EVALUATION OF SERUM MUCOPROTEINS IN PATIENTS SUFFERING FROM LIVER CANCERS.

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ABSTRACT

Mucoproteins are normal and essential constituents of different types of cells, tissues, and organs in our body. Interestingly, an increase in the serum mucoprotein has been described in several malignant disorders in various studies. To study this in the cases of hepatocellular carcinoma thirty five patients suffering from histologically or cytologically confirmed hepatocellular carcinoma were tested for serum mucoprotein in the Department of Biochemistry and the Department of Pathology of North Bengal Medical College for a period of one and a half years against matched healthy controls. The serum mucoprotein was found to be increased significantly in the cases suffering from hepatocellular carcinoma ($p < 0.001$). So, the present study suggests a strong correlation between the mucoprotein level and carcinomatous changes in the hepatocellular tissues.

KEY WORDS

Hepatocellular carcinoma, serum mucoprotein.

INTRODUCTION

Mucoproteins are normal constituents of cell membrane, connective tissue, ground substances, and basement membrane. These are glycoproteins in nature. Increase in serum glycoproteins in various types of malignancies has been described in various works for a substantial period. It has been found that MUC1 mucin forms an important tumour marker for the bladder cancer (1). Evaluation of sialic acid forms and seromucoid fraction showed it to be an effective marker for the patients of head and neck cancer undergoing radiotherapy (2). The reactivity of the antibodies specific against the colon carcinoma cell lines has also been found to be increased by some workers (3). Similarly, it has been noted that breast mucins are expressed by malignant epithelial cells (4). Keeping these facts in mind a study was undertaken to observe any increase in serum mucoproteins in the patients suffering from hepatocellular carcinoma in the departments of Biochemistry and Pathology of North Bengal Medical College. The study was performed through a period of one and a half years during which

thirty-five patients suffering from hepatocellular carcinoma diagnosed either by liver biopsy or by fine needle aspiration cytology (FNAC) under the guidance of ultrasonography were evaluated. The data obtained were compared against age and sex matched healthy controls. The difference was found to be significant, which may suggest that the increase in serum mucoproteins in the cases of hepatocellular carcinoma is due to some broader biochemical and pathological basis responsible for the disease.

Selection of patients

The study was carried out in thirty-five patients with untreated hepatocellular carcinoma. The cases were diagnosed histologically by core biopsy of the lesion or cytologically by FNAC under the guidance of ultrasonography. The patients suffering from any other diseases were excluded from the study. The controls were selected from the age and sex matched healthy people having the mean age (in years) of 53 ± 6.1 (mean + S.D). The mean age (in years) of the cases was 51.35 ± 10.67 (mean + S.D). Thirty patients were male and five patients were female.

METHOD

Serum mucoproteins in both controls and cases were measured by a method provided by Winzler

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(5) in which the normal average value was given as 80mg/dl. In this method after removing the heat coagulable proteins by means of perchloric acid, mucoproteins, which remain in solution, are precipitated by phosphotungstic acid. The amount of mucoprotein thus precipitated has been estimated by determining its carbohydrate content by its tyrosine content using the Folin-Ciocalteu reagent (Winzler *et al.*, 1948; Weimer and Moshin, 1952; Winzler, 1955). 0.5 ml of serum was added to 4.5 ml of 0.85 percent saline and then 2.5 ml. of 1.8M perchloric acid was added to it, dropwise, with shaking. After ten minutes the mixture was filtered through a Whatman No. 50 filter paper and 1 ml. of 5 percent phosphotungstic acid was added to 5 ml. of filtrate. After mixing well and waiting for 10 minutes it was centrifuged at 2000 rpm for 10 minutes, decanted, and the precipitate was washed with 0.6M perchloric acid. The supernatant was decanted and the precipitate was dissolved in 20 percent sodium carbonate solution. Into this 3.5 ml of distilled water and 0.5 ml. of the Folin-Ciocalteu reagent was added. The mixture was placed in a water bath at 37° C for fifteen minutes and read at 680 millimicrons or using a red filter. At the same time a standard was put up by taking 0.5 ml of tyrosine solution in 1 ml of 20 percent sodium carbonate, 3.0 ml of water and 0.5 ml of the Folin-Ciocalteu's phenol reagent. The reading for both was taken against a blank from 3.5 ml of water, 1.0 ml of carbonate and 0.5 ml of the phenol reagent. The calculation was done as follows:

