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Original Article

Long-Term Management of Hepatitis C-Seropositive Subjects with AntiOxidant Biofactor (AOB®), a Fermented Food Supplement

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The efficacy of AntiOxidant Biofactor (AOB®) for the management of apparently healthy subjects with chronic hepatitis C infection was investigated. A total of 60 subjects (35 males, 25 females) participated in the trial. AOB was given orally in 2 packs (3 g per pack) 3 times per day. 17 subjects had taken AOB for 3 years, 31 subjects up to 2 years, and 41 subjects up to one year. The initial mean (SD) serum alamine aminotransferase (ALT) level was 46.3 ± 35.4 IU/L, and significant ($p < 0.05$, paired *t*-test) reductions in the mean serum ALT levels were observed at 6 months (38.6 ± 21.5 IU/L), 18 months (31.9 ± 18.1 IU/L), 2 years (31.2 ± 14.6 IU/L), and 3 years (28.0 ± 15.9 IU/L). Those presenting with high serum ALT levels (30 subjects) demonstrated significant levels ($p < 0.05$, paired *t*-test) of reduction in the mean serum ALT levels at 6, 12, 18, 24, and 36 months of treatment. No side effects were observed and the AOB treatment was well tolerated by all subjects.

Key words: hepatitis C, AntiOxidant Biofactor (AOB®), ALT level

Hepatitis C virus (HCV), an RNA virus, was first identified in the USA in 1989 as a major causative agent of post-transfusion non-A, non-B hepatitis [1]. The World Health Organization (WHO) has estimated that 170 million persons are chronically infected with HCV [2]. Although the acute infection is usually asymptomatic and may not be recognized clinically, the subsequent chronic infection is usually life-long and may lead to chronic liver disease and, ultimately, liver cirrhosis and hepatocellular carcinoma. In fact, in Japan, where the incidence of hepatitis B virus infection is low, HCV infection has been reported as the single most important etiological factor for the development of hepatocellular carcinoma [3].

The problem of transfusion-associated Non-A Non-B (NANB) hepatitis had been investigated in Myanmar since the mid-80s, with one study reporting that nearly 20% of blood transfusion recipients had contracted NANB hepatitis in that country [4]. It has been reported that hepatitis C virus infection is present in one-third of patients with hepatocellular carcinoma and in 2.5% of apparently healthy subjects [5]. The prevalence of hepatitis C seropositivity among 102,632 blood donors at the major blood banks in Yangon was found to be 2.84% (Paing Soe *et al.*,

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presented at the Myanmar Health Research Congress, Yangon, 2000). The prevalence of antibody to hepatitis C virus (anti-HCV) was found to be 2.8% among 569 subjects, aged 3 months to 74 years, residing in Yangon [6]. In recent study of 349 subjects aged 12 months to 70 years in a northeast border town of Myanmar, the overall anti-HCV positivity rate was even higher, or 13.5% [7]. Based on these findings, it could be estimated that there are more than 1 million persons with hepatitis C seropositivity in Myanmar.

Clinically, the proportion of HCV-infected patients who go on to develop chronic liver disease exceeds 80%, with up to 20% of chronic HCV cases progressing to liver cirrhosis, and 1–5% of HCV-infected patients developing HCC over a period of 20–30 years. However, the treatment options for HCV are limited and expensive. Therapy with interferon (IFN) alpha/beta subtypes or in combination with antiviral drugs such as guanosine analogue (Ribavirin) are generally considered as the first line treatment for chronic HCV infection. However, high doses of IFN may be associated with the development of many dose-related side effects [8]. A report that clastogenic factors were present in the plasma of 19 of 20 chronic hepatitis C patients but in none of the control subjects has led to interest in the use of antioxidants in the management of chronic hepatitis C patients [9].

Clastogenic—*i.e.*, chromosome-damage inducing—substances are present in the plasma of patients with a variety of pathological conditions accompanied by oxidative stress. These include irradiated persons, patients with inflammatory diseases of the connective tissue, the gut, the liver or the nervous system, and patients with HIV diseases [10]. Researchers from Paris conducted a clinical trial comparing Anti-Oxidant Biofactor (AOB®) to placebo in patients with chronic hepatitis C. They found that AOB treatment inhibited lipid peroxidation, increased glutathione levels and significantly reduced serum alamine aminotransferase (ALT) levels [11]. This study, together with studies on several other diseases [12, 13], has encouraged scientists to investigate the effects of AOB® in hepatitis infection.

