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Management of Dengue Infection; a Prospective Study Highlighting the Effect of Silymarin on Liver Functions Test

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Abstract

Background: Dengue is a viral illness that globally affects around 50 million people annually and approximately 3.9 billion people are at risk to contract the infection. Liver is the major organ involved during the disease process. Silymarin is being used to cure liver problems for hundreds of years and have been proved to be a hepatoprotactive agent in many studies.

Objectives: 1) To evaluate the effect of Silymarin in dengue infection induced liver problems, primarily liver enzymes and, 2) to assess symptomatological improvement with the treatment of Silymarin in dengue patients.

Methods: A randomized controlled trial was conducted Ziauddin hospital, Clifton, Karachi.92 dengue patients were included in the study who were equally divided into two groups, A and B, which received silymarin and placebo respectively, along with the symptomatic dengue treatment. The laboratory parameters utilized to measure liver function were serum levels of direct and indirect billirubin, alanine amino transferase, gamma glutamyl transferase and alkaline phosphatase. The data were analyzed using SPSS version 20.0. Chi-square and independent samples t-test were used for inferential analysis whereas the significance level was set at 0.05.

Results: The study results revealed a significant difference only in the mean serum levels of sodium and indirect bilirubin in both study groups ($p<0.05$ for both). Moreover, none of the clinical features studied were found to be significantly different in both study groups ($p>0.05$ for all). Furthermore, none of the liver enzymes were found to significantly decrease after the use of Silymarin in the treatment group as compared to the placebo group.

Conclusion: The study showed no positivity in improvising liver function in the treatment group, as determined by the serum levels of liver enzymes. Furthermore, symptomatological improvement with the treatment of Silymarin in dengue patients could not be established definitely.

Key words: Management, Dengue, Silymarin, Liver Function Tests

Introduction

Dengue is a viral illness that has spread its adversity globally [1]. According to a study, around 50 million people are being affected annually [2]. There has been 128 countries which are facing endemic situation for dengue infection and approximately 3.9 billion people are at risk to contract the infection in these areas; furthermore, statistics has shown the rise of cases from 2.2 million to 3.2 million, between 2010 and 2015 [3]. This ailment is taking its substantial toll on the health care system [4], particularly in tropic and sub tropic areas of world [5].There are 4 different serotypes of the dengue virus [6]. Each of them is capable to cause infection [5]. Diagnosis in endemic areas is done on clinical basis [7]. One the other hand, the laboratory confirmation is achieved by using virus detection and serology tests [8]. Dengue virus is a known arbo virus [5], where Aedesaegypti mosquito serves as the main vector [4].The virus is transmitted to human body via bite from infected Aedesaegypti [4]. After incubation period of 4-7 days clinical picture is observed [4], while infection starts in skin [6], with attacking dendritic cells in skin and then spreading to T cells which release cytokines and other inflammatory mediators to fight the infection [9]. Most of the symptoms of the disease occur due to this interaction [9]. Most of the infected individuals remain asymptomatic or show mild febrile illness with approximately 5% presenting with distinctive symptoms and a very few experiencing severe form of disease [4]. The classical

presentation of disease include fever of sudden onset for 3-5 days plus muscle ache, pain at the back of eye, joint pain, severe head ache, nausea and rash [4, 6]. The disease is labeled as severe, if it additionally manifests with sudden severe thrombocytopenia, bleeding, multiple organ's failure or shock [6, 10].

Liver is the major organ involved during the disease process [11, 12]. Liver association in disease course is shown with variable level of contribution, ranging from rise in liver enzymes [13] to hepatic failure in severe cases [14]. Moreover, laboratory analysis of liver enzymes in dengue patients has shown an increase in aspartate amino transferase more than alanine amino transferase, in infected patient [15]. Despite the fact that hepatic association in dengue is greatly described but organ failure rarely occur [12]. Nevertheless severe liver involvement is associated with significant mortality [14]. Studies have shown frequent liver manifestations related to dengue these days [16]. Till present date there has been no approved treatment to target the elimination of infection [13] and all the management protocols revolve around symptomatic treatment to address fever and fluid losses [11].

Silymarin is an extract from Silybummarian, known as milk thistle plant [17]. It has been used for 2000 years in the management of liver diseases [17] and studies have shown significant properties of Silymarin for liver protection, most of which are attributed to its characteristic anti oxidative quality [17,18]. Many studies have proved its significant effects in improving disease states of patients suffering from liver malfunction [19], such as alcoholic liver cirrhosis [20], nonalcoholic fatty liver [21] and poisoning of Amanita phalloides [20].

