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Original Research Article

Biochemical and Immunochemical Status of Patients Receiving Herbal Treatment in Some Traditional Herbal Homes in Rural Nigeria

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Abstract: Herbal treatment is currently attracting more patronage due to the effectiveness and low cost of treatments. Many herbs contains phytochemicals of health benefits. Some traditional ways of administering herbal treatments could bring about cross-infection. This work was designed to determine the biochemical and Immunochemical status of patients receiving herbal treatment in some herbal homes in rural Nigeria. Fifty patients receiving herbal treatments were recruited for this work. The patients were tested for anti-HIV, anti-HCV and HBsAg immunochemically by ELIZA and Westernblot before treatment. 23(46%) of the fifty patients who were anti-HCV, anti-HIV and HBsAg seronegative were recruited for biochemical evaluation of plasma Total Bile Acid, Albumin, Total Antioxidant , Glucose and Creatinine using spectrophotometric technique before and after treatments. **T**he overall immunochemical status obtained in the patients include 10(20%) HBsAg, 4(8%) anti-HCV, 7(14%) HBsAg + anti-HCV, 23(46%) HBsAg /anti-HCV/HIV seronegative, 3(6%) HBsAg + anti-HIV, 2(4%)Anti-HCV+ anti-HIV, 1(2%)Anti-HIV. Gender distribution include: in Female- 4(8%)HBsAg, 1(2%)anti-HCV , 3(6%)HBsAg + anti-HCV, 13(26%)HBsAg /anti-HCV/HIV seronegative, 2(4%)HBsAg + anti-HIV, 1(2%)Anti-HCV+ anti-HIV, 1(2%)Anti-HIV and in male patients : 6(12%)HBsAg, 3(6%)anti-HCV , 4(8%)HBsAg + anti-HCV, 10(20%)HBsAg /anti-HCV/HIV seronegative, 1(2%)HBsAg + anti-HIV, 1(2%)Anti-HCV+ anti-HIV, 0(0%)Anti-HIV. There was a significantly higher plasma value of Total Bile Acid and Total Antioxidant in the herbal home patients after not less than three weeks treatment than before herbal treatment with $p<0.05$. There was a significantly lower plasma level of glucose after not less than three weeks of herbal treatment than before the treatment with $p<0.05$. Monoinfection of HBV, HCV and their coinfections were more frequent in the patients than the mono and coinfection of HIV. HBV, and HCV including their coinfections were more frequent in male than female while HIV was more in female patients. AThere was a significant increase in plasma Total Bile Acid and Total Antioxidant with a decrease in plasma glucose in the patients after than before herbal treatment. Routine immunochemical and biochemical assessment should be carried out on patients of herbal homes before, during and after herbal treatment for effective management.
Keywords: Biochemical, Immunochemical status, patients, herbal treatment, herbal homes, rural Nigeria

INTRODUCTION

Human serum albumin is the version of serum albumin found in human blood. It is the most abundant protein in human blood plasma; it constitutes about half of serum protein. It is produced in the liver. It is soluble and monomeric. Albumin transports hormones, fatty acids, and other compounds, buffers pH [1]. and maintains oncotic pressure, among other functions. Albumin is synthesized in the liver as preproalbumin, which has an N-terminal peptide that is removed before the nascent protein is released from the rough endoplasmic reticulum. The product, proalbumin, is in turn cleaved in the Golgi vesicles to produce the secreted albumin [2]. The gene for albumin is located on chromosome 4 and mutations in this gene can result in anomalous proteins. The human albumin gene is 16,961 nucleotides long from the putative 'cap' site to the first poly (A) addition site. It is split into 15 exons that are symmetrically placed within the 3 domains thought to have arisen by triplication of a single primordial domain [3].

Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. The term "antioxidant" is mainly used for two different groups of substances: industrial chemicals which are added to products to prevent oxidation, and natural chemicals found in foods and body tissue which are said to have beneficial health effects [4].

To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase) produced internally or the dietary antioxidants, vitamin A, vitamin C and vitamin E. The antioxidant defense system has many components. A deficiency in any of these components can cause a reduction in the overall antioxidant status of an individual. Reduction in total antioxidant status has been implicated in several disease states, such as cancer and heart disease [5, 6]. Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Antioxidants are found in many foods, including fruits and vegetables. They are also available as dietary supplements. Examples of antioxidants include; Beta-carotene; Lutein ;Lycopene, Selenium, Vitamin A, Vitamin C, Vitamin E [7].

