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DETERMINATION OF SOME BIOCHEMICAL PARAMETERS IN BLOOD IN A GROUP OF INDIVIDUALS WITH HYPERBILIRUBINEMIA AND HEPATITIS B

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Abstract:

Hyperbilirubinemia is a condition that can be a result of liver damage or liver malfunctioning, and linked to specific disorders or diseases. Bilirubin and some other biochemical parameters are used to test the health conditions in a group of individuals, suspected for hepatitis B. Total bilirubin, indirect and direct bilirubin, ALT and AST were observed and monitored in two different randomly chosen groups. In the first group, were involved all the healthy individuals and in the second one, were included hepatitis B positive individuals.

The purpose of this study was to determine and compare these biochemical parameters between the groups separately, and to further understand the biochemical profile of hepatitis B hyperbilirubinemia.

Data analysis clearly show that men are more likely to have higher levels of Total Bilirubin. It turned out that the most affected age group by Hepatitis B is the one from 18 to 30 years old. A good reason for the achieved results can be the lifestyle of these individuals, alcohol consumption as well as higher sexual activity in this age group. There is no significant difference between the two groups when comparing the levels of Indirect Bilirubin and Direct Bilirubin/Indirect Bilirubin ratio. There is a strong positive correlation between ALT and AST ($r= 0.985$; $p= 0.00$), which is linked to the stage of the liver cells damage.

When analyzing the relationship between the ALT/AST ratio against total Bilirubin, it results that they are not directly related to each other, so, it is important and necessary to measure separately and take into consideration separately Direct and Indirect Bilirubin value. This results to be a very important information when drawing a conclusion about the problem and make the differentiation, because the information we get from the levels of total bilirubin is not always enough.

Keywords: hyperbilirubinemia, total bilirubin, conjugated hyperbilirubinemia, unconjugated hyperbilirubinemia, ALT, AST

Introduction

Hyperbilirubinemia involving the conjugated fraction of bilirubin occurs with conditions affecting the liver and biliary systems, such as infection or congenital abnormality (Jane E C. 2009). When we refer to bilirubin, it is necessary to be mentioned that it is a product that is formed as a result of heme catabolism, which is found in the red blood cells. The porphyrin ring of hemoglobin is opened up during catabolism process and it is converted to bilirubin, a yellow color molecule. It is a toxic molecule that has to be removed from the body. As it is not very water soluble, we find bilirubin molecule in circulation tightly bound to the protein albumin, which carries the molecule to the liver and is rapidly cleared from the body, predominantly by the liver, via glucuronidation and biliary elimination (Sane, R. S. et al. 2014). Once in the liver, UDP-glucuronosyltransferase, transforms this molecule in a less toxic form and more water soluble by adding glucuronic acid, leading to mono and diglucuronide conjugated forms of bilirubin. After this process, bilirubin is effluxed into the bile by multidrug resistance-associated protein (MPR) 2, encoded by the ABCC2 gene (Hashimoto K. et al. 2002).

Hyperbilirubinemia or excess bilirubin in the blood, is also known with the term jaundice and it causes the yellow pigmentation of the skin. Jaundice in adults can be an indicator of significant underlying disease and has to do with specific conditions, when the liver is unable to properly metabolize or excrete bilirubin (Sullivan, J. I., & Rockey, D. C. 2017). It is caused by elevated serum bilirubin levels in the unconjugated or conjugated form (Fargo, M. V. et al (2017). Unconjugated hyperbilirubinemia occurs with increased bilirubin production caused by red blood cell destruction, such as hemolytic disorders, and disorders of impaired bilirubin conjugation, such as Gilbert syndrome. Conjugated hyperbilirubinemia occurs in disorders of hepatocellular damage, such as viral and alcoholic hepatitis, and cholestatic disorders, such as choledocholithiasis and neoplastic obstruction of the biliary tree (Am Fam Physician. 2017; 95(3):164-168. Copyright © 2017 American Academy of Family Physicians). According to other studies, the gold standard method for bilirubin estima-tion is the total and conjugated bilirubin assess-ment based on the van den Bergh reaction (Royal Prince Alfred Hospital 2003; Bosschaart N. et al. 2012).

