RESEARCH COMMUNICATION

Increased Serum Granulocyte Colony Stimulating Factor in Turkish Hepatocellular Carcinoma Patients

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Abstract

    <u>Purpose</u>: To evaluate the serum levels of G-CSF in patients with hepatocellular carcinoma and to compare with values in healthy individuals. <u>Patients and Methods</u>: Thirty-three patients with hepatocellular carcinoma and 30 controls were included in the study. Histological confirmation of hepatocellular carcinoma (HCC) was performed by core needle biopsy and patients with cirrhosis were classified according to the Child-Pugh score. The serum G-CSF levels of individuals in both groups were calculated as pg/ml and compared for Child-Pugh Class A, B and C patients with HCC. <u>Results</u>: Median ages of patients with HCC and control group individuals were 58 (range:47-78) and 56 (range 45-70), respectively. Sex distributions were approximately equal. The mean serum level of G-CSF in patients with HCC was 199.4 ± 112.2, as compared to 24.0 ± 8.8 in the controls ($p < 0.001$). In addition, on subgroup analysis, the serum levels of G CSF were increased with Child-Pugh Class A, B and C, although without statistical significance ($p=0.253$). <u>Conclusion</u>: Increased levels of G-CSF are observed in patients with HCC. Further investigations are necessary to clarify the mechanism of G-CSF production and its effects on outcomes.

Key Words: Hepatocellular carcinoma - G-CSF - cirrhosis - Child-Pugh classification

Introduction

    Granulocyte colony-stimulating factor (G-CSF) is a blood growth factor that stimulates the production of neutrophils through the proliferation and maturation of precursor cells in bone marrow (Lieschke et al.,1992). The increased levels of G-CSF are shown in cases of infection-induced granulocytosis (Kawakami et al.,1990). However, in cases of malignancy with leukocytosis without infection, the production of G-CSF has also been reported in a variety of non-hematological malignancies that include carcinomas of the lung, bladder, stomach, thyroid, buccal mucosa and tongue (Asano et al.,1977; Obara et al.,1985; Ito et al.,1990; 1993; Oka et al.,1993; Matsuo et al.,1994; Sato et al.,1994; Yamano et al.,2007).

    The G-CSF producing HCC cases were rarely reported (Tohyama et al.,1989; Tani et al.,1990; Hori et al.,1998; Yamamoto et al.,1999; Araki et al., 2007). The production of G-CSF by tumor cells without infection had only been reported as case presentation. We calculated the serum levels of G-CSF in patients with hepatocellular carcinomas that were diagnosed with core needle biopsy specimens of liver and compared the results with the serum levels of G-CSF in control group individuals without any liver disease.

Patients and Methods

    Thirty-three patients with hepatocellular carcinoma whose proven cytologically or histologically and 30 healthy individuals were included to this study. Histological confirmation of the hepatocellular carcinoma (HCC) was performed by core needle biopsy. During the diagnosis, the staging of disease was performed at baseline with clinical examination, abdomen sonography and laboratory findings.

    The inclusion criteria were as follows: patients with histologically confirmed HCC, no taken any chemotherapy prior to the study, no established any acute or chronic enfection status, no the history of G-CSF using prior to the study. The process of blood collection was started before the initial treatment for all patients with HCC.

    The serum G-CSF levels of individuals in both groups were calculated as pg/ml in biochemistry laboratory. This results in both groups were compared. All patients with cirrhosis were classified according to Child-Pugh score. At subgroup analysis, the serum levels of G-CSF were compared for Child-Pugh score in patients with HCC. The patients with HCC were also evaluated for underlying reasons that caused liver cirrhosis and chronic hepatitis

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(i.e, hepatitis B, C, D virus infection, alchol intake) . In addition, the levels of serum α-fetoprotein (AFP) were calculated in patients with HCC.

The results of serum G-CSF in both groups were compared using Mann-Whitney U test. At subgroup analysis, the serum levels of G CSF in patients with HCC were compared for Child-Pugh stage using One-Way ANOVA test. p values < 0.05 were considered as significant. Statistical procedures were performed by using SPSS for windows version 11.5 soft ware program.

Results

The patients characteristics and laboratory findings were shown in Table 1. Median ages of patients with HCC and control group individuals were 58 (range:47-78) and 56 (range 45-70), respectively. Of 33 patients with HCC, 20 (60.6 %) patients were male and 13 (39.4 %) patients female. Of 30 control group individuals, 20 (66.7 %) cases were male and 10 cases (33.3 %) female.

The patients with HCC were evaluated for cirrhosis and chronic hepatitis reasons. Hepatitis B virus and C virus were detected in 6 (18.2 %) patients and 10 (30.3 %) patients, respectively. Two (6.1 %) patients had alcholic cirrhosis. The reason of cirrhosis in 15 (45.4 %) patients with HCC was unknown. The evaluation of Child-Pugh score were performed in 26 (88.9 %) patients with cirrhosis who diagnosed hepatocellular carcinoma (see Table 1). The mean serum levels of G-CSF in patients with HCC were 199.4±112.2. The mean serum levels of control group individuals were 24.03±8.8. The levels of G-CSF in patients with HCC were obviously higher than control group. This different between two groups was statistically significant (p < 0.001).

In addition, at subgroup analysis, the serum levels of G-CSF were compared for Child-Pugh score in patients with HCC (Figure 1). The mean serum G-CSF levels of stage A, B and C patients were 142.5±132.5, 225.9 ± 154.9 and 285.4±176.4, respectively. For unknown stage the value was 247.6±118.9. Although this difference between subgroups was not statistically significant (p=0.253), the serum levels of G-CSF in patients with HCC were found positively associated with Child-Pugh Stage A, B and C.