We conducted the present study to determine the efficacy of AOB® for the long-term treatment of healthy hepatitis C-infected patients in Myanmar. The primary objective of the study was to determine the effects of AOB® on liver enzyme levels, and the secondary objectives were to determine the acceptance of AOB by the patients and to observe the side effects.

Subject and Methods

Study design. This open-label, single-arm, non-comparative clinical trial was carried out in Yangon, Myanmar during 2005 and 2008. Prior to the conduct of the study, ethical permission was obtained from the Institutional Ethical Committee of the Department of Medical Research (Lower Myanmar), and the study was conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki.

Subjects. Subjects were asymptomatic healthy individuals attending the Hepatitis Carrier Clinic of the Department of Medical Research (Lower Myanmar), Yangon, Myanmar, with seropositivity to hepatitis C virus. The subjects were adequately informed about the objectives and details of the clinical trial and characteristics of AOB and were offered inclusion in the study. Those willing to participate were asked to sign a consent form. The subjects were screened by history and physical examination before entering the study. All included patients were in apparently good health, were more than 18 years of age, and had been diagnosed HCV antibody-positive. Both men and women, and individuals with a broad range of liver enzyme levels were included. Exclusion criteria were (a) alcohol intake of more than 60 g/day and/or smoking of more than 15 cigarettes or 30 g of tobacco/day, (b) complications of liver disease such as cirrhosis or hepatocellular carcinoma, (c) HIV infection, (d) pregnancy, (e) lactation, and (f) the use of any antioxidant or hepatotoxic drug over the previous 3 months.

AntiOxidant Biofactor (AOB®). AOB® (AOA Company, Kobe, Japan) contains many biofactors and exerts many bioeffects as a result of the roasting and fermentation of the raw materials. The raw materials of AOB are rice germ (18%), rice bean (15%), soybean (20%), adlay millet (7.5%), sesame (4%), wheat (9%), green leaves extract (Japanese radish leaf; 10%), and citrus junos (Chinese lemon juice: 2.5%), which are mixed together with additional sesame extract. The ingredients are roasted

and fermented according to the traditional techniques used in Chinese herbal medicine.

Trial procedure. Each patient was given an explanation regarding the nature of study, and only those who gave voluntary consent were included in the study. Each patient had the right to withdraw from the study at any stage. A complete physical examination was performed and a complete medical history was obtained at baseline using standard proformas. Abdominal ultrasonography findings were recorded and a detailed physical examination was carried out to exclude serious medical problems including liver cirrhosis and hepatocellular carcinoma. Venous blood samples (3 ml) were collected to confirm HCV seropositivity by HCV PAII test kits (Ortho-Clinical Diagnostic; Fujirebio Inc., Tokyo, Japan). Hepatitis B surface antigen was detected using DMR ELISA and ALT levels were measured using ALT kits (Nichimen Company, Tokyo, Japan).

Assessment. Analysis was conducted for subjects who had taken AOB for more than 3 months. The following patients were excluded from the analysis: those who did not adhere to the schedule of clinic visits; those who required additional antiviral therapy; those who received medications with a potential effect on immunity; those who failed to meet the inclusion criteria; and those who met one of the exclusion criteria either before or after the start of the study.

Sample size calculation. Sample size calculation was based on the assumption of a natural reduction of ALT levels in 20% of subjects taking any treatment and a reduction of ALT levels in 70% of subjects taking AOB. A sample size of 36 subjects was considered sufficient with an alpha error of 0.05 and a power of 0.8 [15].

Statistical analysis. Statistical analysis was carried out using MINITAB® 14.1, (Minitab Corporation, State College, PA, USA). For continuous variables, descriptive statistics (mean + standard deviation) and for discrete variables, counts with percentages are presented. Successful treatment was defined as a significant reduction in ALT levels at 12, 24 and 36 months of therapy. ALT levels were compared using a pair-wise *t*-test (values of $p < 0.05$ were considered statistically significant). Those with initial ALT levels higher than or equal to 40 IU/L were regarded as having high initial ALT levels.