As dengue is emerging in prevalence and recently showing variable disease presentations which not only include classic presentation but also targeting multiple organ involvement. Furthermore, considering the evolutionary nature of infection we need to be vigilant in understanding the manifestations as part of the disease and finding an ultimate solution for them. On the other hand, Silymarin is being used to cure liver problems for hundreds of years and have been proved to be a hepatoprotactive agent in many studies. It has shown promising results in not only halting the disease process but also augments healing.

This study intends to evaluate the effect of Silymarin in dengue infection induced liver problems, primarily liver enzymes. Furthermore, it was aimed to assess symptomatological improvement with the treatment of Silymarin in dengue patients. Considering the fact regarding the paucity of data on this subject, this study may open a door to the search of a valid solution for liver problems accompanying this infection.

Material and Methods

This study is a randomized controlled trial and was conducted for 1 year at outpatient department and inpatient setting of Ziauddin hospital, Clifton after taking ethical approval. The sample was collected using non probability sampling technique, with sample size established to be 92.

Informed consent was taken from every patient prior to data collection and all the information of participants has kept confidential. The qualifications for a candidate to enroll in the study were determined as follows: a diagnosed case of dengue on clinical basis; age of patient must be in between 18-70 years; patient must have positive serology test for dengue. All the patients having any of the following clause were disqualified from the study, these include: patients taking silymarin before study; patients experiencing other liver pathology; patients already engaged in any other clinical trial for new drug or device; patient who failed to give consent for the study.

An initial assessment was done to evaluate the eligibility criteria for each patient. This was accomplished using physical examination in addition to the interview for medical history. CRF was used as the guide for this assessment.

The placement of study subjects in one of the two groups ensue the initial evaluation. The study members were equally divided by simple random allotment through computer assistance, in these groups. Group A was labeled as trial group and members of this group received silymarin, in addition to the regular dengue treatment. Group B on the other hand was the control group and its members received placebo along with the dengue treatment. The laboratory parameters utilized to measure liver function were serum levels of direct and indirect billirubin, alanine amino transferase, Gamma glutamyl transferase and alkaline phosphatase.

The data entry was done using Microsoft excel while analysis for statistics was performed with the help of SPSS version 20.0. The frequency and percentage was described for clinical features of disease and the significant discrepancy among groups for these features was calculated using chi square test. On the other hand mean and standard deviation was stated for age, duration of fever and laboratory values. Likewise, t test was used for evaluating statistical significance for these variables in the two groups. Furthermore, level of significance was set to be 0.05.

Results

The data for this study was collected among 92 patients, diagnosed with dengue. After equally placing them in control and trial group, it was established that there were equal males and females in each group having the ratio of 2:1. The mean age of patients in each group was found to be 38.39±17.08 years in group A and 36.32±13.93 years in group B with no significant association (0.527) [table 1]. The duration of fever in both groups was noted as 3.30±1.28 days in group A and 3.84±1.46 days in group B, in addition to that there was an insignificant difference in duration of fever between group A and B (0.061) [table 1].

At the time of presentation, most common compliant observed was body ache with 46(100.0%) patients in group A and 44(95.7%) patients in group B experienced it, while that of 0(0.0%) patient in group A and 2(4.3%) patients in group B were not having body ache, the association was insignificant among groups (0.45) [table 2]. Correspondingly the least observed symptom among the candidates of the study was petechiae,

Table 1: Demographic characteristics and initial laboratory parameters in dengue patients (n=92)

Variables	Group A Mean ±S.D	Group B Mean ±S.D	p-value
Age (years)	38.39±17.08	36.32±13.93	0.527
Duration of Fever (days)	3.30±1.28	3.84±1.46	0.061
Hemoglobin (gm %)	13.89±1.87	13.92±3.04	0.957
Pack Cell Volume (%)	42.10±5.13	41.17±4.68	0.364
Total Leucocytes Count (103 cells / cumm)	3.62±1.55	3.78±1.68	0.644
Platelets(105 cells / cumm)	79.00±44.04	84.60±45.50	0.55
Na Sodium (mEq/L)	134.89±3.42	137.13±3.35	0.002
Potassium (mEq/L)	3.68±0.38	3.70±0.44	0.763
Urea (mg/dl)	19.26±7.53	19.41±6.67	0.919
Creatinine (mg/dl)	0.87±0.23	0.87±0.22	0.925
Serum Billirubin Direct (mg/dl)	0.20±0.12	0.25±0.18	0.172
Serum Billirubin Indirect (mg/dl)	0.251±0.14	0.42±0.42	0.011
Alanine Transaminase (U/L)	132.76±178.03	107.32±93.86	0.394
Gamma GultamylTransferase (U/L)	87.86±59.55	89.39±103.85	0.931
Alkaline Phosphates (U/L)	79.54±37.05	66.65±28.42	0.064