Vegetables and fruits are rich sources of antioxidants. There is good evidence that eating a diet with lots of vegetables and fruits is healthy and lowers risks of certain diseases. But it isn't clear whether this is because of the antioxidants, something else in the foods, or other factors. High-dose supplements of antioxidants may be linked to health risks in some cases. For example, high doses of beta-carotene may increase the risk of lung cancer in smokers. High doses of vitamin E may increase risks of prostate cancer and one type of stroke [8].

Bile acids are steroid acids found predominantly in the bile of mammals and other vertebrates. Different molecular forms of bile acids can be synthesized in the liver by different species. Bile acids are conjugated with taurine or glycine in the liver, forming bile salts may also interact with some medicines. Bile Acids (BA) make 67% of the total composition of Bile. They are 24-carbon steroids generated during cholesterol metabolism. They form conjugates with either glycine or taurine to form bile salts. Five of the BAs account for more than 99% of the total population found in biofluids [9]. The average composition in healthy individuals includes conjugates of cholic, chenodeoxycholic, deoxycholic and lithocholic acids. Bile acids are critical due to their ability to solubilize lipids by forming micelles with cholesterol, and fatty acids. Their synthesis is not only critical for the removal of cholesterol from the body abut they are also needed for proper uptake of dietary lipids into the small intestine [10]. The measurement of circulatory Total Bile Acids (TBA) therefore provides information about hepatic functions and liver diseases such as jaundice, and hepatocellular injury. TBA estimation can detect liver damage during early stages and permits patients to get treatment before hepatic damages become irreversible [11].

HBsAg is present in the sera of patients with viral hepatitis B (with or without clinical symptoms). Patients who developed antibodies against HBsAg (anti-HBsAg seroconversion) are usually considered non-infectious. HBsAg detection by immunoassay is used in blood screening, to establish a diagnosis of hepatitis B infection in the clinical setting (in combination with other disease markers) and to monitor antiviral treatment [12,13].

An HCV antibody test is used to screen for infection. It detects the presence of antibodies to the virus, indicating exposure to HCV. This test cannot distinguish between someone with an active or a previous HCV infection [14]. The human immunodeficiency virus (HIV) is a lentivirus (a subgroup of retrovirus) that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS). Antibody to HIV proteins could be collectively refer to as anti-HIV which are detectectable by immunochemical techniques [15].

The accurate measurement of glucose in serum or plasma is important in the diagnosis and treatment of carbohydrate metabolism disorders such as diabetes mellitus, neonatal hypoglycemia and idiopathic hypoglycemia.

High levels of glucose in the blood usually indicate diabetes however levels can be raised in many other disorders including kidney disease, hyperthyroidism, pancreatitis and pancreatic cancer. Glucose is often measured in conjunction with various tolerance tests after the administration of doses of leucine, insulin, glucagon or glucose [16].

Creatinine is derived from creatine and creatine phosphate in muscle tissue and may be defined as a nitrogenous waste product. Creatinine is not reutilized but is excreted from the body in the urine via the kidney. It is produced and excreted at a constant rate which is proportional to the body muscle mass. As a consequence of the way in which creatinine is excreted by the kidney, creatinine measurement is used almost exclusively in the assessment of kidney function. Creatinine is regarded as the most useful endogenous marker in the diagnosis and treatment of kidney disease.

Creatinine is measured primarily to assess kidney function and has certain advantages over the measurement of urea. The plasma level of creatinine is relatively independent of protein ingestion, water intake, rate of urine production and exercise. Since its rate of production is constant, elevation of plasma creatinine is indicative of under-excretion, suggesting kidney impairment. Depressed levels of plasma creatinine are rare and not clinically significant [17, 18].

This work was designed to determine the Biochemical and Immunochemical status of patients

receiving herbal treatment in some herbal homes in rural Nigeria.

