RESEARCH COMMUNICATION

Hemoglobinopathies, Iron Overload and Chronic Viral Hepatitis in Patients with Hepatocellular Carcinoma in Myanmar

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Abstract

To assess the relevance of altered iron metabolism, hemoglobin electrophoresis by isoelectric focusing was performed for 16 cases of hepatocellular carcinoma (HCC) from the Liver Unit, Yangon General Hospital. Serum iron, total iron binding capacity, serum ferritin and free iron were also determined. Hemoglobin A (HbA) was found in all of the cases. Four cases had one extra band, hemoglobin A_2 in three cases, and hemoglobin F in one case. No abnormal hemoglobin was detected. Anemias due to chronic disorders or associated with liver disease were observed in all of the cases. Iron overload was documented in 83% and free iron was detected in all cases. Viral markers like HBsAg, AntiHBc, and AntiHCV singly or in combination were found in all cases. HCC occurring at young age was seen in this study; the youngest patient was 23 years old and four cases (25%) were under 40 years, with a mean age of 49 years. The findings support the hypothesis that free iron and iron overload is a potential promoter of the development of HCC, especially if underlying chronic viral infection is present.

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Key words: hepatocellular carcinoma, isoelectric focusing, hemoglobin electrophoresis, viral markers, iron overload, Myanmar

Introduction

Detection of abnormal hemoglobins and/or variants, which induce iron overload, is one of the essential component of our collaborative project between Monbusho Special Cancer Research Unit, Japan and the Pathology Research Division, Department of Medical Research, Myanmar. The main study area of this collaboration is the significance of free iron-induced free radicals for hepatocarcinogenesis.

In comparison with other common electrophoretic techniques, in particular cellulose acetate membrane electrophoresis, thin layer isoelectric focusing (IEF) is preferred for separation of hemoglobin variants and thus an ideal method for screening (Brozovic and Henthorn, 1995). IEF provides most information from a single test. Although many hemoglobin variants are of genetic interest only, an increasing number have been associated with diseases. IEF was performed for abnormal hemoglobins and/or variants in all of the cases entered into this study.

Here we report hemoglobin phenotypes and their relation to serum iron status distributed among cases with hepatocellular carcinoma (HCC).

Materials and Methods

A total of 16 cases of HCC from the Liver Unit, Yangon General Hospital, were studied. Four cases were females and 12 cases were males. The age distribution was from a 23-year-old male to a 70-year-old female. Four cases were under 40 years, 6 were between 40 -50 years, 2 were between 51 - 60 years and 4 were between 60 - 70 years of age. Diagnosis of HCC was based on clinical findings and ultrasonography, confirmed by histopathological examination.

Five ml aliquots of intravenous blood were collected in new plastic tubes. Serum was separated by centrifugation and cell pellets were used for hemoglobin electrophoresis by isoelectric focusing as described in the manual of the commercial gel kit (Joko Co., Tokyo, Japan). Serum iron, total iron binding capacity (TIBC), and serum ferritin were determined with a Hitachi 7150 autoanalyser. Free iron was

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determined for 15 cases by Gutteridge's method (Gutteridge and Halliwell, 1987). Hepatitis B core antibody (HBcAb), hepatitis B surface antigen (HBsAg) and Hepatitis C viral antibodies (Anti-HCV) were also studied (Serodia-HCV, Fujirebio Inc. Tokyo, Japan).

Results

Hemoglobin phenotypes: HbA band were detected in all cases, along with bands for HbA_2 in 3 cases and HbF in one case. No abnormal hemoglobin was observed.

Iron metabolism: Serum iron level ranged from 25.6 to 170.2 μg/100 ml. Eleven cases had reduced serum iron level (< 70 mg/100 ml) and remaining five cases had values within the normal range (70-180 μg/100 ml). Serum TIBC was within the normal range (250 - 400 μg/100 ml) in 10 cases. It was reduced in six cases. There was no case with high serum TIBC. Serum ferritin was within the normal range (20-200 ng/ml) in three cases and high in 13 cases. Serum free iron (not detectable in normal individuals) was detected in all of the cases except one. The levels ranged from 0.03 to 0.105 ng/ml.

Viral markers: Viral markers were studied in 14 cases. HbcAb (which indicates a past history of hepatitis B virus infection only) was detected in all cases. HBsAg (which indicates an active hepatitis B disease) and Anti-HCV (which mostly indicates active hepatitis C disease) were positive in eight and seven cases, respectively. All three markers were positive in one case.

Discussion

The iron status of the body, that is, presence/absence of iron deficiency and the amount of iron storage, can be deduced from serum iron, TIBC and serum ferritin levels. Anemia due to chronic diseases and iron deficiency anemia sometimes pose a diagnostic difficulties, but can be differentiated by the use of these parameters. In iron deficiency anemia, serum iron and ferritin are reduced and TIBC is increased. In the anemia accompanying tumor/HCC, serum iron and TIBC are reduced and serum ferritin is increased. The capacity to sequester free iron in the serum released from injured cells is impaired with chronic disorder anemia. Ferritin is an index for iron storage and becomes lower if iron is depleted. It was high in 13 of our cases (81 %).

Raised TIBC is a characteristic feature of iron deficiency. In this study there was no case with increased serum TIBC. However transferrin iron saturation was markedly reduced (less than 10%) in two cases and slightly (near lower limit of 15% saturation) reduced in five cases. Serum iron level was low in the majority of cases (11 cases; 69%). Thus it is assumed that anemia found in HCC is predominantly of chronic disorder type and not a result of iron deficiency. Raised transferrin saturation (more than 60%) usually indicates iron overload. In this study only one case was found to have transferrin saturation of 70%. Interestingly, all three viral markers were positive in that case.

