity, the higher the enzyme levels (Massobrio *et al.*, 1973; Oesterling *et al.*, 1977; Ashokraj *et al.*, 1985; Silva *et al.*, 1985a; Okpere *et al.*, 1986). Also, the positive correlation between ALPP levels in the serum and in the placenta is obviously expected since the ALPP enzyme in the serum is originated from the placenta.

Table I - The distribution of parturients according to parity and birthweight within each placenta alkaline phosphatase (ALPP) activity group in the placental tissue.

Parity birthweight	Primipara		Multipara	
	< 2500 g	> 2500 g	< 2500 g	> 2500 g
Group I	16	69	26	201
Group II	1	17	0	24

Group I and II: ALPP activity in placental tissue up to and higher than 280 mg phenol/g/15', respectively.

Statistical analysis for adherence between variables.

For parity and groups: $\chi^2 = 4.38$; $p < 0.05$. There is an excess of primipara in group II.

For birthweight and groups: $\chi^2 = 4.26$; $p < 0.05$. There is an excess of heavier newborns in group II.

For birthweight and parity: $\chi^2 = 2.60$; $p > 0.20$. There is no effect of parity on birthweight.

The intriguing finding in group I is the negative association between placental weight and ALPP levels in the placenta itself. Considering, however, that a great number of statistical analyses was carried out on the present data, some tests may yield significant results by pure chance.

The results regarding smoking habits, in the present parturient sample, confirmed the well known association with low birthweight (Fishman *et al.*, 1972). However, smoking habits seem to have no effect on ALPP levels in either serum (Silva *et al.*, 1985a) or placenta, as reported here. In spite of birthweight being dependent on smoking, and serum ALPP being dependent on birthweight, there is no association between smoking and ALPP when birthweight is held constant (Silva *et al.*, 1985a). In the reported increased serum placental-like alkaline phosphatase activity in smokers, both male and female, the enzyme is originated from the lung (Kallioniemi *et al.*, 1987). This ALPP from the lung may have special sensitivity to smoking which ALPP from the placenta does not, or the effect of smoking in placental ALPP is too small to be measurable during pregnancy.

The high Black admixture in the population studied by us had been found to be significantly associated with ALPP gene frequencies (Silva *et al.*, 1985b; Silva *et al.*, 1991) but not with ALPP levels of activity in the serum (Silva *et al.*, 1985a). The present paper shows that Black admixture also has no effect on ALPP levels in the placental tissue. These findings are in agreement with the similarity of serum ALPP levels reported for African and Caucasian women (Silva *et al.*, 1985a).

Group II - This group comprises those parturients having the highest ALPP levels in the placenta. In this group, ALPP activity does not increase with birthweight, nor with fetal maturity, and shows no effect of electrophoretic phenotypes. However, in this group, every newborn, but one, weighed over 2500 g, and there was an excess of primiparas. Fishman *et al.* (1972) reported a prepartum decline in the serum ALPP which occurred in two out of three parturients and appeared more frequently in multipara than primipara. Assuming that the prepartum decline in the serum means a decline in the placenta, the excess of primipara, as observed here, explains the high levels of ALPP, at partum time, in group II and fits with Fishman *et al.*'s (1972) observation (the prepartum decline is less frequent in primipara). It seems that primiparas carrying heavy babies makes the appropriate combination to yield the highest ALPP levels.

In conclusion, the present data suggest that the activity of ALPP in placental tissue behaves as

belonging to two groups of parturients. The cut-off point between groups is suggested by the histogram of enzyme levels itself, and is corroborated by statistical tests and behavior of biological variables. The main characteristics showing differences between the groups, are: lower levels of enzyme, positive correlation of enzyme levels with birthweight, fetal maturity and phenotypes in group I; higher enzyme levels, heavier babies and excess of primiparity in group II.

ACKNOWLEDGMENTS

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico for support.

RESUMO

Atividade da enzima fosfatase alcalina placentária (ALPP) foi determinada no soro materno e extrato de tecido placentar de 325 parturientes de uma população brasileira com miscigenação racial negra. Baseada na distribuição bimodal da atividade da enzima no extrato de placenta foram definidos dois grupos: grupo I (312 pacientes com valores abaixo de 280 mg fenol/g/15') e grupo II (42 pacientes com valores acima de 280). O ponto de corte foi sugerido pelo histograma dos níveis enzimáticos. Os níveis da enzima foram correlacionados com diversos parâmetros materno e fetais. Os resultados das análises realizadas em cada grupo separadamente reforçam a independência dos dois grupos. As principais diferenças observadas entre os grupos foram: correlação positiva dos níveis de ALPP com peso ao nascer, maturidade fetal e fenótipo, observados apenas no grupo I, e maior peso fetal e excesso de primiparidade observado no grupo II. O desconhecimento da função da ALPP dificulta a interpretação biológica destes resultados.

