

## Check and aflatoxin total B degeneration of the liver after hepatitis

Maryam Tavakkoli Lakeh

Department of Microbiology, Faculty of Basic Sciences, Lahijan Branch, Islamic Azad University (IAU), Lahijan, Iran  
Email: [aktavlakeh@yahoo.com](mailto:aktavlakeh@yahoo.com)

### ABSTRACT

The aflatoxins are a group of mycotoxins produced by certain *Aspergillus* species and can contaminate foodstuff especially in developing countries. In this study, 25 serum samples from hepatitis-positive from hospital in the city of Rasht collected by ELISA was tested in the meantime, out of 25 samples of which contained aflatoxin total B (B1 and B2) had. Then to learn possible liver disease situation and the impact of aflatoxin on the situation, using the results of the ELISA and the values of some blood markers (ALT, AST, ALP, BIL) derived from the patient's blood test results, with the help of statistical calculations and SPSS software to analyze data and communicate and influence between the factors involved there. The results showed that among a group of these factors were significant. The results showed that the activity of liver enzymes (ALT, AST, ALP) in hepatitis have increased significantly over time, which can damage liver cells and to confirm or cholestasis. Also according to the comparisons made to the conclusion that the bilirubin test, the appropriate test to assess the condition of the liver is not. Because usually cause changes in liver failure. However, the amounts of aflatoxin B, from the ELISA test, a synergistic effect of the toxin with hepatitis B and C was confirmed. The result can be total aflatoxin B as a contributing factor in the progression of hepatitis title.

**Keyword:** Aflatoxin Total B, Degeneration, Liver, Hepatitis

### INTRODUCTION

Aflatoxins are mycotoxins produced by *Aspergillus* are the most important. Aflatoxins are due to reasons such as relatively long history, extensive use of empirical research and a lot of visibility in nature known as the most famous mycotoxins have been introduced. The word aflatoxin letters A and F, respectively, representative of fungal genera *Aspergillus flavus*, which are its species or combined with toxins dictionary [3,6], hepatitis, liver inflammation that will cause pain and swelling. Liver, blood waste is dismantled. When the liver becomes inflamed, your task is not doing well in cleansing the blood. Initial civilized societies in the area of Mesopotamia knew the liver, the heart of life. Because it seemed that the liver is the accumulation of blood in the body. Causes of hepatitis are numerous. Hepatitis is caused mainly by one of the five hepatitis viruses [1]. hepatocellular carcinoma (HCC), one of the most common malignancies worldwide. The annual incidence of about one million cases of cancer worldwide between men and women are affected four times. It seems the strongest carcinogens, natural products, plants, fungi and bacteria are [5]. Many studies a strong relationship between the carriers and chronic hepatitis B increased incidence of HCC have shown. Several non-viral causes of hepatitis and cancer of the liver cells are involved in the development process. One of the most important factors in the development of effective non-viral hepatitis, are aflatoxins. aflatoxicosis reduced growth, reduced production, reduce calcification of the bones, increase blood coagulation time and the effects of the carcinogen. In general aflatoxins on most systems affect the human body. Among the significant impact, the effect on the urinary system through inflammation and kidney failure, affecting the digestive system by reducing the digestion and absorption of protein and fat, the effect on the nervous system by causing depression and abnormal behavior, the effects on the reproductive system by reducing spermatogenesis and infertility and low birth weight, fetal defects and an increased incidence of cancer is caused by abnormalities in gene expression system and reduce resistance and susceptibility to infection and opportunistic infections due to immune suppression dose Bashd. aflatoxicosis high acute illness and death from cirrhosis of the liver and cause chronic disease with low doses through the results of the immunological and nutritional. But all doses, in turn, have a

cumulative effect on cancer [6, 5], so we in this study, tissue degeneration of the liver after hepatitis and its relation to the amount of aflatoxin B, described in Gilan province.

