I. INTRODUCTION

A. Statement of problem

Alkaline phosphatase (Orthophosphoric-Monoester Phosphohydrolase, ALP, E.C.3.1.3.1) is a membrane - bound enzyme that consists of a group of true isoenzymes, all are glycoproteins and encoded for by separate genetic loci. Other isoenzymes arise by post-translational modification (Crofton, 1992; Van Hoof and De Broe, 1994).

The isoenzymes of alkaline phosphatase exhibit optimal activity at alkaline pH (8.8-10.3) *in vitro*. Optimum pH, however, varies with the substrate, buffer, and nature of each isoenzyme. By the study of hypophosphatasemia, an inborn error of metabolism which lacks of all forms of tissue non specific ALP activity, three phosphocompounds : phosphoethanolamine (PEA), inorganic pyrophosphate (PPi), and pyridoxal 5'-phosphate (PLP) have been proposed as the natural substrates (Whyte *et al.*, 1995).

The enzyme is present in most body tissues and is generally localized in the membranes of cells. It presents at particularly high levels in the liver, bone, intestinal epithelium, kidney and placenta. The significant amount of individual isoenzyme can be identified in patient serum with various diseases. In addition to the liver, bone, intestinal and placental isoenzymes, other ALP isoenzymes have been identified. These include fast liver, Regan, Nagao and renal isoenzymes. However, the predominant forms present in normal serum can be characterized as the liver and bone ALP (Kachmar and Donal, 1976).

The form present in normal adult serum originates mainly in the liver or the bone (Schiele *et al*, 1983). A small amount of biliary tract component may also present (Van Hoof *et al*, 1990). Liver ALP isoenzyme is expressed at the sinusoidal and bile canalicular membrane. Bone ALP activity is present on the plasma membrane of the osteoblasts. Increasing of liver and bone isoenzyme activities in serum have been used as markers for altered conditions of these organs (Mc Comb *et al.*, 1979).

Interpretation of serum alkaline phosphatase measurement is complicated by the fact that the enzyme activity can increase in the absence of diseases. Serum alkaline phosphatase activity is risen during puberty because of rapid bone growth and during the third trimester of

pregnancy because of the increase in maternal syncytiotrophoblast microvilli activity which help to transport nutrients to the fetus (Abu-Hasen and Sutcliffe, 1985).

The most common disorders, causing elevation of alkaline phosphatase, are liver and bone diseases. Liver disease include hepato-cellular and cholestatic disease, such as acute and chronic hepatitis, cirrhosis, carcinoma of the liver, metastatic carcinoma of the liver, and acute or chronic biliary obstruction, are all associated with increase liver ALP activity (Rhone and Mizuno, 1973) and, frequently, with high-molecular-mass (high-Mr) ALP (Jennings *et al.*, 1970). High-Mr ALP is also known as fast-liver ALP (Moss, 1982), koinozyme (De Broe *et al.*, 1975), and bile or biliary ALP (Price and Sammon, 1974). The liver disease resulting in parenchymal cell necrosis does not elevate serum ALP unless that disease is associated with damage to the canaliculi or biliary stasis.

The value of characterizing ALP isoenzymes in serum as a diagnostic aid is becoming better established as improved methods to better differentiate the various ALP forms become available. Specific ALP isoenzymes measurement, as compared with total ALP measurements, are at least twofold more sensitive for assessment of both bone and liver diseases. The measurement of bone and liver isoenzymes so far has proved clinically useful for diagnosing and monitoring certain diseases of these organs.

ALP isoenzymes from various tissue sources display considerable heterogeneity with respect to net molecular charge, differential sensitivity to heat, different in response to the presence of selected inhibitors or urea, and differences in immunochemical characteristics. These characteristics have provided the basis for the development of a variety of techniques for the separation and identification of ALP isoenzymes (Kaplan and Rogers, 1969 ; Price, 1993 ; Hendrix *et al.*, 1990)

The ideal separating technique should be easy to handle and allow a sensitive and reproducible quantification of the different isoenzymes. One of its most important features should be a good and unambiguous separation of bone and liver ALP. It is more difficult to distinguish bone from liver ALP activity, because both isoenzymes are the products of a single gene and differ only with respect to post-translational glycosylation (De Broe and Moss, 1992). Although these glycosyl differences are sufficient for qualitatively distinguishing bone from liver ALP in serum by electrophoretic mobility (Lum *et al.*, 1983), heat inactivation (Farley *et*

al., 1981, Valenzuela *et al.*, 1987), liquid chromatography (Schoenau *et al.*, 1986), immunoaffinity (Lawson *et al.*, 1985), wheat germ agglutinin (WGA) precipitation (Behr and Barnert, 1986 ; Rosalki and Foo, 1984), none of these techniques completely resolves the two isoenzyme activities. Thus, a combination of more than one technique may be needed to ensure reliable discrimination.

Although electrophoretic technique is convenient for qualitative examination of ALP isoenzymes, bone and liver ALP are generally insufficiently resolved by conventional electrophoresis to permit their quantification by densitometric scanning. Thus, studying the carbohydrate side chains heterogeneities of ALP isoenzymes will lead to a better understanding of human ALP. The knowledge may become applicable as improved electrophoretic method to better differentiate the ALP isoenzymes. The distinct electrophoretic patterns of ALP isoenzymes will lead to express identities of ALP isoenzymes. Finally, it may be important for diagnosing and monitoring patients with liver disease to evaluate the patient's response to therapy.

Modifications of glycosylation patterns in sera of patients with various forms of liver disease as compare to the normal control are also reported in this study. Results obtained offer some valuable information for the analytical purposes such as the separation of serum ALP isoenzymes by WGA lectin precipitation or by some of electrophoretic techniques.

B. Objectives of this study

1. To characterize the carbohydrate side chains of ALP isoenzymes in sera of patients with differential diagnosis of liver disease and compare with those in the normal control.

2. To examine effects of alteration of carbohydrate side chains of ALP isoenzyme molecules on the quantification methods used for separating ALP isoenzymes in serum samples.

3. To search for factors affecting the separation of modified ALP isoenzyme molecules by cellulose acetate (CA) electrophoretic technique.

4. To identify patterns and identities of ALP isoenzymes in serum of patients with various conditions of liver disease by cellulose acetate electrophoretic method.