REFERENCES

- Abu-Hasan, N.S. and Sutcliffe, R.G. (1984). Purification and analysis of the microvillous (M) and a form of placental alkaline phosphatase. *Placenta* 5: 71-82.
- Ashokraj, G., Sharma, S.D., Jainawalla, S.F., Sagreiya, K. and Bhatia, A. (1985). Placental phosphatases in normal and abnormal pregnancies. Part I. *Indian Pathol. Microbiol.* 28: 39-44.
- Azevêdo, E.S. (1980a). The anthropological and cultural meaning of family names in Bahia, Brazil. *Curr. Anthropol.* 21: 360-363.
- Azevêdo, E.S. (1980b). Subgroups studies of black admixture within a mixed population of Bahia, Brazil. *Ann. Hum. Genet.* 44: 55-60.
- Azevêdo, E.S., Silva, K.M.C., Silva, M.C.B.O., Lima, A.M.V.M.D., Fortuna, C.M.M. and Santos, M.G. (1981). Genetic and anthropological studies in the Island of Itaparica, Bahia, Brazil. *Hum. Hered.* 31: 353-357.

- Azevêdo, E.S., Fortuna, C.M.M., Silva, K.M.C., Sousa, M.G.F., Machado, M.A.M.L., Lima, A.M.V.M.D., Aguiar, M.E., Abé, K., Eulálio, M.C.M.N., Conceição, M.M. and Silva, M.C.B.O.** (1982). Spread and diversity of human population in Bahia, Brazil. *Hum. Biol.* 54: 329-341.
- Azevêdo, E.S., Chautard-Freire-Maia, E., Freire-Maia, N., Fortuna, C.M.M., Abé, K., Santos, M.G., Barbosa, A.A.L., Silva, M.E. and Costa, A.F.** (1986). Mating types in a mixed and multicultural population of Salvador, Brazil. *Rev. Brasil. Genet.* 9: 487-496.
- Brock, D.J.H. and Barron, L.** (1988). Measurement of placental alkaline phosphatase in maternal plasma as an indicator of subsequent low birthweight outcome. *Br. Obstet. Gynaecol.* 95: 79-83.
- Capurro, H., Konichezky, S., Fonseca, D. and Caldeyro-Barcia, R.** (1978). A simplified method for diagnosis of gestational age in the newborn infant. *Pediatr.* July: 120-122.
- Fishman, W.H., Bardawil, W.A., Habib, H.G., Antiss, C.L. and Green, S.** (1972). The placental isoenzyme of alkaline phosphatase in sera of normal pregnancy. *Clin. Pathol.* 57: 65-74.
- Gogolin, K.J., Slaughter, C.A. and Harris, H.** (1981). Electrophoresis of enzyme-mono-clonal antibody complexes: studies of human placental alkaline phosphatases polymorphism. *Proc. Natl. Acad. Sci.* 78: 5061-5065.
- Harris, H. and Hopkinson, D.A.** (1976). *Handbook of Enzyme Electrophoresis in Human Genetics*. Amsterdam, North Holland, Chapter 4 (3.1.3.1), pp. 1-4.
- Henthorn, P.S., Raducha, M., Edwards, Y.H., Weiss, M.J., Slaughter, C., Lafferty, M.A. and Harris, H.** (1987). Nucleotide and amino acid sequence of human intestinal alkaline phosphatase: close homology to placental alkaline phosphatase. *Proc. Natl. Acad. Sci.* 84: 1234-1238.
- Jones, C.J.P. and Fox, H.** (1976). An ultrahistochemical study of the distribution of acid and alkaline phosphatases in placentae from normal and complicated pregnancies. *J. Pathol.* 118: 143-151.
- Kallioniemi, O.-P., Nieminen, M.M., Lehtinen, J., Veneshoski, T. and Koivula, T.** (1987). Increased serum placental-like alkaline phosphatase activity in smokers originates from the lungs. *Eur. J. Respir. Dis.* 71: 170-176.
- Kam, W., Clauser, E., Kim, Y.S., Kan, Y.W. and Rutter, W.J.** (1985). Cloning sequencing and chromosomal localization of human term placental alkaline phosphatase cDNA. *Proc. Natl. Acad. Sci.* 82: 8715-8719.
- Kanzaki, Y., Obara, H. and Yoshioka, T.** (1977). The change of alkaline phosphatase binding conditions with trophoblast membrane at different stages of human placenta. *Acta Obstet. and Gynecol. Scand.* 56: 459-461.