High levels of ferritin usually indicate iron overload in the

Table 1 Patients under Study, Iron Status, and Viral Markers

Code	Age (Yr)	Sex M/F	Iron mg/dL	TIBC mg/dL	Ferritin ng/mL	Free iron ng/mL	Viral markers HBsAg	HBcAb	HCV-Ab
L71	47	M	27.0	193.3	257.5	0.030	+	+	-
L73	30	F	40.3	282.9	058.9	0.048	-	+	+
L74	67	F	128.6	251.6	326.7	0.039	-	+	+
L79	48	M	35.9	195.1	786.0	0.000	+	+	-
L82	67	M	98.6	223.4	372.9	0.077	-	+	+
L84	54	M	65.6	319.2	303.5	0.049	-	+	+
L85	27	M	46.6	301.0	1829.0	0.093	-	-	-
L87	49	M	170.2	242.9	662.1	0.061	+	+	+
L88	23	M	53.2	208.8	433.1	0.068	+	+	-
L89	48	M	106.7	383.5	156.8	0.045	+	+	-
L91	46	M	28.4	384.6	477.1	0.046	+	+	-
L92	61	M	42.5	284.9	547.6	0.089	-	+	+
L93	57	M	152.7	384.9	598.6	0.105	-	+	+
L95	34	M	27.1	287.5	130.1	0.050	+	+	-
L99	70	F	25.6	223.3	505.4	0.032	-	-	-
L103	49	F	35.5	262.4	352.3	0.045	+	+	-

Yr: year, M/F: male or female, Iron: serum iron (normal range: male 80-180, female 60-160 mg/dL), TIBC: total iron binding capacity (normal range: 250-466 mg/dL), Ferritin: serum ferritin (normal range: 18-300 ng/dL), Free iron: normally not detected, HBsAg: hepatitis B surface antigen, HbcAb: hepatitis B core antibody, HCV-Ab: hepatitis C viral antibody

study population. Although the exact mechanism(s) has yet to be established, a high plasma ferritin level may also be due to malignant conditions and liver disease itself. Free iron was detected in all of the cases in the present study. This finding supported the deleterious effect of iron on the liver which is either toxic or carcinogenic (Okada, 1998).

HbA_2 increases in iron deficiency anemia. There are three cases with HbA_2 in this study. Serum iron was reduced and serum ferritin was increased in all of these cases, whereas TIBC was reduced in two and normal in one. Thus the A_2 bands found in these cases were not likely due to iron deficiency, but rather associated with the b thalassemia trait. Hb A_2 quantitation, which was not done in this study, is required to confirm diagnosis of the letter.

A faint band of Hb F was observed in one case. Although the cause in this case was not identified, the milder form of thalassemia intermedia is most likely. In this case serum iron was reduced but TIBC and ferritin were within the normal range, and free iron was detected. Hb A_2 in three cases and Hb F in one case thus pointed to an overall prevalence rate for thalassemia of 25% among the HCC cases under this study. This prevalence rate was not significantly different to that of among general population in Myanmar (Aung-Than-Ba-Tu. 1968). All of these four cases were males.

Viral markers were studied in 14 cases of HCC in this study. HbcAb was detected in all of the cases. HBsAg was positive in eight cases. Anti-HCV was detected in seven cases. All three viral markers were positive in one case. This finding provides supportive evidence for the hypothesis that the local conditions that promote the release of free radicals such as in chronic inflammation will favor the de novo production of neoplasms. Increased stored iron promotes propagation of damaging free radical reactions (Okada, 1996., Okada, 1998).

In conclusion, iron overload in combination with chronic viral infection is almost always associated with HCCs in Myanmar. The cause of such iron overload is not settled but most probably features increased absorption from the intestine. ß-thalassemia in minor forms was only detected in 25% of Myanmar HCCs. Iron may play definite roles in hepatocarcinogenesis although the pathogenic mechanism(s) remains to be established. Clinical trials of phlebotomy to reduce iron in patients with chronic active hepatitis C are now in progress with remarkable improvement of serum chemistry (Hayashi et al., 1995; Shedlofsky, 1998). These therapeutic trials are also worthy of consideration in Myanmar.

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Personal Profiles: see overleaf

Personal Profile: **Ne-Win**

Dr Ne-Win was born in Yangon in 1951 and graduated from the Institute of Medicine I, Yangon, in 1976.He has been a research scientist/consultant pathologist in the Department of Medical Research, Ministry of Health, Yangon, Myanmar, since 1993. He has the qualifications of M.B., B.Sc., M.Med.Sc. (Clinical Pathology) and his main research interests are hematology, coagulopathies and general pathology. He is now presently a Ph.D. candidate in Myanmar.

Personal Profile: **Shigeru Okada**

Shigeru OPkada was born in Okayama in 1940. He graduated from Okayama Medical School in 1963 (MD) and obtained his Ph.D. in 1969. He has been Professor of the Department of Pathology I, Okayama Medical School, since 1990. His activity in research into iron metablism and free radical induced carcinogenesis is relected in a large number of publications. He ahs been active as the leader of the Japan-Myanmar collaboration on the subject of iron overload in thalassemics and its link to hepatocellular carcinoma since 1996.

Dr Khin-Saw-Aye, Ms Hauk-Kying, Prof Okada and Dr Ne-Win (left to right) in the laboratory.