MATERIALS AND METHODS

Study Area

Study Population

Fifty patients receiving treatment at 5 traditional homes in Saki-west local Government area of Oyo State-Nigeria were studied. Immunochemical status was determined in all the patients (50) were while 23(46%) of the patients who were sero-negative to HCV, HIV, HBsAg were biochemically assessed following their claim that they did not eat overnight till the morning of presentation to the herbal homes. None of the patient recruited was known to be taking alcohol or cigarette and were not on any medication before presenting to the herbal homes for treatment. In addition, patients who have been successfully and previously treated by the herbal homes before the current clinical conditions were included.

Biological Sample

After an overnight fasting as claimed by patients before herbal medication,, Five milliliters of venous blood was obtained from each of the patients into Lithium heparinized bottles for evaluation of plasma Total Bile Acid, Glucose, Creatinine, Albumin , Total antioxidant and determination of anti-HCV, HBsAg and anti-HIV.

Duration of Study

Three months between May and July, 2016.

Methods

Total antioxidant assay

Randox total antioxidant status kit enables assessment of the integrated antioxidant system which encompasses all biological components with antioxidant activity.

The Randox Total Antioxidant Status kit can also be used to determine the phenolic content of beverages such as wine, beer and fruit juice. Antioxidants present in red wine, tea and other beverages have been shown to give a cardio-protective effect. The compounds in beverages, which contribute to this effect, are Phenolics, of which Polymeric Phenols are the largest subgroup. Phenolics also contribute towards the taste, color, odor and preservative of the beverage.

Principle

2, 2'-Azino-di-3-ethylbenzthiazoline sulphonate is incubated with a peroxidase (metmyoglobin) and H2O2 to produce the radical cation This has a relatively stable blue-green color, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this color production to a degree which is proportional to their concentration.

Total Bile Acid

Principle

Two reactions are combined in this kinetic enzyme cycling method. In the first reaction bile acids are oxidised by 3-α hydroxysteroid dehydrogenase with the subsequent reduction of Thio-NAD to Thio-NADH. In the second reaction the oxidised bile acids are reduced by the same enzyme with the subsequent oxidation of NADH to NAD. The rate of formation of Thio-NADH is determined by measuring the specific absorbance change at 405nm. (Abreviations: NADH, NAD, Thio-NADH, Thio-NAD)

Determination of plasma ALBUMIN

Randox kit was used for the determination of albumin in the plasma.

Principle

The measurement of serum albumin is based on its quantitative binding to the indicator 3,3',5,5'-tetrabromo-m cresol sulphonephthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578nm, the absorbance being directly proportional to the concentration of albumin in the sample.

Creatinine Estimation using Randox kit

Principle

Creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Glucose Estimation using Randox kit

Principle

Colorimetric method without deproteinisation. Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide produced, reacts with phenol and 4-aminophenazone to form a red - violet quinoneimine dye. The intensity of the final color is directly proportional to the glucose concentration and is measured at 505 nm.

Hepatitis B surface antigen (HBsAg) test

Hepatitis B surface antigen (HBsAg) test was carried out on the patient volunteers by using a one step enzyme immunoassay technique of the sandwich type for the detection of HBsAg in human serum or plasma using the reagent kit of BIO –RAD Raymond Poincare, Marnes La Coquette.

Principle

MONOLISA AgHBs PLUS is a one enzyme technique of the sandwich type using three monoclonal antibodies selected for their ability to bind themselves to the various subtypes of HBsAg now recognized by World Health Organisation.

The solid phase is made up 0f 12 strips of 8 polystyrene wells coated with the first monoclonal antibody.the two other monoclonal antibodies are bound to the peroxidase.

The assay procedure includes the following reaction step;

1. Distribution of samples into the wells of the microplates.

This distribution can be visually controlled;there is a clear difference of colouration between empty well and well with sample.This distributioncan also be controlledautomaticallyby reading at 450/620 – 700 nm(optional).

2. Distribution of the conjugate into the wells: This distribution can also be visually controlled; the conjugate which is initially orange becomes red after addition into the well. It is possible to control automatically this distribution by spectrophotometric reading at 450/620–700nm[optional].The sample deposition can also be controlled at this step of the manipulation by automatic reading at 450/620-700nm.