Calculation: Since 100 mg mucoprotein contain 4.2 mg of tyrosine, the factor for converting mg tyrosine to mg mucoprotein is 23.8; so

mg. mucoprotein per 100 ml of serum

= Reading of unknown/ Reading of standard x 100/0.33 x 0.05 x 23.8

= Reading of unknown/Reading of standard x 357.

RESULTS

Serum mucoprotein levels (in mg/dl) in the controls was (mean + S.D) 89 + 7.7. In patients suffering from hepatocellular carcinoma it was (Mean + S.D.) 302.3+ 40.97 (Table 1). The difference between the two is found to be significant as apparent from the value of significance (p value) of the difference. The difference between the cases and the reference value is also significant, while the difference between the controls and the reference value is found to be

insignificant. These differences are also evident from the graphical representation provided in Fig.1.

DISCUSSION

Serum mucoprotein is a type of glycoprotein that forms an important constituent of cell membrane and cell architecture. This is also an important constituent of connective tissues and basement membranes. In liver the mucoproteins play an important role in forming ground substances where several cells e.g. perisinusoidal cells of Ito, have been subscribed the function of secreting significant amount of proteoglycans(6). Normally, they are found in blood in low concentrations(7). But in malignancies, the tissue proliferation, depolymerisation of ground substances of connective tissue adjacent to tumour, increased local production and direct release into circulation tend to increase the serum mucoproteins(8). Moreover, increased proliferation of cells, and the cell membrane damage subsequent to oxidative stresses are also responsible for the increase of serum mucoproteins in patients suffering from cancers (9, 10). Thus, being important constituents of cell membrane, cellular architecture, and ground substances, the mucoproteins are frequently increased in serum in various cancers. Several observations and ideas have been put forward in an effort to analyze and explain the cause of this increase. It has been postulated that growth and differentiation of the cancer cells have been observed to be involved in altered expression of mucin mRNA synthesis, and so a positive correlation has been described between mucin synthesis, tumorigenicity, and a poor prognosis in some cancers e.g. colorectal cancers (11). Falletti *et al* have described an increase in serum alpha 1 –acid glycoprotein despite the decline of liver synthetic function in cirrhotics with hepatocellular carcinoma(12). In the present study the increase in the serum mucoprotein in the patients suffering from hepatocellular carcinoma also supports the view that as in various other cancers, the increase of serum mucoprotein in hepatocellular carcinoma strengthens the opinion that mucoprotein metabolism is affected grossly in many malignant disorders. This indicates an inherent change in the turnover of the mucoprotein in the hepatocellular tissues which have undergone malignant changes. Moreover, as serum mucoproteins measurement is an easy and cheap laboratory method, along with other clinical and laboratory assays it may provide an adjunct for an evaluation of the suspected cases of liver cancers in future too.

Table 1

Data showing the difference between the serum mucoprotein levels of the cases, controls, and the normal reference values.

Serum mucoprotein(mg/dl) Cases (n=35) Mean $\pm$ S.D	Serum mucoprotein(mg/dl) Controls (n=35) Mean $\pm$ S.D	Serm mucoprotein(mg/dl) Reference value
302.3 $\pm$ 40.97	89.4 $\pm$ 7.7	80

- i) The difference between the serum mucoprotein levels of the cases and controls is significant ($p < 0.001$).
- ii) The difference between the serum mucoprotein levels of the cases and the normal reference value is also significant ($p < 0.001$).
- iii) The difference between the serum mucoprotein levels of the controls and the reference value is insignificant ($p > 0.05$).

The scatter diagram showing the comparison between the serum mucoprotein levels of the cases, controls, and the normal reference values.

Fig.2

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