A specific and very important issue to be considered is hyperbilirubinemia or jaundice in newborns as it is classified as a life threatening disorder. It is a multifactorial disorder with many symptoms. Generally, the physiological jaundice is the most prevalent type (Ullah, S. Et al.2016). According to other studies jaundice is present in 4 of 5 (84%) healthy newborns (Vinod K. et al. 2013). Other studies report that nearly 8% to 11% of neonates develop hyperbilirubinemia (Young Infants Clinical Signs Study Group 2008; Burke BL. Et al. 2009). Neonatal hyperbilirubinemia is a common clinical problem encountered during the neonatal period, especially in the first week of life (Bhutani VK. et al. 2013; American Academy of Pediatrics Practice Parameter 1994).

There are different types of neonatal hyperbilirubinemia including physiological jaundice, pathological jaundice, jaundice due to breastfeeding or breast milk and hemolytic jaundice including three subtypes due to Rh factor incompatibility, ABO blood group incompatibility and Jaundice associated with Glucose-6-phosphate dehydrogenase (G6PD) deficiency (Mishra S. et al. 2008).

Regarding the laboratory evaluation it is recommended that the initial laboratory evaluation of jaundice in adults should include fractionated bilirubin, complete blood count, alanine transaminase, aspartate transaminase, alkaline phosphatase, γ-glutamyltransferase, prothrombin

time and/or international normalized ratio, albumin, and protein (Winger J, Michelfelder A. 2011). Different liver function tests (LFTs) are used to show restoration of bilirubin levels and AST, ALT and ALP values are evaluated also as indicators of hepatic damage/injury due to the infection (Tetteh A.K.; Asamoah L.K. 2017).

Method

Blood samples were collected from 135 individuals who were tested for hepatitis B in the biochemical laboratory "ALK" in Berat city in Albania during a two-year period study, from 2017-2019. The individuals included in this study were presented in the laboratory for routine tests or by doctor's recommendation.

Student test was used to compare the distribution of data between groups. Before determining the value of the Student test for Indirect Bilirubin and the BD/BI ratio, we look at the homogenization of the variances, through the Levene test. For each of the patients included in the study, plasma was extracted to determine the viral load of hepatitis B, and serum was used for the determination of transaminases ALT, AST, Total bilirubin, Direct and Indirect bilirubin. Minitecna photometric method and End-Point method have been used in measuring the biochemical parameters.

All the data received from samples testing are organized in tables and statistical analysis is done by SPSS 23 program for windows. Distribution of the parameters were evaluated by the Kolmogorov-Smirnov test in order to see if they have normal distribution or not. It was noticed that datas had abnormal distribution ($p < 0.05$) with the exception of Indirect Bilirubin and DB/IB ratio that had normal distribution ($p = 0.078$).

To test if there is a difference between the groups, for Indirect Bilirubin and the BD/BI ratio we use the Student test for independent samples (Independent samples T-test). For the other parameters, the non-parametric Mann Whitney test was this case we do not have homogenization of variances ($p = 0.005$). In terms of significance we noticed a different distributions between the groups (healthy and unhealthy) included in this study in all the parameters ($p < 0.05$).

Results and Discussion

Two different groups of individuals are inculded in this study. The first group represents all the healthy individuals who have been presented in the laboratory for routine tests and the second one is a hepatitis B suspect group of individuals (who have been presented in the laboratory for blood tests after doctor recommendation). Descriptive statistics about the group included in this study, taking into consideration age and sex and a group of biochemical parameters, is presented in table 1. The age of the individuals vary from 3 to 74 years old. According to the received data, we noticed that 47.7 % of the individuals included in this study are healthy or not infected with hepatitis B and 52.22 % resulted to be positive for hepatitis B. According to other studies for the albanian population, despite the estimated two-fold reduction of HBsAg prevalence in the general population from about 18%-19% to 9.5%, Albania remains a highly endemic country (i.e. over 8% of HBsAg prevalence rate) (Resuli, B. et al. 2009).