Results

Characteristics of the subjects. A total of 77 subjects volunteered to participate in the study, and 60 of these subjects fulfilled the criteria for assessment. Their mean age ± SD was 44.52 ± 10.45 years. There were 35 males and 25 females. There was no significant difference in the mean age (years) of males and females (43.03 ± 9.9 vs 46.6 ± 11.0). So far, 17 subjects have finished 36 months of therapy, and 31 subjects have finished 24 months of therapy (Table 1).

Serum ALT levels. The mean serum ALT

Table 1 Changes of serum ALT level by months of AOB therapy with special reference to the initially high ALT subjects

Mean serum ALT levels by months of AOB therapy						Subjects with initial ALT > =40 IU/L				
Months of therapy	Total number of subjects	Mean ALT levels ± SD (IU/L)	Paired "*t*" test T-value	*P*-value	Number of subjects with ALT < 40 IU/L (Proportion to total number %)	Initial number of subjects	Mean ALT levels ± SD (IU/L)	Paired "*t*" test T-value	*P*-value	Number of subjects shifted to ALT < 40 IU/L (Proportion to initial number %)
Initial	60	46.3 ± 35.4			30 (50.0)	30	65.4 ± 42.0			
3	60	41.3 ± 20.3	1.37	NS	35 (58.3)	30	52.2 ± 22.1	1.98	NS	10 (33.3)
6	50	38.6 ± 21.6	2.05	0.046	33 (66.0)	24	48.4 ± 26.2	2.76	0.011	12 (50.0)
9	43	39.3 ± 23.4	2.19	0.034	23 (53.5)	23	47.1 ± 26.0	2.91	0.008	8 (34.8)
12	41	39.6 ± 20.3	1.98	NS	25 (61.0)	22	46.0 ± 22.3	2.81	0.011	8 (36.4)
18	34	31.9 ± 18.5	3.94	0.005	25 (73.5)	18	38.4 ± 21.0	4.51	0.005	11 (61.0)
24	31	31.2 ± 14.6	2.92	0.007	24 (77.4)	17	32.1 ± 15.3	4.09	0.001	13 (76.5)
30	20	33.8 ± 17.2	1.67	NS	15 (75.0)	12	30.7 ± 11.4	3.25	0.008	9 (75.0)
36	17	28.0 ± 16	2.59	0.02	14 (82.4)	11	26.8 ± 13.4	3.65	0.004	9 (81.8)

SD, Standard deviation; ALT, alanine aminotransferase.

level on admission was 46.25 ± 35.41 IU/L with a minimum of 15 IU/L and a maximum of 253 IU/L. The mean ± SD ALT levels by months of AOB therapy are shown in Table 1.

A detailed analysis was carried out to determine the effect of AOB on subjects with high initial ALT levels. A significant decrease in the mean serum ALT levels was observed from 6 months to 36 months of AOB treatment (Table 1, Fig. 1).

Proportion of subjects achieving low levels of ALT. The proportion of subjects with a serum ALT level less than 40 IU/L was assessed. At the start of the trial 30 subjects (50%) had an ALT level less than 40 IU/L. An additional increase in the proportion of subjects with an ALT less than 40 IU/L was seen at each successive treatment time point. At 24 months of treatment more than 75% of the subjects had achieved low ALT levels (Table 1).

Discussion

Hepatitis C infection is an emerging health problem in Myanmar. Although significant results have been obtained by the national Control of Hepatitis C Project, a considerable population of hepatitis C-infected individuals still exists in Myanmar. Based on the prevalence of 2.8% HCV seropositivity in blood donors (Paing Soe *et al.*, presented at the Myanmar Health Research Congress, Yangon, 2000), it can be estimated that there are more than one million subjects with HCV infection in Myanmar. Although HCV infection can lead to chronic liver diseases and hepatocellular carcinoma, the treatment options are very limited. In addition, existing treatments are difficult and compliance is essential to achieve results. Current regimens are rigid and certain laboratory tests needs to be carried out during management. Dual therapy with interferon and antiviral combinations are the standard treatment regimens, but they are very expensive and are not 100% effective [8]. Moreover, interferon has to be given by subcutaneous injection and is associated with a long list of side-effects including flu-like symptoms, fever and depression.