with only 1(2.2%) member in group A and 2(4.3%) members in group B had petechiae to begin with. Furthermore, 45(97.8%) of group A and 44(95.7%) of group B members were restrained from these lesions, also there was no significant association found among these groups on the basis of this symptom (0.50) [table 2]. Likewise, 37(80.4%) participants in group A and that of 28(60.9%) in group B experienced nausea and vomiting as a presenting complaint while 9(19.6%) of group A and 18(39.1%) of group B participants had no such symptom initially and this symptom has no significance in association among both the groups (0.247) [table 2]. Nonetheless, hepatomegaly was not at all observed in group A with only 1(2.2%) patient in group B had hepatomegaly primarily, at the same time 46(100.0%) candidates in group A and 45(97.8%) candidates in group B were found to be free from enlarged liver, moreover, no associated difference was seen in group A and group B with respect to hepatomegaly (0.50) [table 2].

Table 2: Initial clinical features in dengue patients (n=92)

Clinical Features		Group A		Group B		P-value
		N	%	N	%	
Nausea and Vomiting)	Yes	37	80.40%	28	60.90%	0.247
	No	9	19.60%	18	39.10%	
Bodyache	Yes	46	100.00%	44	95.70%	0.45
	No	0	0.00%	2	4.30%	
Headache	Yes	38	82.60%	36	78.30%	0.397
	No	8	17.40%	10	21.70%	
Retro Orbital Pain	Yes	26	56.50%	28	60.90%	0.416
	No	20	43.50%	18	39.10%	
Rash	Yes	36	78.30%	40	87.00%	0.205
	No	10	21.70%	6	13.00%	
Petechiae	Yes	1	2.20%	2	4.30%	0.5
	No	45	97.80%	44	95.70%	
Facial Flushing	Yes	31	67.40%	36	78.30%	0.174
	No	15	32.60%	10	21.70%	
Injected Conjunctiva	Yes	22	47.80%	26	56.50%	0.266
	No	24	52.20%	20	43.50%	
Hepatomegaly	Yes	0	0.00%	1	2.20%	0.5
	No	46	100.00%	45	97.80%	
Splenomegaly	Yes	0	0.00%	0	0.00%	0.5
	No	46	100.00%	46	100.00%	

Where presenting symptoms had shown no significant association, some of the initially recorded parameter showed significant association among group A and group B; these include serum sodium levels and serum indirect bilirubin levels. To be specific, initial serum sodium level in group A was assessed to be 134.89±3.42 mEq/L similarly, group B had the level of 137.13±3.35 mEq/L and a significant difference (0.002) was observed between serum levels of sodium in 2 groups [table 1]. Other laboratory parameter that showed significance (0.011) in difference of values in group A and B was serum indirect bilirubin level, where lab value was noted as 0.251±0.14 mg/dl in group A and 0.42±0.42 mg/dl in group B. Besides these, all other laboratory parameters monitored had no significance associated between the two groups [table 1].

The indirect bilirubin level was observed to be 0.24±0.14 mg/dl in group A and 0.45±0.50 mg/dl in group B at the start of study. While the values after 5 days of drug and placebo administration were 0.30±0.18 mg/dl in group A and 0.39±0.28 mg/dl in group B

Table 3: Effect of Silymarin on liver function tests in dengue patients on 5thday (n=92)

Groups	Variables		Mean	SD	p-value
Silymarin Group A	Serum Bilirubin Indirect (mg/dl)	Before	0.24	0.14	0.097
		After 5th Day	0.3	0.18	
	Serum Billirubin Direct (mg/dl)	Before	0.19	0.11	0.029
		After 5th Day	0.27	0.19	
	Alanine Transaminase (U/L)	Before	140.76	181.08	0.466
		After 5th Day	168.3	178.72	
	Alkaline Phosphates (U/L)	Before	79.52	37.56	0.487
		After 5th Day	85.89	49.55	
	Gamma Gultamyl Transferase (U/L)	Before	89.08	60.08	0.003
		After 5th Day	155.52	119.84	
Placebo Group B	Serum Bilirubin Indirect (mg/dl)	Before	0.45	0.5	0.412
		After 5th Day	0.39	0.28	
	Serum Billirubin Direct (mg/dl)	Before	0.25	0.19	0.338
		After 5th Day	0.28	0.14	
	Alanine Transaminase (U/L)	Before	114.39	99.67	0.222
		After 5th Day	145.45	146.27	
	Alkaline Phosphates (U/L)	Before	66.82	27.81	0.001
		After 5th Day	93.5	48.71	
	Gamma ltamyl Transferase (U/L)	Before	89.3	103.56	0.001
		After 5th Day	179.41	147.3	

with no statistical difference before and after drug administration (0.097). Nevertheless, the placebo administration in group B also did not show statistically significant alterations in indirect billirubin levels (0.412) [table 3].