Table 1. Biochemical parameters in the group of healthy and hepatitis B positive individuals

Biochemical parameters	H/N *	Minimum value		Maximum value		Average (± St Dev)	
		Male	Female	Male	Female	Male	Female
Age	H	28	17	70	46	44.70 (±11.4)	34.19 (±8.093)
	N	17	3	73	74	39.9 (±15.12)	33.3 (±16.51)
ALT	H	11.7	13.2	641.0	224	121.8 (±140.41)	76.93 (±58.08)
	N	16.0	11.0	2000	994.5	162.2 (±476.62)	82.56 (±195.73)
AST	H	17.4	16.0	590	206	100.9 (±122.86)	65.72 (±51.24)
	N	21.0	21.0	1819.0	1819	143.08 (±432.31)	143.07 (±432.31)
Total Bilirubin	H	0.3	0.3	22.4	3.8	2.76 (±4.19)	1.80 (±1.24)
	N	0.3	0.3	10.5	10.5	2.48 (±2.27)	2.48 (±2.27)
Direct Bilirubin	H	0.1	0.2	18.4	5.4	2.06 (±3.54)	0.94 (±1.23)
	N	0.2	0.2	9.0	9	1.3 (±2.16)	1.3 (±2.16)
Indirect Bilirubin	H	0.20	0.1	4.0	0.8	0.70 (±0.70)	0.46 (±0.21)
	N	0.10	0.2	2.2	3	1.183 (±0.70)	1.55 (±0.73)
ALT\AST ratio	H	0.60	0.6	3.0	2.01	1.194 (±0.54)	1.14 (±0.42)
	N	55.5	0.4	162.5	2.40	110.2 (±33.58)	0.81 (±0.42)
DB\IB ratio	H	0.30	37	8.6	7.75	2.68 (±2.35)	3.01 (±2.25)
	N	0.23	0.4	7.0	5	1.96 (±2.41)	0.85 (±1.17)

**(H/N-Hepatitis/Normal)*

From the descriptive statistics, presented at table 1 we can clearly see the difference in parameters between the healthy group and the infected individuals with hepatitis B. In the group of males, total bilirubin is observed in the levels from 0.3 to 22.4 mg/dl. Except the concentration of total bilirubin there is observed difference in concentration in the other parameters too. In the group of females a narrow interval of total bilirubin is observed, from 0.3 to 3.8 mg/dl. Different studies on sex and age relationship with bilirubin levels (Rosenthal, P. et al.1984), claim that males generally exhibit much higher serum bilirubin than females and this was confirmed in the current study too. According to recent research, men tend to have slightly higher bilirubin levels than women (American Association of Clinical Chemistry, 2021).

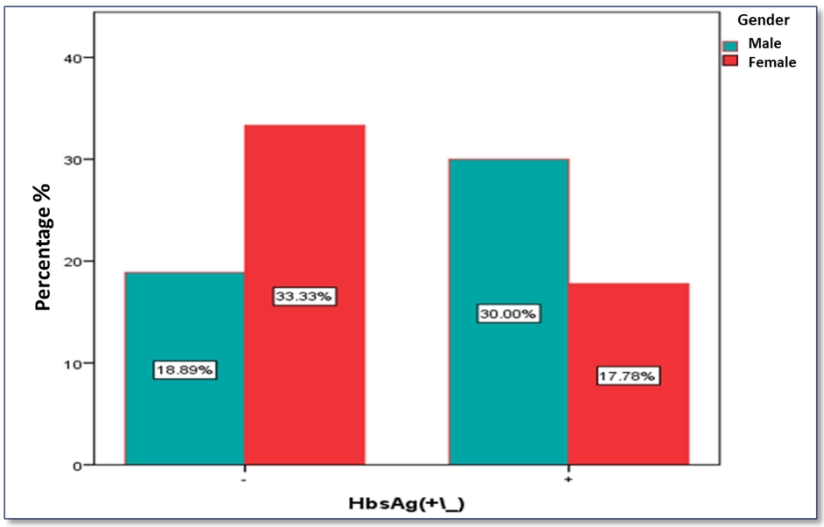

Figure 1. Gender based distribution of HbsAg (Hepatitis B surface antigen)

From figure 1, we can see that 18.89 % of males resulted to be negative for hepatitis B, while 30 % of them resulted positive. Regarding the group of females, 33.33 % of them resulted negative and 17.78% were hepatitis B positive. There can be different reasons that lead to this distribution of cases. Firstly, the distribution of males and females in our sample is not equal and another important reason is linked to the higher sexual activity in men compared to women. Another important fact that should be taken into consideration is that there are more male consumers of alchohol and drugs compared to females. Other studies report a higher number of cases in males compared to females, with a male to female ratio of 3.8:1 (Baig S., 2009).