## MATERIALS AND METHODS

In order to do experimental projects require serum samples were positive for hepatitis. For this purpose, the samples in the period March 2014 to May 2015, were collected from hospitals in the city of Rasht. The number of these samples, 25 (including HCV Ab and HBsAg), respectively. In addition, serum samples, test results CBC of others, to evaluate the possibility of using some of the factors affecting the liver, serum Shd.nmvnh get ice and freeze-dried using the Xbox transferred from the hospital to the laboratory was. After receiving serum samples, the samples to determine the presence or absence of aflatoxin B and determine the dose in samples containing aflatoxin B, were tested by ELISA.

### Kit Specifications

Total Aflatoxin Elisa Kit ,ProductEuroclone and made in Italy. The kit works by ELISA to detect quantitative aflatoxin B1, B2, G1 and G2 in cereals, grains, cotton seed and animal feed used. This plate contains 96 wells or 12 columns or strip is left. End of each of the wells coated with rabbit IgG that during the test, the antibodies are anti-aflatoxin band.

### ELISA test procedures

Due to the number of samples, we isolated 32 wells. To safety in disposable gloves were used during this test. Using a micro-pipette, 200 ml of distilled water and 50 ml standard solutions prepared in the first well to excursive in wells second to seventh, respectively We send to dilute concentrated. Using a micropipette, 50 microliter of serum hepatitis into each sample well, We send. In order to avoid contamination and prevent such errors in the results, apart from the samplers for each sample.the next step, 50 ml of enzyme conjugate into each well, except for the first well (Blank) We send. Then, 100 ml of aflatoxin antibody to each well, except the first wells added. Gently shake plates so that the material inside the wells were mixed well. After this process, the plates for 20 minutes at room temperature (20-25 ° C), away from direct sunlight made. After the desired time, pre-diluted washing buffer (at a ratio of 1:10 was diluted with distilled water) into all the wells using the washing machine showers washed At this point add all ingredients and plates went out. Do this 4 times and finally to ensure the complete removal of these substances, slowly and with taps, plate upside down on absorbent paper went Laboratory. Stage washing should be done carefully, otherwise it will cause errors in the results. After washing, using a micropipette, 200 microliters of chromogenic solution is added to each well for 20 minutes and at room temperature, away from direct sunlight made. And enzymatic reaction by adding chromogenic color was blue. Intensity of the color produced is directly related to the amount of aflatoxin in the samples. In the final stage, 50 ml of stop solution to each of the wells added. After adding this solution, change the color was changed to yellow and blue. Finally plates to put in Elisa reader at 450 nm. The device is based on the absorption rate, total aflatoxin absorption values and calculate the logarithmic curve associated with it. Finally, to determine the status of liver tissue obtained from patients through values ELISA test and blood test results the appropriate patients, the relationship between paid results.

### Statistic analysis

In order to do statistical analysis, 25 patients in five different age groups (<35, 35-45, 45-55, 55-65,> 65) were classified. The mean, maximum and minimum values of aminotransferases (ALT and AST), alkaline phosphatase (ALP), bilirubin (BIL), total aflatoxin B in HBsAg positive, total aflatoxin B in patients with HCV Ab positive, total aflatoxin B in the general population review and results CUT-OFF patients based on the HBsAg and HCV Ab, was calculated according to 5 age group. The statistical analysis of these values and the presence of a possible link to the foregoing, we used SPSS software. The results of the statistical analysis, a significant association of these factors indicate that we continue to investigate this relationship.

## RESULTS

ELISA results showed that 15 of 25 samples positive for serum hepatitis (B and C), with total aflatoxin B, respectively. After analysis of the data obtained from the ELISA values and the values of a group of blood markers (ALT, AST, ALP, BIL) patients, to compare the factors involved there. Some of these factors were associated with statistically significant relationships for this chart (fig.1), shown below.

