

# **Chitotriosidase activity in different stages of hepatitis C. It may a possible tumor marker for hepatocellular carcinoma**

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**Abstract:** Chitotriosidase is synthesized and secreted especially in activated macrophages. The aim of this study was to evaluate chitotriosidase activity in patients with various stages of hepatitis C. The study included a total of 90 patients. The patients were divided into four groups. Group 1 included 54 patients with chronic active hepatitis, Group 2 included 20 patients with recovered HCV, Group 3 included 6 patients with HCV induced hepatocellular carcinoma, and Group 4 included 10 patients with HCV cirrhosis. Chitotriosidase activity was measured with spectrophotometry (SigmaAldrich®). The mean chitotriosidase activity of the four groups was 0,927u/L (0.804u/L in Group 1, 0.521u/L in Group 2, 3.211u/L in Group 3 and 1.030u/L in Group 4). Chitotriosidase activity was significantly higher in Group 3. ROC analysis, used to evaluate chitotriosidase activity for the diagnosis of hepatocellular carcinoma, showed that chitotriosidase activity of 0,935 was below the curve (CI: 95%; 0,862 - 0,976), which was statistically significant (p= 0,0001). The cut-off value was >1,098 with a sensitivity of 100% and a specificity of 81%. Chitotriosidase activity can be a marker with a high sensitivity and specificity for the diagnosis of hepatocellular carcinoma.

**Keywords:** Chitotriosidase, Hepatitis C, Hepatocellular Carcinoma

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## **1. Introduction**

Hepatocellular carcinoma (HCC) is the fifth most common type of cancer and the third most common cause of death due to cancer [1,2]. HCC is a type of cancer, the treatment of which is quite difficult. Liver cirrhosis is an important risk factor for HCC and as a precursor it allows surveillance of HCC [3]. Therefore, surveillance of all cirrhosis patients is recommended [4]. A liver lesion presenting with arterial vascularization, becoming indistinct gradually when contrast medium is given and disappearing in late phases on computed tomography and magnetic resonance imaging is suggestive of HCC [5]. If there is an atypical appearance on sectional dynamic radiological examinations, histopathological examination is required to confirm the diagnosis of HCC. Serum alpha-fetoprotein (AFP) levels and ultrasonography are the most frequent

techniques used in diagnosis and surveillance of HCC [6]. One of the main problems in diagnosis and follow up of HCC is lack of reliable tumor markers.

Although AFP is the most frequent tumor marker used in surveillance of HCC, not all HCCs produce AFP. In addition, serum AFP levels can be elevated in chronic liver disease without HCC. Recent review articles showed that there was not strong evidence for the use of AFP as a diagnostic test for HCC [7,8]. There have been studies on other tumor markers for HCC. One of them is des-gamma carboxy prothrombin (DCP), commonly used for diagnosis and surveillance of HCC in Japan [9]. DCP is an abnormal prothrombin molecule produced as a result of an impairment acquired during posttranslational carboxylation of the prothrombin precursor in malignant cells [10,11].

This abnormal prothrombin molecule is similar to the one produced in vitamin K deficiency [9].

Apart from AFP and DCP, other tumor markers for HCC have been investigated. Chitotriosidase, an enzyme the serum levels of which are increased in some liver diseases, can be an appropriate candidate for a tumor marker of HCC. It (CHIT, EC 3.1.4.12) is a member of a group of chitinase enzymes able to hydrolyze chitin. 18-chitinase-family hydrolyzes chitin, which is an N acetyl glucosamine. These enzymes have been described in bacteria, fungi, insects, plants, viruses and protozoan parasites [12,13,14,15,16,17].

Chitotriosidases are produced by activated macrophages in tissues and polymorphonuclear leukocytes in humans [18]. Although physiological functions of chitotriosidases have not been sufficiently described, they are thought to play a role in natural immunization based on their production typical of phagocytes. Chitotriosidases and acidic mammalian chitinases (AMC) are real enzymes which have a chain binding C-terminal chitin and which can hydrolyze chitin. YKL-40, YKL-39, SI-CLP and murine YM 1/2 proteins only have glyco-18 chain and do not have a hydrolytic activity [18]. The main sources of proteins having glyco-18 are macrophages, neutrophils, epithelial cells, chondrocytes, synovial cells and cancer cells. Both macrophages and neutrophils employ regulatory secretory mechanisms for the release of proteins with glyco-18 [18].