From our observations, we noticed that different age groups have different sensitivity against this pathology. Starting from that, we tried to compare the data between different age groups and evaluate the higher prevalence of Hepatitis B between the two groups.

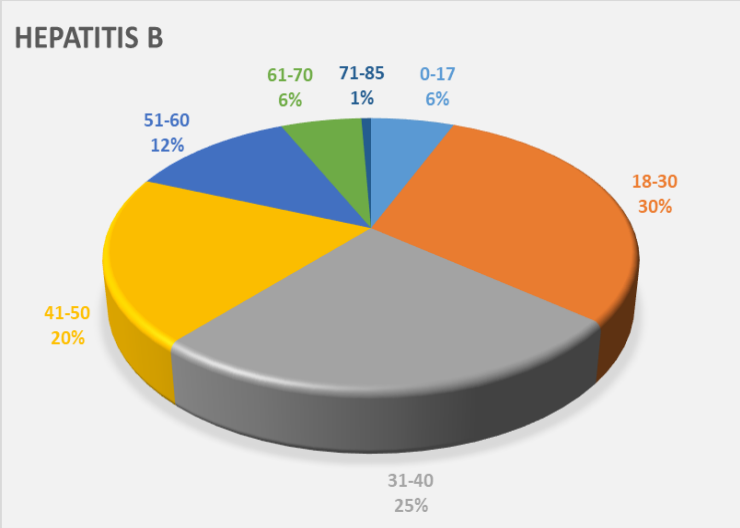

Figure 2. Distribution of cases based on their age group

Looking at the data obtained from this study and the bar chart in figure 2 the most affected age group by Hepatitis B is the second one, from 18-30 years old, followed by the third age group 31-40 years old. The lowest prevalence is observed in individuals over 51 years old. Higher levels of HBsAg are usually connected with a higher risk and lower levels of HBsAg are usually connected with a lower risk. However, negative results for serum HBsAg tests do not always represent a clearance or inactivating status of HBV (Hepatitis B Virus) viruses. HCC could still develop in the absence of detectable HBsAg in serum. This situation is called occult hepatitis B virus infection (OBI) (Chen, L. et al. 2014).

From earlier studies in the Albanian population we notice similar results regarding the incidence in different age groups, where anti-HBs prevalence is higher in students when compared to other groups and prevalence of HBsAg was higher in the young generation (16-20 years old) (Resuli, B. et al. 2009). Studies from other authors in Togo (Kolou et al. 2017) confirm the high prevalence of Hepatitis B among young people. According to this study 1200 individuals were tested for HBsAg from 2009-2011. The overall prevalence of HBV infection was 19.08%. This prevalence was significantly higher in males (25.00 %) than in females (14.80 %). The highest prevalence of HBV was observed in the age range 20-29 years and 30-39 years with 26.33 % and 21.67 % respectively. The lowest prevalence was 6.08 %, found in individuals over 50 years of age.

Relationship of biochemical parameters is observed and evaluated in both groups (healthy and Hepatitis B group), in order to better understand the trend of the disease and to point the importance of different parameters assessment in the diagnosis and treatment of the disease.

