SHORT COMMUNICATION

α_1-Microglobulin (HC protein) in human hepatocellular carcinoma

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α_1-Microglobulin (α_1-m) is a highly glycosylated polypeptide of 182 amino acids associated with a brown chromophore (Ekström et al., 1975; Lopez Otin et al., 1984). The molecule is heterogeneous in charge and was described as HC protein (Tejler & Grubb, 1976) or α_1-microglycoprotein (Seon & Pressmann, 1978). It occurs in biological fluids as a 31 kDa monomer and as a 90 kDa component covalently associated to one α chain of monomeric immunoglobulin A (IgA) (Grubb et al., 1983; Vincent et al., 1985). The α_1-m gene is associated with a gene coding for the HI-30 domain of inter-α-trypsin inhibitor, one of the acute phase proteins (Kaumeyer et al., 1986). The human 31 kDa α_1-m was shown to be synthesised by fetal liver explants (Tejler et al., 1978) and we recently reported that the molecule was produced in vitro by several human hepatoma cell lines but not by lymphoma or plasmocytoma cell lines (Vincent et al., 1987). For these reasons we have determined serum levels of α_1-m in a large series of patients suffering from primary hepatocellular carcinoma in order to evaluate the possible use of this molecule as an additional tumour marker. The data show that 31 kDa α_1-m levels are usually not increased in this disease, whereas the 90 kDa component levels are elevated in some cases.

Our study involved a hundred South African Blacks with histologically proven hepatocellular carcinoma. They included 11 women and 89 men whose ages ranged from 20 to 73 years (median 39). Seventy-seven healthy South African Black subjects served as controls. All serum samples were sent frozen in dry ice from South Africa and were stored at −40°C until use.

The differential quantitation of 31 kDa and IgA-associated 90 kDa α_1-m in unfractionated sera was performed by enzyme linked immunosorbent assay (ELISA) as previously described (Vincent & Revillard, 1985). Briefly, the monoclonal antibody 832 ALB which binds both forms of α_1-m with high affinity (1.4×10^{10} L/M) was used as solid phase. The binding of serum α_1-m was assessed by addition of polyconal immunopurified biotinylated antibodies specific for α_1-m or for the heavy chain of IgA, then by incubation with peroxidase-streptavidin, addition of the substrate and recording of the absorbance at 280 nm. Interassay coefficients of variation did not exceed 16%. Normal levels of 31 kDa in 25 French adults were 9.54 ± 2.73 mg l^{-1} (mean ± s.d.). As regards 90 kDa α_1-m, normal levels in the same control group were 101.9 ± 36.6 kU l^{-1} (Vincent & Revillard, 1987).

Albumin concentrations were measured by radial immunodiffusion, β_2-microglobulin and IgA by competitive ELISA (Vincent & Revillard, 1986; Vincent et al., 1985). α-Fetoprotein and HBs antigen were determined by solid phase radio-immunoassay, γ-glutamyl transpeptidase and alkaline phosphatase activities by routine biochemical methods. The upper limit values for serum 31 kDa and 90 kDa α_1-m were defined by the 90th percentile of the control group (Soleberg, 1985). Comparisons between groups were based on the Mann–Whitney U test.

Individual serum levels of 31 kDa and 90 kDa α_1-m are presented in Figure 1. In healthy subjects from South Africa serum levels of 31 kDa α_1-m were similar to that of European controls whereas those of the 90 kDa form appeared to be slightly lower. Twenty-two patients had 31 kDa α_1-m levels above the upper limit of control values but the distribution in patients did not differ from that of controls. As regards 90 kDa α_1-m levels, 35 patients had elevated
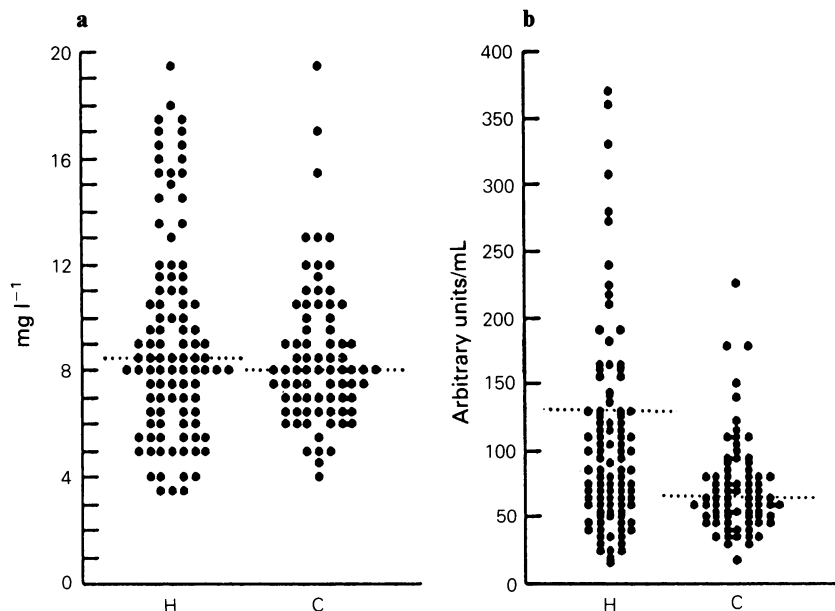
Figure 1 Serum levels of 31 kDa α_1-m (**a**) and IgA-associated 90 kDa μ_1-m (**b**) in 100 patients with hepatocellular carcinoma (H) and 77 controls (C). The dashed lines indicate the median of the distribution in each group.

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values and the overall distribution differed from that of healthy controls ($P<0.002$, Mann–Whitney U test). Serum IgA concentrations were elevated in four of the patients and were not correlated with 90 kDa α_1-m levels. No difference in α_1-m levels was found between the 54 HBs antigen-positive and HBs antigen-negative patients. The concentrations of serum albumin were not correlated with those of α_1-m. Finally, α-fetoprotein was increased in 91% of patients' sera, β_2-microglobulin in 59%, alkaline phosphatase activity in 78% and γ-glutamyltranspeptidase in 94% but none of these biological parameters was correlated with α_1-m levels.

Serum levels of 31 kDa α_1-m were reported to rise in renal insufficiency (Itoh *et al.*, 1983; Yu *et al.*, 1983) and in a few patients with malignant tumours, including the only two cases of hepatoma that were investigated (Tagaki *et al.*,

1980). Immunohistochemical studies of normal human tissues showed that hepatocytes were intensely stained by anti-α_1-m antibodies along with macrophages and some lymphocytes (Bouic *et al.*, 1984). Studies of human fetal livers revealed that fetal hepatocytes but not haematopoietic stem cells were strongly labelled by anti-α_1-m antibodies (Bouic *et al.*, unpublished data). Furthermore, the protein was shown to be synthesised by human fetal liver explants (Tejler *et al.*, 1978) and produced in high amounts by some human hepatocarcinoma cell lines (Vincent *et al.*, 1987). Therefore α_1-m was considered as a potential biologic marker of hepatocellular carcinoma. However, the present study demonstrates that this prediction was not confirmed, despite the significant elevation of 90 kDa α_1-m in the patients group as compared with controls.

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