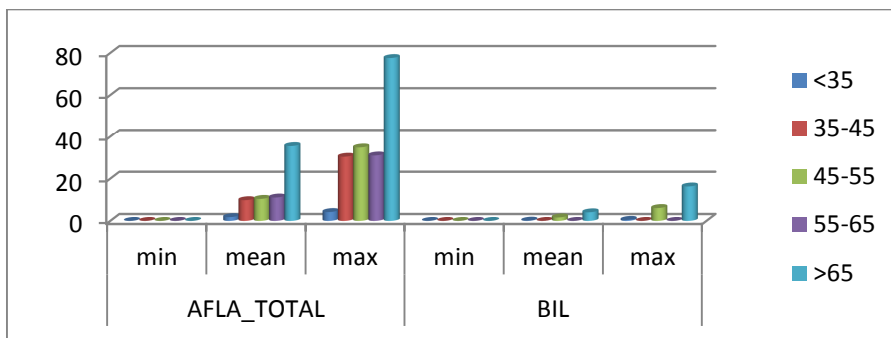


Fig 1.age with the BIL and amount of aflatoxin B charts in the total population under investigation

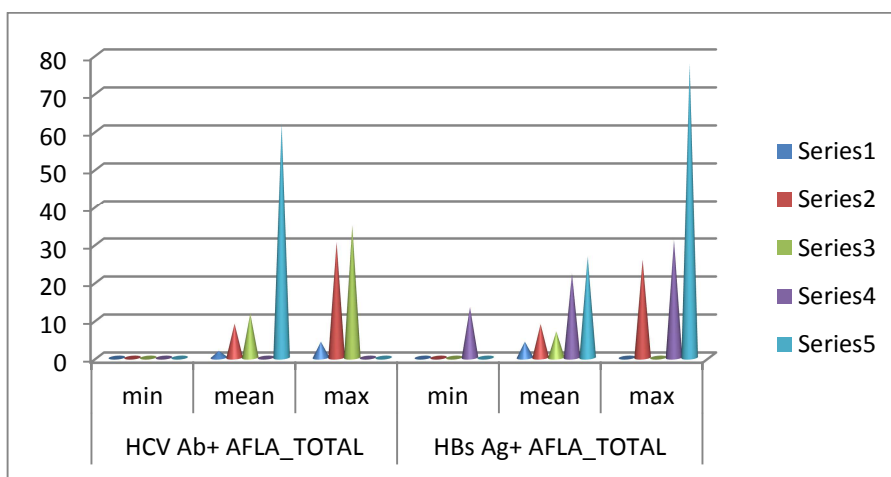


Figure 2. Aflatoxin B in HIV Ab HBs Ag positive patients

Correlation analysis and numerical difference as the total amount of aflatoxin B, in people who are HBsAg and serum HCV Ab, has been suffering from a viral infection is statistically significant. ( $Z = 2.041$ ) (Sig = 0.04), whereas this correlation in the study population, represents a serious relationship between the presence of aflatoxin B, average serum total has not been in a range from one person to another, very different numerical changes can have.

In people with infectious hepatitis B, ALT and AST levels were total aflatoxin B, showed no significant correlation with size and need to be investigated further ( $Z = 1.753$ ) (Sig = 0.080)(fig.2).