3. Incubation at 37^0C

4. Washing and development of the enzyme activity bound solid phase by the addition of substrate.

5. Stopping development, then reading of the optical densities at 450/620 – 700nm and interpretation of the results.

HIV screening

HIV screening was carried out on the patient volunteers after pre-test counseling by using the reagent kit of Abbot Laboratories Co.LTD, Japan. The Abott Determine HIV-1/2 is an invitro, visually read qualitative immunoassay for the detection of antibodies to HIV-1 and HIV-2 in human serum plasma or whole blood.The test is intended as an aid to detect antibodies to HIV-1/HIV-2 from infected individuals.

Summary And Explanation Of The Test

AIDS [acquired Immunodeficiency Syndrome] is characterized by changes in the population of T-cell lymphocytes. In an infected individual, the virus causes depletion of helper –T cells, which leave the person susceptible to opportunistic infections and some malignancies. The virus that causes AIDS exists as two related types known as HIV-1 and HIV-2 .The presence of the AIDS virus elicits the production of specific antibodies to enter to either HIV-1/HIV-2.

Biological Principle of the Procedure

Determine HIV – 1/2 is an Immunochromatographic test for the detection of antibodies to HIV-1/HIV-2. Sample is added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid – antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site. If antibodies to HIV-1 and or HIV-2 are present in the sample, the antibodies bind to the antigen – selenium colloid and to the antigen at the patient window forming a red line at the patient window site. If antibodies to HIV-1 and HIV-2 are absent, the antigen – selenium colloid flows past the patient window and no red line is formed at the patient window site. To ensure assay validity a procedural control bar is incoorperated in the assay device.

Western blot assay

The HIV confirmatory test was carried out on all the volunteers by Western blot assay, using reagent kit of Immunoetics, Inc., 27 Dryclock Avenue, Boston, USA. http//www.immunoetics.com

Principle

The QualicodeHIV1/2 kit is a qualitative immunoblot assay based on the Western Blot principle. The assay is performed on immunoblot membrane containing HIV-1 viral lysate protein (HLTVIII B stain) and a recombinant HIV-2 protein. To produce the membrane HIV-1 viral protein are fractionated according to molecular weight on a Polyacrylamide slab gel (PAGE) in the presence of Sodium deodecyl sulphate (SDS). The separated HIV-1 is then transferred through electrophoretic blotting from the gel to a nitrocellulose membrane two bands are directly sriped on the membrane

1) A control band containing staphylococci protein A.
2) A recombinant HIV-2 specific envelope antigen.

The membrane is then cut into strips for individual sample testing. During the procedure, the strips containing HIV1/2 are reacted with the serum specimen and washed to remove unbound antibodies. Visualization of human globulin specifically bound to HIV-1 or HIV-2 proteins is performed by sequential reaction with goat anti-human immunoglobulin-alkaline phosphatase conjugate and BCIP/NBT substrate. Band positions are compared to those on the Reference Card developed using the HIV -1/2 Positive Control Serum. The intensity of the bands is monitored by comparism to the HIV-1/2 Weakly Reactive Control.

Anti – HCV test

Anti – HCV test on all the volunteers was carried out by a third generation enzyme immunoassay for the determination of antibodies to hepatitis C virus in plasma using the reagent kit of

DIA.PRO; Diagnostic Bioprobes Srl; Via Columella, Milano – Italy

Principle

Microplates are coated with HCV specific antigens derived from core and ‘ns’ regions encoding for conservative and immunodominant antigenic determinants (Core, NS3, NS4 and NS5). The solid

phase is first treated with the diluted sample and HCV antibody are captured,if present, by the antigens.After washing out all other components of the sample,in the 2nd incubation bound anti - HCV are detected by the addition of anti – human immunoglobin G &M antibody ,labeled with peroxidase . The enzyme captured on the solid phase, acting on the substrate / chromogen mixture, generates an optical signal that is proportional to the amount of anti – HCV antibodies present in the sample.

Data Analysis

The values of the parameters obtained were subjected to statistical analysis using SPSS 18.0 to determine level of significance of differences at 0.05.

Ethical Consideration

The proposal of this work was presented to the Research and Ethical committee of Baptist Medical Centre, Saki, Oyo state-Nigeria. It was reviewed and approved before the commencement of the work. Only volunteers were studied.