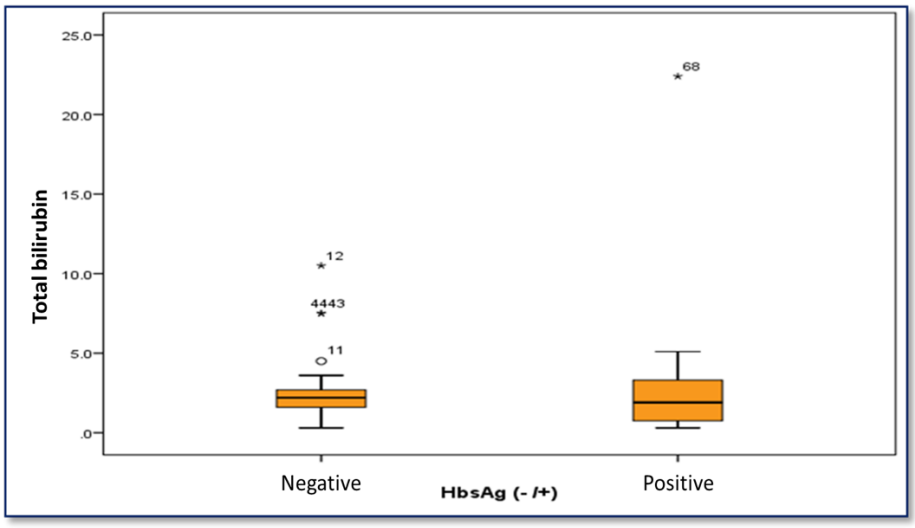

Figure 3. Total bilirubin distribution

A high level of total Bilirubin was considered, so it is expected to find a significant difference for both groups (p = 0.05). The Mann Whitney test showed that U = 821.50, with values range from 0.3-22.4 mg/dl. The maximum level (22.4mg/dl) was observed in a male patient at the age of 46 years. Although not necessarily high levels of total bilirubin indicates liver damage as it is observed in the study group also, this is a key parameter that warns of changes in Direct or Indirect Bilirubin, a parameter which will later determine and differentiate the diagnose.