Chitotriosidase levels are used in the diagnosis and surveillance of some liver diseases such as Gaucher disease [18,19]. Chitotriosidase is produced excessively by macrophages in some diseases presenting with accumulation of glycosphingolipids, iron and glycogen in macrophage lysosomes [18,19]. The aim of this study was to evaluate chitotriosidase activity in various stages of hepatitis C.

## 2. Material and Methods

This study included 90 patients diagnosed with chronic hepatitis C and followed up in our gastroenterology clinic. The mean age of the patients was 52,62±8,89 years (range: 23-69 years). Chronic hepatitis C was diagnosed based on liver biopsy and histopathological examination in anti-HCV and HCV-RNA positive patients. Liver cirrhosis was diagnosed based on clinical, radiological and/histopathological examinations in anti-HCV positive patients. The patients found to be anti-HCV positive but HCV-RNA negative on two measurements six months apart indicated a prior HCV infection. Hepatoma was diagnosed when a hypervascular mass on the basis of HCV related liver disease was shown on at least two dynamic imaging techniques or when AFP was >300 ng/ml or when histopathological examination showed evidence. Fifty-four patients with chronic hepatitis were assigned into Group 1, 20 patients with sustained viral response and chronic hepatitis C were assigned into Group 2, six patients with hepatocellular cancer were assigned into Group 3 and 10

patients with HCV related cirrhosis were assigned into Group 4.

ELISA (SigmaAldrich ®) was used to measure serum chitotriosidase activity. For this purpose, first, venous blood samples were taken into tubes containing gel. Then, the samples were centrifuged to obtain their serum and obtained sera were kept at -80 °C until they were analyzed.

The analysis was based on formation of color nitrophenylate which results from ionization of p-nitrophenol as consequence of enzymatic hydrolysis of the substrate 4-nitrophenyl-β-D-N'-N''-triacetyl chitotriose in the acidic environment (pH: ~4,8) at 37 °C when stop solution (sodium carbonate) is added. Substrate solution (1mg/ml) 4-nitrophenyl β-D-N'-N''-triacetyl chitotriose was dissolved in tampon solution. Standard solution 5μl 10 mM p-Nitrophenol was dissolved in 995μl stop solution. Stop solution 1 gr. sodium carbonate was dissolved in 24 ml distilled water. The final concentration of the chitinase control enzyme 5ml PBS was 0.2mg/ ml. Blank solution (40μl substrate solution), standard solution (300 μl), control solution (40 μl substrate and 5μl chitinase control enzyme) and sample solution (40μl substrate and 5μl patient serum) were incubated at 37 °C for 30 min. Following incubation, except for standard solution, all solutions were added stop solution (sodium carbonate). Thirty minutes later, absorbance of the resultant color was measured with spectrometry. Specimens were not diluted.

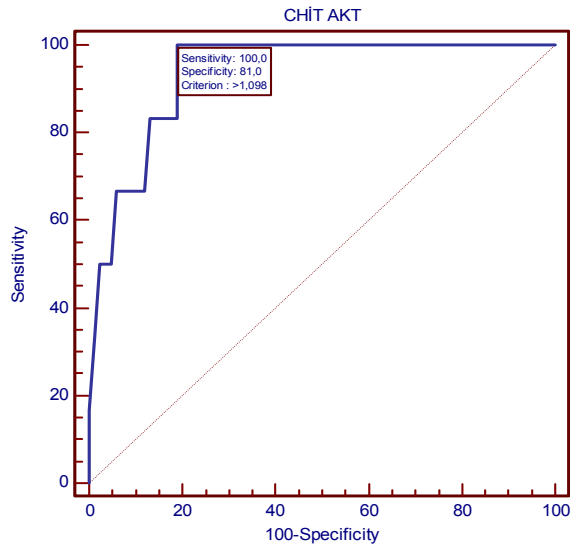
ANOVA and Kruskal Wallis test were used to compare the groups. Receiver Operating Curve (ROC) analysis was used to evaluate the role of chitotriosidase activity in the diagnosis of HCC.