RESULTS

The overall immunochemical status obtained in the patients include 10(20%) HBsAg, 4(8%) anti-HCV , 7(14%) HBsAg + anti-HCV, 23(46%) HBsAg /anti-HCV/HIV seronegative, 3(6%) HBsAg + anti-HIV, 2(4%)Anti-HCV+ anti-HIV, 1(2%)Anti-HIV. Gender distribution include: in Female- 4(8%)HBsAg, 1(2%)anti-HCV , 3(6%)HBsAg + anti-HCV, 13(26%)HBsAg /anti-HCV/HIV seronegative, 2(4%)HBsAg + anti-HIV, 1(2%)Anti-HCV+ anti-HIV, 1(2%)Anti-HIV and in male patients : 6(12%)HBsAg, 3(6%)anti-HCV , 4(8%)HBsAg + anti-HCV, 10(20%)HBsAg /anti-HCV/HIV seronegative, 1(2%)HBsAg + anti-HIV, 1(2%)Anti-HCV+ anti-HIV, 0(0%)Anti-HIV(Table 1)

There was a significantly higher plasma value of Total Bile Acid and Total Antioxidant in the herbal home patients after not less than three weeks treatment than before herbal treatment with $p<0.05$. There was a significantly lower plasma level of glucose after not less than three weeks of herbal treatment than before the treatment with $p<0.05$. However no significant difference was obtained in the plasma level of Albumin and Creatinine in the patients before and after not less than three weeks of herbal treatment with $p>0.05$(Table 2 & 3).

Table1: Immunochemical Status of Patients Receiving herbal Treatments in Herbal Homes

		HBsAg seropositive patients	anti-HCV seropositive patients	HBsAg + anti-HCV seropositive patients	HBsAg /anti-HCV/HIV seronegative patients	HBsAg + anti-HIV seropositive	Anti-HCV + anti-HIV seropositive	Anti-HIV seropositive
Frequency	Total	10(20%)	4(8%)	7(14%)	23(46%)	3(6%)	2(4%)	1(2%)
	Female	4(8%)	1(2%)	3(6%)	13(26%)	2(4%)	1(2%)	1(2%)
	Male	6(12%)	3(6%)	4(8%)	10(20%)	1(2%)	1(2%)	0(0%)

Table 2: Plasma value of Glucose, Creatinine, Total Bile Acid, Total Antioxidant and Albumin in HIV, HCV and HBsAg seronegative patients before and after herbal treatment

	Plasma Level of the biochemical parameters in HIV,HCV and HBsAg seronegative patients Before Treatment	Plasma Level of the biochemical parameters in HIV,HCV and HBsAg seronegative patients After Treatment
Total Bile Acid (µmol/L)	5.5±1.0	17±2.0
Total antioxidant (µM)	262±6.0	548±5.0
Albumin (g/L)	40±3.0	30±2.0
Plasma Glucose (mg/dl)	92±5.0	70±5.0
Creatinine (mg/dl)	0.7±0.05	1.8±0.6

Table 3: Comparative analysis of Plasma Total Bile Acid, Total Antioxidant, Albumin, Glucose and Creatinine

		Plasma Level of the biochemical parameters in HIV,HCV and HBsAg seronegative patients Before Treatment Vs Plasma Level of the biochemical parameters in HIV,HCV and HBsAg seronegative patients After treatment
Total Bile Acid (µmol/L)	't'	-5.1
	'p'	0.02*
Total antioxidant (µM)	't'	-35.34
	'p'	0.0004*
Albumin (g/L)	't'	2.77
	'p'	0.06
Plasma Glucose (mg/dl)	't'	3.11
	'p'	0.045*
Creatinine (mg/dl)	't'	-1.83
	'p'	0.10