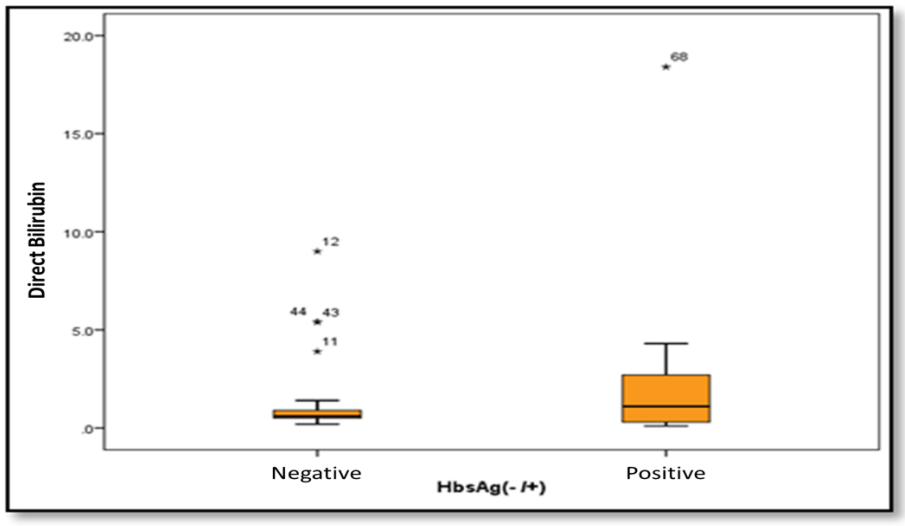

Figure 4. Direct bilirubin distribution

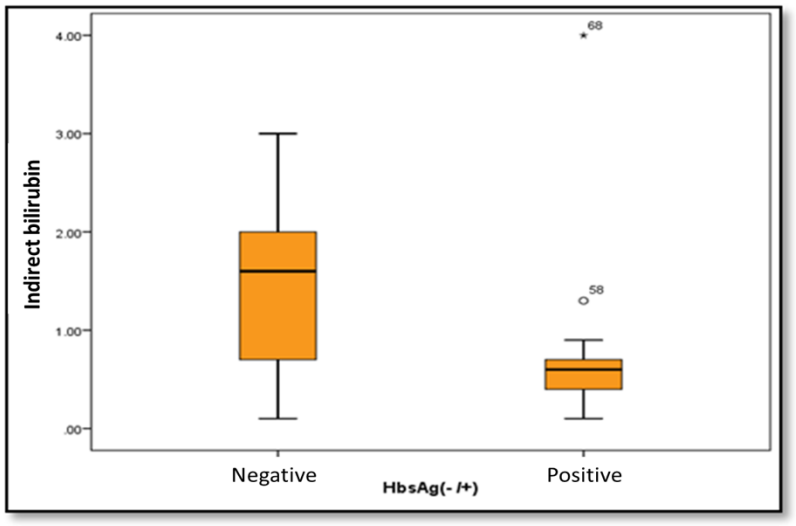

Figure 5. Indirect bilirubin distribution

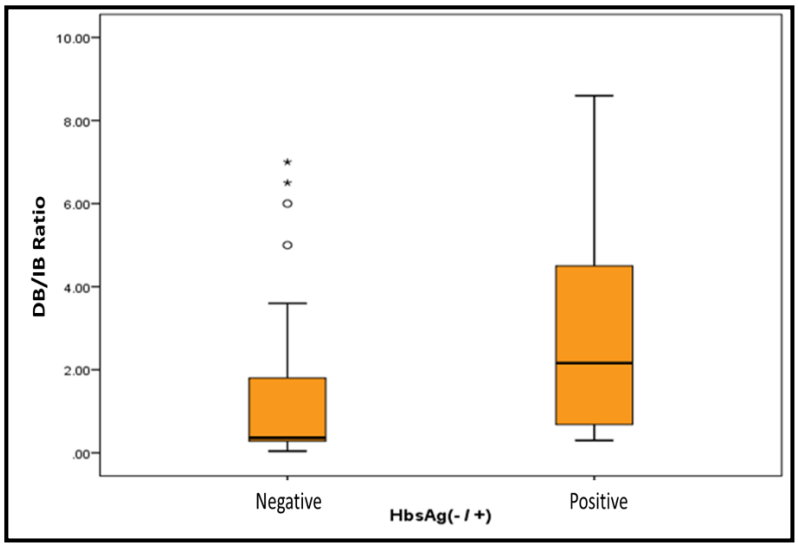

Figure 6. Direct/Indirect bilirubin ratio distribution

Data distribution for Direct Bilirubin varies between the two groups as shown by the value of U (U = 927.50). Student test for Indirect Bilirubin (t = 5.704) confirms the differences between the two groups. There is a significant difference in mean`s value between the two groups p <0.05 (p = 0.005) and this difference is in the value of 0.805. Usually, indirect bilirubin culminates (excluding bilirubinuria) in the case of hemolytic anemia, especially when bilirubin concentrations are <3-5 mg/dL, as mentioned above.

According to the statistical analysis of data distribution for the individuals included in the study, there is a significant difference in DB\IB ratio between Hepatitis B negative and Hepatitis B positive individuals. This is confirmed by Student Test (t = -3.594) for the comparison of the two groups, where p <0.05 (p = 0.001) (difference in the value of -1.548)..