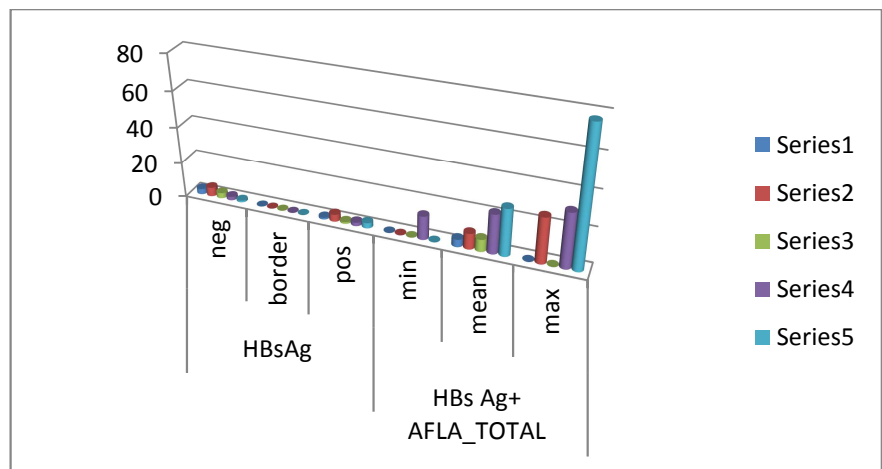


Figure 3. Aflatoxin B in HCV Ab and HBs Ag negative patients

In the population studied in people who are HBsAg negative, numerical values and its correlation with the total aflatoxin B in people who have had positive HBsAg, has been significant. ( $Z = 2.023$ ) (Sig = 0.043), while the situation in a positive HCV Ab, has not been proved (fig.3). ( $Z = 1.214$ ) (Sig = 0.225) It is noteworthy that the correlation in patients with HBsAg negative and mean aflatoxin total B, patients population requires more study ( $Z = 1.753$ ) (Sig = 0.080). In HBsAg positive individuals have had a significant amount of aflatoxin B, toxic relationship. In people who are HBsAg positive correlation with an average population of aflatoxin B, numerical difference is significant. ( $Z = 2.023$ ) (Sig = 0.043) in those with HCV Ab were positive significant relationship with total aflatoxin B, average serum size of the study population was not statistically significant and need to be investigated further ( $Z = 0.080$ ) (Sig = 1.753). In people who have negative HCV Ab, has always been compared to people who are HBsAg positive, the quantity of aflatoxin B, serum total and the average serum aflatoxin study population had a statistically significant difference ( $Z = 2.023$ ) (Sig = 0.043).

## DISCUSSION

One of the most important factors in the development of effective non-viral hepatitis, are aflatoxins. Aflatoxins are a group of strong fungal toxins that are causing adverse effects on biological systems. These toxins through ingestion, inhalation and absorption back into the body and blood and accumulate in various organs, among which most are metabolized in the liver and condensed, so that the concentration of toxins in the liver can be 10 times the amount of muscles [2].

A common set of blood tests that can be used for preliminary evaluation of liver disease, including measurement of serum alanine aminotransferase (ALT or SGPT), aspartate aminotransferase (AST or SGOT), alkaline phosphatase (ALP), serum Bailey Rubin (BIL) albumin and prothrombin time is measured in this study, we examined four factors. Measuring the level of serum aminotransferases, particularly ALT levels as easy and non-invasive method used to track the activities of liver disease, but it looks. These enzymes are always for determining the severity or prognosis, it can not be trusted. Increased levels of alkaline phosphatase of liver origin is not entirely appropriate for almost any type of cholestatic liver disease, urinary NAG less than 3 times the normal value may be seen. In cases where there is no increase in bilirubin or elevated aminotransferase levels, increased levels of alkaline phosphatase of liver origin, often but not always, non-infectious and non-toxic cholestasis early stages raises. Although the rate of increase in serum bilirubin level as a prognostic marker is not intended to be, but can be valuable in a number of cases [1,4, 6].

In a study in 1390, by doctor Tavakoli and colleagues to investigate the relationship between serum markers of liver fibrosis severity and the number of patients with chronic hepatitis took place, the significance of this relationship proved and increased levels of liver enzymes was observed. Today we will try to further studies on the possibility of using reproducible, in expensive and non-invasive especially less complications such as checking blood serum levels of some markers, such as ALT, AST, ALP, BIL and the associated with the severity of liver fibrosis are done. Had to interpret the results of this study to investigate the relationship between total aflatoxin B, and liver patients pay status. Based on what the results were in, the age of patients with the ALP and bilirubin levels there is a significant relationship. (Sig = 0.043) In other words, the age of patients has had an impact on the activity of ALP and bilirubin levels. In patients with hepatitis B and C, aged 45-55 years had the highest ALP activity and low activity aged less than 35 years. This could indicate that the age less than 35 years, ALP can not be a good test for assessment of liver activity, because although there hepatitis, the activity of this enzyme has not increased. But for the amount of bilirubin, the maximum value of the corresponding age is 65 years. This suggests that in patients with hepatitis, increased ALP, you should increase the amount of bilirubin or vice versa. On the other hand, because usually the amount of bilirubin in the later period of time increases liver failure, can be partly explained the value in old age. In another comparison of age with the disease represents values based on the index value HBsAg and HCV Ab (Sig = 0.043), as well as the total amount of aflatoxin B, in patients infected with HBsAg and HCV Ab There was a significant relationship. there is a significant correlation. Thus, the total amount of measurable toxin, a logical correlation was observed with increasing patient age. (Sig = 0.043), in a study in 1381, by doctor Alavi and colleagues to investigate the prevalence of hepatitis C infection and its association with liver enzymes in patients with thalassemia major were done, we have a positive relationship between age and the prevalence of anti-HCV there were significant. In a study in 2010, by Crough and colleagues in Australia to predict fibrosis in HBV-positive patients was conducted, between age and reflects the values of the disease (HBeAg), a significant relationship was observed.