## 3. Results

There was not a significant difference in age between the groups ( $p=0,279$ ) (see table 1). The male to female ratio was not significantly different in all four groups ( $p=0,084$ ). The mean enzyme activity was 0.927u/L. It was 0.804u/L in Group 1, 0.521u/L in Group 2, 3.211u/L in Group 3 and 1.030u/L in Group 4. Chitotriosidase activity was not significantly different between Group 1 and Group 2 and between Group 1 and Group 4. However, the difference was significantly different between Group 1 and Group 3 ( $p=0,138$ ,  $p=0,387$  and  $p=0,000$  respectively). Chitotriosidase activity was lower in Group 2 than in Group 3 and Group 4 ( $p=0,000$  and  $p=0,003$  respectively). It was higher in Group 3 than in Group 4 ( $P=0,015$ ) (Table 1). The enzyme activity was significantly higher only in Group 3 (HCC Group) ( $p=0,0001$ ). In fact, it was significantly higher in Group 3 (3,21±2,51) than in Groups 1, 2 and 4 (0,76±0,69) ( $p<0,0001$ ) (see table 1). The ROC analysis, made to evaluate chitotriosidase activity for the diagnosis of HCC, revealed an area of 0,935 (95%CI: 0,862 - 0,976) below the curve, which was significant ( $p=0,0001$ ). The sensitivity was 100%, the specificity was 81% and the cut-off value was >1,098 (Figure 1).

**Table 1.** Distribution of Chitotriosidase Levels by Age

|                          | Group 1 n=54 | Group 2 n =20 | Group 3 n =6 | Group 4 n =10 | Total N=90 | p=    |
|--------------------------|--------------|---------------|--------------|---------------|------------|-------|
| Age                      | 51,72±9,35   | 51,90±8,60    | 56,00±7,77   | 56,90±6,52    | 52,62±8,88 | 0,279 |
| Chitotriosidase activity | 0,80±0,80    | 0,52±0,42     | 3,21±2,51    | 1,03±0,35     | 0,92±1,08  | 0,00  |

**Figure 1.** ROC Curve for Chitotriosidase Activity in all Groups. Sensitivity of 100%, specificity of 81% and cut-off value of >1,098

## 4. Discussion

We evaluated chitotriosidase activity in patients with hepatitis C and found the enzyme activity higher only in the patients with HCC (3,2112±2,510). The difference resulted from the group ( $p=0.001$ ). ROC analysis made to evaluate the role of chitotriosidase activity in HCC diagnosis revealed an area of 0,935 (%95CI: 0,862 - 0,976) below the curve, which was statistically significant ( $p= 0,0001$ ). The sensitivity was 100%, the specificity was 81% and the cut-off value was >1,098.

Proteins containing gliko-18 can be used as biomarkers in human diseases. Chitotriosidase is produced excessively by lipid loaded macrophages in Gaucher disease and it is a basic marker for hereditary lysosomal storage disease [19]. The synthesis of AMC and murine lectin YMI on the Th2 base is increased and enzymatic activity of AMC is involved in the pathogenesis of asthma [20]. YKL proteins function as mediators able to be dissolved in cell proliferation and migration and have been shown to be associated with rheumatoid arthritis, inflammatory bowel disease and cirrhosis [21]. Chitotriosidase and YKL-40 show activation of macrophages in atherosclerotic plaques. Serum YKL-40 levels are diagnostic and a prognostic marker for many solid tumors [22]. Human chitinase and chitinase like proteins exist in tissues and the circulation and can be measured with noninvasive methods [18].

Although alpha-fetoprotein is the most frequently used tumor marker in surveillance and diagnosis of ACC, not all types of HCC produce alpha-fetoprotein. In addition, serum

alpha-fetoprotein levels can increase in chronic liver disease in the absence of HCC. Recent systematic literature reviews have shown that there is not strong evidence for the use of alpha-fetoprotein in surveillance and diagnosis of HCC [7,8].

## 5. Conclusion

Chitinase proteins can be used as markers for many inflammatory and malignant diseases. In the present study, chitotriosidase activity was considerably higher in patients with various stages of hepatitis C accompanied by hepatocellular carcinoma. Therefore, chitotriosidase can be considered as a prospective marker with high sensitivity and specificity for the diagnosis of hepatocellular carcinoma. However, further studies should be performed to evaluate chitotriosidase activity in large samples including patients with other types of cancer, metastatic liver disease and hepatocellular carcinoma.

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