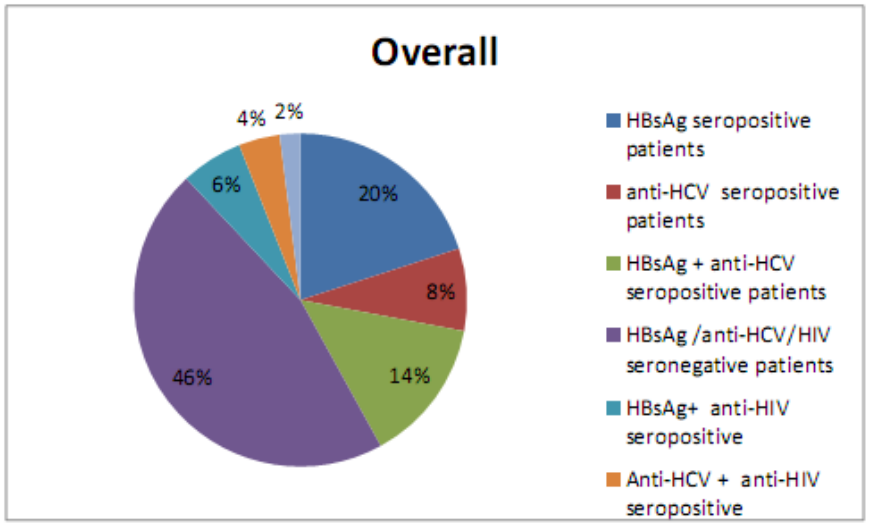

Fig-1: Frequency of Anti-HCV, Anti-HIV and HBsAg in the Patients

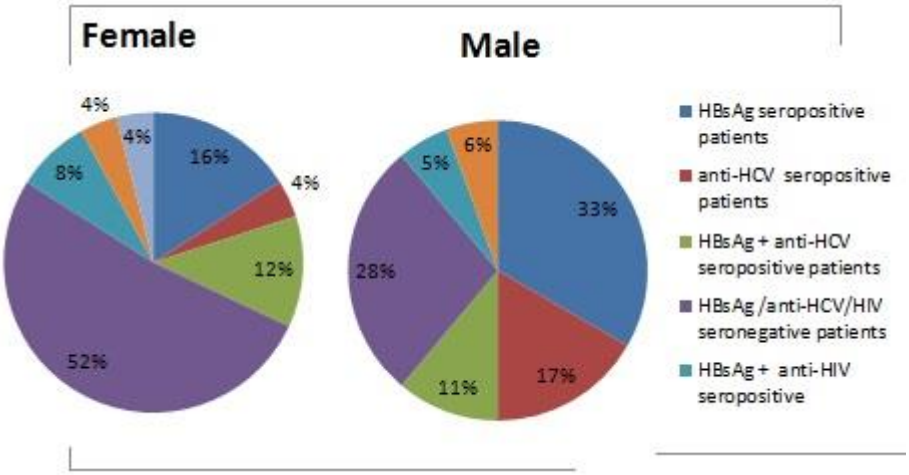

Fig-2: Gender distribution of Anti-HCV, Anti-HIV and HBsAg in the Patients

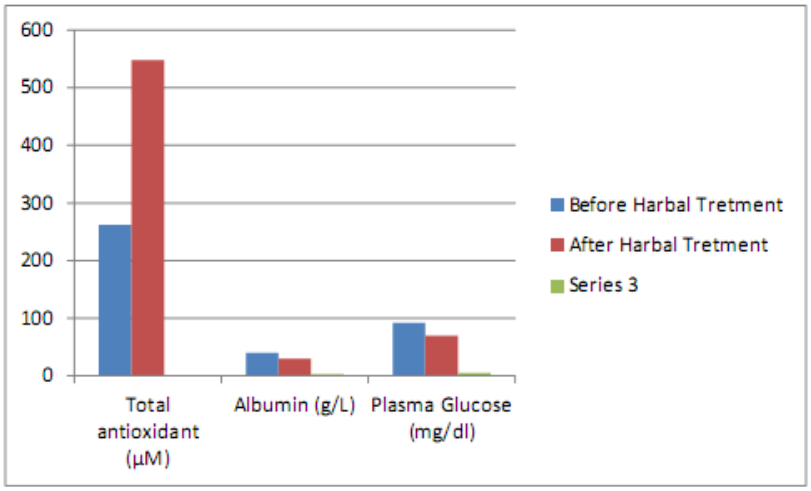

Fig-3: Plasma Value of Total antioxidant, Albumin and Plasma Glucose of Patient Before and After Herbal Treatment