The correlation of total aflatoxin B, infection in patients with HBsAg and HCV Ab significantly (Sig = 0.045) , indicating that it would be synergistic relationship with hepatitis B and C is aflatoxin. On the other hand, we can take this issue as between the amount of total aflatoxin B in people infected with hepatitis B and C with non-infectious, there is a significant difference. While this correlation in the study population, represents a serious

relationship between the presence of aflatoxin B, in a range not mean serum levels are variable from person to person. As a result, we can make it as was shown in patients who have this connection, factors other than toxins including virus strains and have been involved in the development of the disease. While the total amount of aflatoxin B, ALT and AST levels in people infected with HBsAg is not significant and needs to be investigated further (Sig = 0.080), which it studies with more samples and recommends a closer look.

Correlation analysis between the values of total aflatoxin B, in patients infected with HCV Ab and values of ALT and AST, a significant relationship was observed (Sig = 0.043), with the increasing concentration of aflatoxin, increased levels of transaminases have also explains that the synergistic effect of aflatoxin with hepatitis C can also be made. Perhaps the size of numerical difference in serum ALT and AST ALP with a distinctly non-specific findings for more accurate analysis considered serum diagnostic laboratories, could be intercepted as a reliable indicator of complications from liver damage as well as provide a valid prognosis of patients benefited from it. Given the significant size of the numerical difference between AST and total aflatoxin B, average in the population of patients can be regardless of ALT, AST variation range and also, if possible, ALT and AST as a measure of the size of numerical difference in assessment B's total aflatoxin-induced liver toxicity and used (Sig = 0.044) , for increased concentrations of aflatoxins as a destructive agent, increases the permeability of the cell membrane and thus increase the output of liver enzymes AST into the blood stream. So the initial examination and follow-up after treatment, the levels of these enzymes can be used to detect an increase or decrease potential damage to the liver, may be used.

And also, according to the numerical value of serum ALT levels of aflatoxin total has been associated with viral hepatitis B in patients with type B and C as well as statistically significant correlation with serum B had an average total aflatoxin, aflatoxin as a destructive agent , causes swelling of hepatic cells. Inflation pushes the bile canaliculi and bile flow is impaired. Under the ALP attached to the cell membrane of liver dysfunction were released and the amount of activity, blood flow increases. So as a distinctly non-specific ALP reliable, both in the hepatic infection and toxin-induced liver toxicity in determining can be used. (Sig = 0.043), Given the significance of the values of total aflatoxin B in HBsAg positive and negative, obviously proves the existence of specific and nonspecific clinical signs of Hpatoxicosis fungal considered in patients with hepatitis effect is decisive (Sig = 0.043), while the situation in a positive HCV Ab, has not been proven (Sig = 0.225), that it would be a witness that factors other than total aflatoxin B were involved in the progression of hepatitis C or hepatitis C, hepatocytes did not hurt that the metabolism of aflatoxin B, as well as into M be seriously affected.

### CONCLUSION

The study was carried out and interpret the results using the results of this study and comparison with other studies, researchers in recent years, it can be concluded that total aflatoxin B is one of the important factors affecting the health of human liver is. The fungal toxins play a role in the progression of hepatitis and liver cells and can even lead to cancer.

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