RESEARCH NOTE

# Alkaline Phosphatase Isoenzymes in Plasma of Chagasic and Healthy Pregnant Women

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Placental alkaline phosphatase (PLALP) (EC 3.1.3.1) is a dimeric sialoprotein that is expressed in the membrane of the syncytiotrophoblast of the human placenta (WH Fishman et al. 1971 *Am J Clin Pathol 57*: 65-74). During pregnancy, PLALP is synthesized after the 12th week and is shed into the maternal circulation. Its serum concentration is correlated with the placental growth, increases as pregnancy proceeds and reaches its maximum at the last three months. Then, PLALP activity in plasma might be used as an indicator of placental function. It has been demonstrated that, in the toxemia of pregnancy, the multiple infarctions of the placental tissue produce high plasmatic levels of PLALP. On the other hand, the delay of the uterine growth is associated with low levels of PLALP (P Holmgren et al. 1979 *Obst Gynecol 54*: 631-634). The parasitic diseases are also a source of placental lesions, for example, high levels of plasmatic PLALP have been observed in patient with bilharziasis (INC Ahmed et al. 1994 *Egypt J Pharmacol Sciences 35*: 1-6).

Although Chagas disease has a low frequency of mother-fetus transmission, it is known that the *Trypanosoma cruzi* can invade the placental tissue. The lesions can be mild (scarce inflammatory infiltrate with or without presence of amastigotes), or serious (destruction of placental tissue). These lesions can be located in decidua, amniochorionic plate, chorionic villii and umbilical cord (M Thin et al. 1978 *Am J Trop Med Hyg 27*: 1108-1115, P Moya 1992 Updates in the disease of Chagas, p. 229-236, Satellite Symposium ).

We determine the activity of total-ALP, soluble-ALP, PLALP, liver-ALP and high molecular weight ALP (high Mr-ALP), in plasma of healthy and chagasic pregnant women during the last three months of gestation, in order to know if the plasmatic levels of alkaline phosphatase (ALP) and their isoenzymes are modified in Chagas disease.

Plasma samples were obtained from 44 healthy pregnant women (control group) and 64 pregnant women with chronic Chagas disease, without manifestations of other pathologies (chagasic group). Samples were obtained during the last three months of gestation. A 10 ml sample of citrated blood was centrifuged immediately after extraction at 4,000 xg for 10 min. The plasma was centrifuged again at 13,000 xg for 15 min to separate the remaining cells and debris. A 4 ml aliquot was then ultracentrifuged at 100,000 x g for 2 hr. The supernatant was decanted and pellets were suspended in 200 µl of 0.04M Tris-HCl buffer pH 7.8. ALP activity in supernatant and pellet suspension were measured at 37ºC by the method of Bessey modified to have a final assay volume of 2 ml (OA Bessey et al. 1946 *Biochem Biophys Res Comm 164:* 321-329). The high Mr-ALP was determined in the pellet suspension (J Moreno et al. 1992 *Clin Chem 38*: 319-320). Soluble-ALP was determined in the supernatant and an aliquot of the supernatant was heat-inactivated at 65ºC during 5 min to determine placental-ALP, the enzyme assay was also carried out with an aliquot of the supernatant in the presence of 0.1 mM levamisole to determine the percentage of inhibition of liver-ALP (WH Fishman 1974 *Am J Med 56*: 617-650). The enzymatic activity was expressed in U/l. One unit is the amount of enzyme catalyzing the conversion of a µmol of substrate per min in the assay conditions.

Statistical analysis was performed by ANOVA test for media comparisons and it was calculated correlation coefficient (r) to correlate the studied variables.

The Table shows mean levels of ALP isoenzymes activity in plasma. There is a significantly smaller ($p<0.05$) placental-ALP activity in plasma from the chagasic group. On the contrary, the activities of total-ALP, soluble-ALP, liver-ALP and

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TABLE

Plasmatic alkaline phosphatase activity in healthy and chagasic pregnant women

| Alkaline phosphatase isoenzymes | Healthy pregnant women (44) | Chagasic pregnant women (64) |
|---|---|---|
| Total-ALP | 262±10 | 270±12 |
| Soluble-ALP | 254±9.6 | 237±10 |
| High Mr-ALP | 4.76±0.4 | 4.2±0.35 |
| Liver-ALP | 142.6±9.4 | 155±7.7 |
| Placental-ALP | 110.9±7.7 | 97.1±7[a] |

*a:* $p<0.05$; values of enzimatic activity are mean U/l±SE; number of cases between parentheses.

high Mr-ALP do not show significant differences among the studied groups. Also, correlation coefficient among isoenzymes activity values has been calculated. There is a positive correlation between total-ALP and liver-ALP (r=0.81) and between soluble-ALP and liver-ALP (r=0.82) in plasma from healthy women. Probably, it would be attributable to gestational cholestasis. On the contrary, these isoenzymes are not correlated in plasma from chagasic women.

It has been reported that human term placenta tissue cultures infected with *T. cruzi* show a decrease in placental-ALP activity (RE Fretes et al. 1990 *Rev Inst Med Trop São Paulo 32*: 403-408). The diminished PLALP production of the infected placental tissue could be reflected in a reduction of enzimatic levels in plasma. Then, we suppose that this finding could be related with the decreased plasmatic PLALP activity shown by chagasic pregnant women.

In conclusion, our data indicate that chronic Chagas disease is associated with a decreased activity of plasmatic placental alkaline phosphatase and an altered behavior of the remaining studied isoenzymes. Further investigations are needed to elucidate if these findings are attributable to placental injuries in chagasic pregnant women.

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