- Kapoor, U. and Melita, H.C.** (1973). Serum heat stable alkaline phosphatase (HSAP) in the hypertensive disorders of pregnancy. *Ind. J. Med. Res.* 61: 749-760.
- Krieger, H., Morton, N.E., Mi, M.P., Azevêdo, E.S., Freire-Maia, A. and Yasuda, N.** (1965). Racial admixture in northeastern Brazil. *Ann. Hum. Genet.* 29: 113-125.
- Marshall, B.R. and Parisi, F.** (1975). Maternal serum heat-stable alkaline phosphatase in normal and high-risk pregnancies. *Obstet. Gynecol.* 45: 136-141.
- Massobrio, M., Svanosio, M. and Calleri, W.** (1973). Enzimi sierici placentari in gravidanza: le fosfatasi alcaline termoresistenti nella gravidanza normale e patologica. *Ann. Ost. Gin. Med. Perin.* 94: 11-12.
- McLaughlin, P.J., Gee, H. and Johnson, P.M.** (1983). Placental-type alkaline phosphatase in pregnancy and malignancy plasma: specific estimation using a monoclonal antibody in a solid phase enzyme immunoassay. *Clin. Chim. Acta* 130: 199-209.
- Millán, J.L.** (1986). Molecular cloning and sequence analysis of human placental alkaline phosphatase. *J. Biol. Chem.* 261: 3112-3115.
- Oesterling, M.J., Cox, S.E. and Carrington, E.R.** (1977). Placental phosphatase of maternal serum: relationship to pregravid weight, prenatal weight gain, and infant birthweight in normal human pregnancies. *Am. J. Clin. Nutr.* 30: 182-190.
- Okpere, E., Okorodudu, A., Gbinigie, O. and Lackman, C.** (1986). Is maternal serum heat stable alkaline phosphatase an index of fetal maturity? *Med. Hypothesis* 21: 225-230.
- Orenberg, J.B., Schaffert, J.M. and Sussman, H.H.** (1981). Human placental alkaline phosphatase: effects on conformation by ligands which alter catalytic activity. *Arch. Biochem. Biophys.* 211: 327-337.
- Robson, E.B. and Harris, H.** (1965). Genetics of alkaline phosphatase polymorphism of the human placenta. *Nature* 207: 1257-1259.
- Sakiyama, T., Mano, R. and Chou, J.Y.** (1980). Synthesis of first trimester placental alkaline phosphatase in cultured human term placental cells. *J. Biol. Chem.* 255: 9399-9403.
- Silva, M.C.B.O., Passos-Bueno, M.R., Sousa, M.G.F., Carvalho, R.D.S. and Azevêdo, E.S.** (1985a). Níveis de fosfatase alcalina placentária no soro de gestantes: estudo em Salvador, Bahia. *Rev. Paul. Med.* 103: 280-283.
- Silva, M.C.B.O., Lima, A.M.V.M.D., Santiago, C.A.S. and Azevêdo, E.S.** (1985b). Alkaline phosphatase polymorphism of the human placenta: a study in Brazilians. *Rev. Brasil. Genet.* 8: 79-87.
- Silva, M.C.B.O., Sousa, M.G.F., Carvalho, R.D.S., Passos-Bueno, M.R. and Azevêdo, E.S.** (1991). Null allele in a human polymorphism restricted to the placenta: call for a search. *Hum. Biol.* 63: 167-178.
- Swinscout, T.D.V.** (1978). *Statistics at Square One*. Dawson and Goodall Ltd., England, 7.
- Takami, N., Ogata, S., Oda, K., Misumi, Y. and Ikehara, Y.** (1988). Biosynthesis of placental alkaline phosphatase and its post-translational modification by glycopospholipid for membrane-anchoring. *J. Biol. Chem.* 263: 3016-3021.
- Tavares-Neto, J. and Azevêdo, E.S.** (1977). Racial origin and historical aspects of family names in Bahia, Brazil. *Hum. Biol.* 49: 287-299.
- Yamaguchi, R. and Shimozato, N.** (1978). Diagnosis of placental function by prediction curves for heat-stable alkaline phosphatase (HSAP). *Tohoku J. Exp. Med.* 124: 73-82.

(Received January 10, 1994)