As for direct bilirubin, the initial serum levels were recorded to be 0.19±0.11 mg/dl among group A and 0.25±0.19 mg/dl among group B. These levels were significantly altered (0.029) after Silymarin therapy in trial group, as the level in trials group on 5th day of drug administration was noted as 0.27±0.19 mg/dl. In contrast, the level in placebo group on 5th day was found to be 0.28±0.14 mg/dl which showed no significant alteration before and after (0.338) [table 3].

The serum Alanine amino transferase level in initial samples of group A was 140.76±181.08 U/L and after the drug trial it was measured as 168.30±178.72 U/L, hence no significant effect of drug was observed (0.466) [table 3]. Similarly group B had Alanine amino transferase levels of 114.39±99.67 U/L before and 145.45±146.27 U/Lat 5th day, which demonstrates no significant impact on Alanine amino transferase level due to placebo effect (0.222) [table 3].

Serum levels of Alkaline phosphatase showed no significant effect (0.487) of Sillymarin administration, as the measured level at the start were 79.52±37.56 U/L, whereas 85.89±49.55 U/L was the level measured at the end of the study [table 3].In contrast, the Alkaline phosphatase levels among the controlled group showed significant alteration (0.001) before and after the study with initial levels of 66.82±27.81U/L were found to be raised and recorded as 93.50±48.71 U/L after the study.

The trial group candidates had value of Gamma glutamyl transferase as 89.08±60.08 U/L in the beginning, which was raised with statistical significance (0.003), after the drug administration and was recorded as 155.52±119.84 U/L. Likewise, patients in placebo group, whom initially were having 89.30±103.56 U/L serum value of Gamma glutamyl transferase, showed to have a statistically significant impact of Placebo administration (0.001) and the level at the end of study was measured as 179.41±147.30 U/L.

Discussion

This clinical trial intended to study the effectiveness of silymarin's pharmacology to combat the disturbed liver profile in dengue patients. For the purpose, 92 dengue patients were enrolled in the drug trial and were divided in to two groups equally on the basis of demographic variables of age and gender. Group A served as a trial group for silymarin administration, while group B was a control group and received placebo.

Before commencing the drug trial, a thorough base line workup was done to get a clear picture of initial clinical presentation of patients. This workup concluded that dengue infection surely affected the liver function, which was demonstrated by elevation in alanine amino transferase and gama glutamyl transferase levels in serum of patients. For alanine amino transferase, group A showed value of 140.76±181.08U/L while group B showed 114.39±99.67 U/L. Similarly the serum levels of gama glutamyl transferase were 89.08±60.08 U/L in group A and 89.30±103.56 U/L in group B. These results are in congruence with the results drawn by Gandhi K et al, in which 27 diagnosed patients participated in the study and approximately everyone was found to have raised amino transferase levels in their serum [22].

During the observation for serum levels of bilirubin in dengue patients, Narasimhan D et al, found out that 5% patients had

increased levels of bilirubin [23]. Another study done on bilirubin levels in dengue infection reported to have a rise, in 3.1% patients [24].Our study in this regards demonstrated no deviation in serum bilirubin from normal levels at the start of the drug trial. In support of similar phenomenon, Fernando S et al discovered no noteworthy elevated bilirubin levels in samples of dengue patients, 33 of them having no sever disease were enrolled for the study and 31 (96.87 %) were found to have total bilirubin of <20µmol/L [25].

While discussing therapeutic significance of silymarin in promoting recovery of hepatic dysfunction there are different reviews. A meta analysis in this subject revealed that silymarin has shown efficacious results in viral hepatitis; non alcoholic steato hepatitis; drugs, toxins and amanitine related liver damage, whereas outcomes in primary biliary cirrhosis and hepatic cancer are not commendable [26]. Similarly, a drug trial on 170 patients with liver cirrhosis, whom were given 140 mg of silymarin in once daily dose for a period of 41 months, showed positive effects of the drug [27]. A different meta-analysis showed no positivity of the drug in curing hepatitis caused by viruses specifically hepatitis C virus [28].