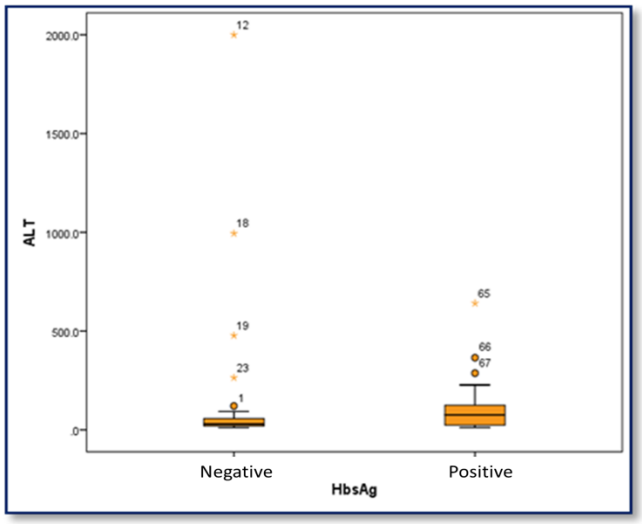

Figure 7. ALT distribution between groups

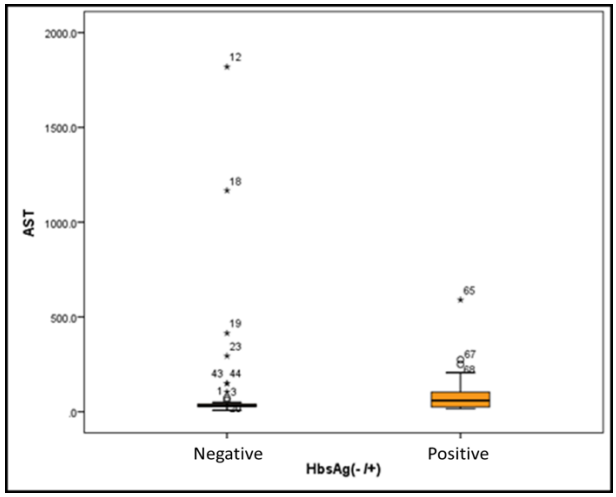

Figure 8. AST distribution between groups

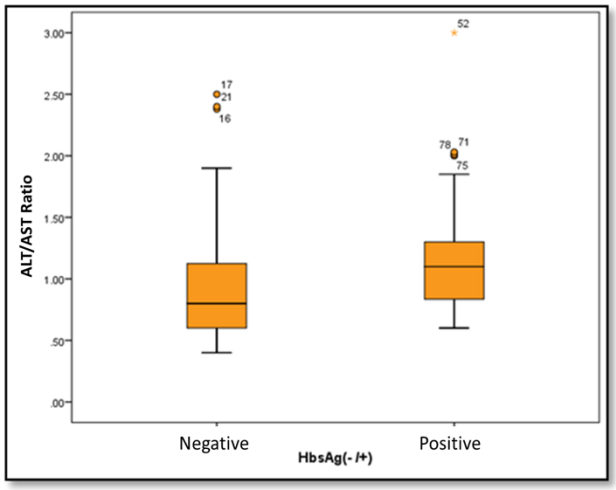

Figure 9. ALT/AST ratio distribution between groups

Mann Whitney test (for non-normal distribution values) was performed for ALT and AST distribution (for ALT distribution U = 683000 and AST distribution U=819.50). There was observed a significant difference in ALT and AST distribution (p = 0.005) between groups. According to other studies and reviews (Malakouti, M. Et al. 2017), common causes of mildly raised aminotransferases levels except hepatitis, can be also alcohol, medication, nonalcoholic fatty liver disease, autoimmune disease, Congestive heart failure, Ischemic hepatitis, Budd-Chiari syndrome, etc. In order to make it easier to differentiate the cause and to set the diagnose except other parameters, ALT, AST and Direct bilirubin levels are necessary for this group of patients.

There is a significant difference in the ALT/AST ratio between the two groups (p = 0.002). Mann Whitney test is also applied for these data (U = 626,500). Based on the statistical data, we noticed that in the study group the ALT/AST ratio is less than 1 in hepatitis B negative individuals, while in hepatitis B positive individuals it is greater than 1. Different studies and observations have shown that if ALT level is greater than that of AST, patients are diagnosed with viral hepatitis, whereas when the level of AST is greater than ALT, patients are diagnosed with alcoholic liver disease. AST/ALT ratios below 1.0 are also typical for chronic viral hepatitis (e.g. hepatitis B and C), however slightly higher ratios than 1.0 can be found in chronic viral hepatitis, but this can be present, especially when there is progression to fibrosis and cirrhosis (Geriatric Clinical Advisor, 2007). The predominance of AST over ALT in alcohol-related liver disease was first reported by Harinasuta et al. in 1967, and it became more widely recognized only with the paper by Cohen and Kaplan in 1979 (H. Nyblom. Et al. 2004). Many authors suggested alcoholic hepatitis when described AST/ALT ratios greater than 1.5 or greater than 2.0 (Adrian B. & Roy A. Sh., 2014). AST/ALT ratio greater than 4 combined with high bilirubinemia is important in situations like Fulminant Hepatic Failure and Hemolysis (Amit K., Vijay K.Sh., 2017).

We tested the relationship between ALT/AST ratio and the DB/IB ratio (p = 0.19), but there was no significant relationship between them as well as between the ALT/AST ratio and total Bilirubin.

Spearmann coefficient was used to evaluate the relationship between ALT and AST (r = 0.985; p = 0.00). There was found a positive correlation between them, showing a very strong relationship between these parameters (r^2= 0.971).

Conclusion

Men are more prone to higher levels of Total Bilirubin and 30% of them are Hepatitis B positive. It turned out that the age group most affected by Hepatitis B is from 18-30 years old. Possible reason is lifestyle, which includes alcohol consumption, a factor that greatly increases Bilirubin, as well as higher sexual activity in this age group.

Taking into consideration the parameters relationship and their importance in the determination of hepatitis and other diseases, we consider very important the measurement of direct and indirect bilirubin.

Recommendations

The ALT\AST report is very helpful for an initial diagnosis, but this diagnosis will be clarified with the measurement of Direct and/or Indirect bilirubin. In that condition we recommend

laboratory measurements of these fractions of direct and indirect bilirubin, that clarify the diagnosis in all the suspect individuals.

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