AOB® is composed mainly of natural products and no side effects have been reported. It has been shown to inhibit liver injury in endotoxemic rats exposed to lipopolysaccharide (LPS). In that study, AOB suppressed LPS-induced superoxide generation and inhibited the decrease in glutathione levels in the rat liver. The liver enzyme levels were reduced and the histology of liver sections showed neither focal necrosis nor neutrophil infiltration in contrast to the untreated controls. The administration of AOB significantly decreased the mortality rate of the endotoxemic animals [15]. In another study, researchers from Paris conducted a clinical trial comparing AOB® to placebo in patients with chronic hepatitis C 15 patients with chronic hepatitis C infection received AOB and 15 received a placebo. AOB® was taken daily at a dose of 2 sachets (3 g of powder per sachet)

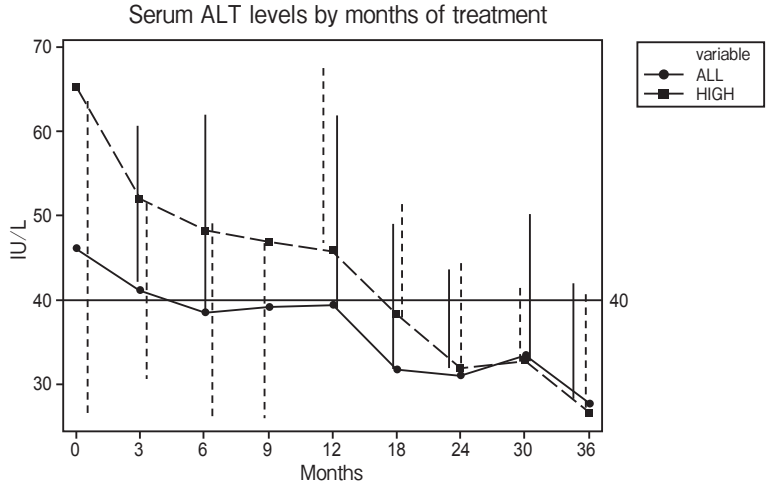

Fig. 1 The decline in ALT values by the months of AOB treatment in subjects with high initial ALT (dotted line) values and in all subjects (straight line) along with the standard deviation.

3 times a day for a period of 3 months. ALT and AST (aspartate aminotransferase) levels decreased in 11 of the 15 patients. Oxidative stress biomarkers, aminotransferase levels and viral load were determined immediately before and after treatment. Although a decrease in viral load was not observed, ALT and oxidative stress markers were reduced [11].

Our study has limitations. Assays for detection of oxidative stress, such as lipid peroxidation, total plasma thiols, and clastogenic factor tests were not available in our laboratory and were not carried out. Thus the effect of AOB® on oxidative stress could not be demonstrated. Also, we were not able to determine the changes in liver histopathology, as liver biopsies are not routinely carried out in Myanmar. Since AOB® is not an anti-viral agent, changes in viral load are not expected and were not determined in this study. Moreover, a previous clinical trial did not observe any reductions in the levels of viral load [11]. Although our paper is preliminary, it clearly showed a reduction of ALP level in association with the duration of AOB therapy, and the proportion was fairly remarkable after 24 months of therapy in subjects with an initially high ALP level (Table 1).

Because AOB is a fermented grain supplement, we speculate that one of the possible mechanisms by which it combats the progression of hepatitis C is through chelation effects on redox active free iron. That is, AOB contains a large proportion of low molecular weight components as a result of fermentation, and those components—for example, amino acids, nucleic acids and their derivatives, which are other low molecular weight organic acids—are well-known metal chelators, and they will remove or stabilize redox active iron in the liver. As hepatic iron has an unfavorable prognostic value and iron-reduction therapy by phlebotomy has a beneficial effect in patients with chronic hepatitis C [16, 17], it is worth investigating whether the chelation effect of AOB may have beneficial effects in patients with high ALT levels.

Drinking tea is a common practice in Myanmar and AOB is well accepted as a food supplement by the Myanmar people. The majority of subjects in the present study reported that AOB had a pleasant taste and increased their sense of well being. This is an important issue, as it is a known fact that many chronically infected HCV patients are severely disabled by the non-hepatic manifestations of HCV infection, mainly fatigue [18]. The lack of side effects also helped to increase the acceptance of AOB among our study population. From this study, it could be concluded that AOB is beneficial for the management of subjects with chronic hepatitis C infection, especially in subjects with high ALT levels. The high acceptance and lack of side effects could help to reduce the consequences of hepatitis C infection and would be of great benefit to individual patients.

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