Discussion

Some malignancies produce certain humoral factors including cytokines such as G-CSF, GM-CSF, erythropoietin or parathyroid hormone (PTH)-related peptide which are caused paraneoplastic syndrome (Ueno et al.,1989; Tani et al.,1990; Kubo-Shimasaki et al.,1992; Asahi et al.,1996). The production of G-CSF in patients with hepatocellular carcinoma was only reported as several cases (Tohyama et al.,1989; Tani et al.,1990; Hori et al.,1998; Yamamoto et al.,1999; Araki et al.,2007).

We investigated the levels of G-CSF in patients with HCC and compared the results with control group individuals. In our study, the levels of G-CSF were obviously found higher in patients with HCC than control group individuals (p< 0.001). In addition, the levels of G-CSF in patients with HCC were compared for the Child-

Table 1. Characteristics and Laboratory Findings of HCC Patients and Control Group Individuals

Characteristics		HCC patients	Healthy controls
Number (n.)		33	30
Age, median (range)		58 (47-78)	56 (45-70)
Sex (n,%)	Female	13 (39.4)	10 (33.3)
	Male	20 (60.6)	20 (66.7)
Cirrhosis reasons			
	B virus	6 (18.2)	-
	C virus	10 (30.3)	-
	Alcohol	2 (6.1)	-
	Unknown	15 (45.4)	-
Child-Pugh Classification			
	A	8 (12.7)	-
	B	11 (17.5)	-
	C	7 (11.1)	-
	Unknown	7 (11.1)	-
Serum G CSF (mean ±SD)		199.4±112.2	24.0±8.8*
Serum AFP (mean±SD)		268.3±132.2	-

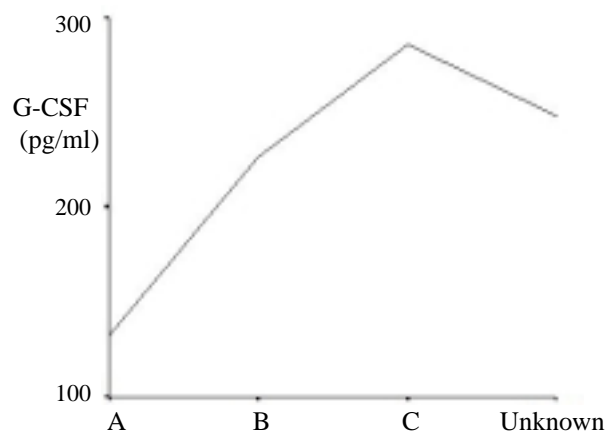*Significantly different at p<001

Figure 1. Mean serum levels of G CSF in patients with Child-Pugh stage A, B, C and unknown stage. The different in subgroups was not statistically significant (p= 0.253)

Pugh Classification (A,B,C). Although this difference between subgroups was not statistically significant (p=0.253), the serum levels of G-CSF in patients with HCC were found positively associated with Child-Pugh Stage A, B and C. As the stage of cirrhosis increases, the serum levels of G-CSF were also rised. This increase may be the correlation with the degree of cirrhosis. In our study, prognostic analysis wasn't performed due to the number of case is not enough.

The increased levels of G-CSF have been reported to be associated with an aggressive clinical course in cases with HCC. The elevation of G-CSF in these cases have been found positively correlated with the increased blood leukocyte count (Tohyama et al.,1989; Hori et al.,1998; Yamamoto et al.,1998; Araki et al.,2007).

The mechanism of G-CSF production by HCC cells has not been elucidated, but it was reported there was a relationship between the G-CSF production in cancer cells and the differentiation status of cancer cells (Yamamoto et al.,1999). In addition, Wang et al (1996) demonstrated the production of GM-CSF in poorly differentiated HCC cell lines and not in well-differentiated cell lines. Also, Yamamoto et al (1999) reported the increased levels of

G-CSF in case with poorly differentiation by microscopic examination. In another case, Hori et al (1998) reported granulocytosis and positive immunostaining of G-CSF in patient with HCC that have the moderately differentiation by cytologic or histologic examination. Therefore, the relationship between the differentiation grade and G-CSF production in HCC remains to be elucidated.

G-CSF producing tumor cells express a G-CSF receptor which stimulates tumor cell growth by G-CSF via the autocrine pathways (Tachibana et al.,1995). Therefore, G-CSF producing tumors usually cause rapid tumor growth along with chronic and progressive inflammation which deteriorates the general condition of the patients and they have poor prognosis. However, it has been reported that the G-CSF producing tumors secrete various cytokines such as IL-1 and IL-6 (Suzuki et al.,1992; Hayashi et al., 2001). IL-1 is also a hemopoetin-1, which potentiates G-CSF induced leukocytosis. Sato et al (1989) reported that the IL-1 have dual effect on the development of leukocytosis and hypercalcemia. Therefore, IL-1 may play a important role in the regulation of G-CSF and IL-6 production in this G-CSF producing liver cancer.

Because there was no found similar study to ours in the English language literature, the results of our study could not been compared with any trial. Such as cases mentioned above, the levels of G-CSF in our study were obviously found higher in patients with HCC than control group individuals.

In conclusion, G-CSF production by liver tumor cells was observed in patients with HCC. All these findings suggest that the increased levels of G-CSF may be associated with poor outcomes in liver cancer. Further investigations are necessary to clarify the mechanism of G-CSF production and its effects on outcomes in patients with HCC.

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