The herbal medicine silymarin when specifically studied for its pharmacological benefits in recovering normal liver function as evaluated via laboratory parameters, also showed diverse illustrations in patients with significant derangements of liver function tests. Federico A et al. executed a drug trial on 85 patients; of them 59 were suffering from non alcoholic fatty liver disease while 26 were having chronic hepatitis due to HCV in addition to the non alcoholic fatty liver disease. This trial revealed improvement in serum levels of trans aminase and gamma glutamyl transferase, distinguishably in patients with only non alcoholic fatty liver disease[29]. Similar results were observed by Wellington K et al. for hepatitis due to multiple reasons, and a dose of 420 mg per day of silymarin, resulted in reducing the deranged values to normal, of serum amino transferease, bilirubin, gamma glutamyl transferase and alkaline phosphatase levels. The study also expresses the finding that these positive effects are not visible for hepatitis due to viral cause [30]. A prospective study for the period of 4 months was conducted in 190 patients whom were prescribed silymarin for raised amino transferase levels due to drug related toxicity. The observations drawn by the study shows pronounced decrease in serum levels of total bilirubin; alkaline phosphatase; alanine amino transferase; aspartate amino transferase and gamma glutamyl transferease, with majority of patients had normal liver enzymes levels at the end of the study [31]. Adrug controlled trial performed for the period of six months, in order to study the advantages of using a herbal medicine silymarin for the cure of liver disease. 36 participants suffering from chronic liver disease because of alcohol consumption were enrolled to the study and the trial group used silymarin in the dose of 420 mg/day for 6 months. The outcomes of this study differ from other studies and showed a remarkable effect in normalizing the liver enzymes, specifically gamma glutamyl transferase, trans aminase and bilirubin levels in serum [32]. The conclusions of these studies are incongruent to the outcomes of our study. We have not encountered any outstanding improvement at the end of the study, in the serum levels of direct bilirubin, indirect bilirubin, alkaline phosphatase and alanine amino transferease in participants of the study, whom were given silymarin. Various researches have described alike results. A double blinded controlled drug trial was done on therapeutic effects of silymarin in 105 cases of acute clinical hepatitis due to any pathology. The study concluded that silymarin bears no beneficence in improving liver function tests in these patients [33]. The silymarin drug trial on one hundred and five patients having acute hepatitis, conducted by El-Kamary et al, also supported the idea of ineffectiveness of the drug in addressing the deranged levels of amino transferase and direct bilirubin [33]. A research work executed by Saller R et al. on alcoholic liver disease describes no optimistic consequences of silymarin treatment in managing disturbed serum alkaline phosphatase value in comparison to the placebo [28].

On the other hand this drug trial disclosed a significant (0.003) raise in gamma glutamyl transferase in serum samples of group A participants. This remark is consistent with a meta-analysis on pharmacological benefits of silymarin in alcoholics, hepatitis B and a hepatitis C patient was done using 13 drug trials. The conclusions drawn reflect inefficiency of drug in benefiting the liver diseases, further it may cause some add on damage to the liver [34].

The group B of the study was given placebo and the results at the end of the study showed a remarkable increase (p value=0.001) in gamma glutamyl transferase levels in these patients. This finding may reflect the effect of infection, as Fernando S et al. studied the gamma glutamyl transferase levels in dengue patients and found that there is a persistently elevated serum of gamma glutamyl transferase in dengue patients the whole time of disease course [25].

Similar pattern of significant rise (p value=0.001) in the readings of serum alkaline phosphatase, at the end of study, were seen in placebo group. This may also be due to the effect of disease course alone. Narasimhan D et al. supported this idea with their study, in which 25% of dengue infected patients had a raised alkaline phosphatase levels[23].

The mixed approach of our study has assured that we have assessed the extensive range of patients with dengue infection and the effect of silymarin. However, the study might not be immune from observer and selection bias. Considering the views of our study and to what extent the effect of silymarin is consistent with the extensive follow up of the patients will be revealing to uncover more facts about the clinical features of the infection.

Conclusion

Our study demonstrated no positivity in improvising liver function in dengue patients, as determined by the serum levels of liver enzymes. Furthermore we also encountered some derangements in these parameters as the study continued. Furthermore, due to paucity of the enough study work on the very domain of silymarin effect it will be immature to declare its beneficial or detrimental effect in this regard and further studies are needed to be done to evaluate the potential of the drug.

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