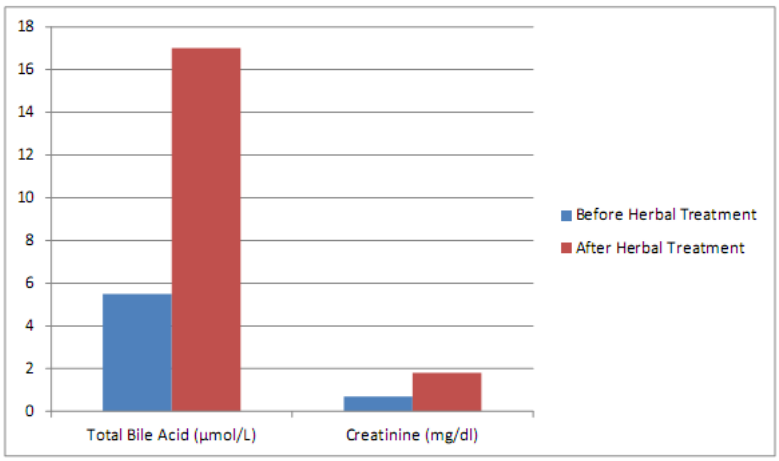

Fig-4: Plasma Value of Total bile and Creatinine of Patient Before and After Herbal Treatment

DISCUSSION

The overall immunochemical status obtained in the patients include 10(20%) HBsAg, 4(8%) anti-HCV , 7(14%) HBsAg + anti-HCV, 23(46%) HBsAg /anti-HCV/HIV seronegative, 3(6%) HBsAg + anti-HIV, 2(4%)Anti-HCV+ anti-HIV, 1(2%)Anti-HIV. Monoinfection of HBV, HCV and their coinfections were more frequent in the patients than the mono and coinfection of HIV. HBV, and HCV including their coinfections were more frequent in male than female while HIV was more in female patients.

The overall prevalence of anti-HCV among the students in this study was higher than those reported by Abiodun *et al.* [19] that of the 1572 Among Undergraduates in Ogbomoso, Southwestern Nigeria, 6 tested positive, giving an overall prevalence of 0.40%..

The prevalence of HBsAg and anti-HCV in this study was also higher than those reported by Chiekulie *et al.* [20] in a suburban University Teaching Hospital in South-East Nigeria that HBsAg positive participants were 9 (2.2%) while 3 (0.7%) were positive for HCV. No participant had triple infection of HIV/HBV/HCV. They concluded that Seroprevalence of HBV and HCV is low among HIV patients in Orlu. However there is a need for HBV and HCV testing of all HIV positive patients to reduce morbidities and mortalities from liver diseases. These could be due to traditional interventions without considering universal precautions. Another contributive factor is that the subjects were patients with clinical conditions which may not be well established by the herbal homes as most of their treatments as obtained from the herbal homes were majorly on measles, chickenpox, coughs, malaria, hypertension and Diabetes.

There was a significant increase in plasma Total Bile Acid and Total Antioxidant with a decrease in plasma glucose in the patients after than before herbal treatment.

Increase in Total Bile acid after treatment could result from toxicant phytoconstituents of the herb that could affect liver normal function and could also cause liver damage because the measurement of circulatory Total Bile Acids (TBA) provides information about hepatic functions and liver diseases such as jaundice, and hepatocellular injury. TBA estimation can detect liver damage during early stages and permits patients to get treatment before hepatic damages become irreversible [11].

The plasma increase in Total Antioxidant could be attributed to antioxidant constituents of the herbs like flavonoids. Antioxidants help to control or elimination of free radicals which can reduce cellular oxidation in the body providing an important defense against degenerate diseases caused by oxidative stress. Spices and herbs are rich sources of powerful antioxidants [21].

Decrease in plasma glucose after herbal treatment could be attributed to the antidiabetic activity of medicinal plants due to the presence of polyphenols, flavonoids, terpenoids, coumarins and other constituents which show reduction in blood glucose levels as reported by Patel *et al* [21].

CONCLUSION AND RECOMMENDATIONS

Monoinfection of HBV, HCV and their coinfections were found to be more frequent in the patients than the mono and coinfection of HIV. HBV, and HCV including their coinfections were more frequent in male than female while HIV was more in female patients. There was a significant increase in plasma Total Bile Acid and Total Antioxidant with a decrease in plasma glucose in the patients after than before herbal treatment. Routine immunochemical and biochemical assessment should be carried out on patients of herbal homes before, during and after herbal treatment for effective management.

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