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Research Article

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Prevalence and identification of fatty liver (FL) risk markers in local Pakistani population

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ABSTRACT

Nonalcoholic fatty liver (NAFLD) is an original disease, firstly recognized at the beginning of the second millennium in Western world, due to advanced life style and dietary habits. It is associated with less or no intake of alcohol and a chronic liver disease, also linked with several features of metabolic abnormalities. NAFLD has grown to epidemic proportion and is currently the most common cause of abnormal liver leading to cryptogenic cirrhosis. Moreover it is a frequent indication for liver transplantation. Extensive research work on NAFLD had been conducted in the Western world, however, its etiology and presentation has not yet been well studied in Asian populations including Pakistan. So, present study was designed to find the prevalence and identification of fatty liver (FL) risk markers in local Pakistani population.A well designed questionnaire and written informed consent was used to gather physical data and blood samples from patients showing symptoms of NAFLD. The set objectives were achieved by recording extensive physical data and biochemical parameters based on international standards like; fasting blood sugar and lipid profile. Liver specific biochemical and physical tests had performed using standard protocols and kit based methods. In conclusion, present study is helpful to find prevalence of NAFLD in local Pakistani population and identification of risk markers leading towards the development of this disease. This preliminary study made a strong basis for the correct diagnosis, treatment and management of FL.

Key words: Nonalcoholic fatty liver, Pakistani population, epidemiology.

INTRODUCTION

Fatty liver disease is a worldwide growing problem. It is a progressive disease leading to more severe forms of liver injuries. Normally liver contain some fat but if it exceeds 5% - 10% percent of the total weight then it is named as fatty liver (steatosis). The accumulation of triglycerides in the liver, in the absence of excess alcohol intake, has been described in the early sixties. Ludwig in 1980 named this condition nonalcoholic steatohepatitis (NASH)[1]. Formally it was considered the disease of adults but now it critically extends to lower age population as well. NAFLD includes conditions ranging from simple steatosis through steatohepatitis to end-stage liver disease (cirrhosis). It has also become the third most important indication for liver transplantation [2, 3]. Possible causes of

nonalcoholic fatty liver disease are metabolic, nutritional, inflammatory, use of drugs, environmental hepatoxins, and autoimmune hepatitis. Nonalcoholic fatty liver disease is also interrelated with a number of other diseases.

Beside nonalcoholic, alcoholic fatty liver disease (AFLD) includes the consumption of unnecessary alcohol that damages liver. There is strong and unvarying evidence on ethanol induced liver damage it has been reported that 30 gram of alcohol intake per day is the doorstep of alcohol induced liver damage[4]. Increased fat accumulation in liver or fatty liver disease can also be secondary to a number of causes, including excessive alcohol consumption, drugs (especiallychemotherapeutic agents e.g. methotrexate, tamoxifen), hepatic toxins (e.g. arsenic,carbon tetrachloride), chronic viral hepatitis (e.g. hepatitis B and hepatitis C viralinfection) and congenital storage diseases (e.g. Wilson's disease, hemochromatosis) [5].

A subset of diseases occurring in the same patient has been known as the metabolic syndrome that is the main cause for the development of nonalcoholic fatty liver disease[6]. Due to unhealthy dietary habits and a sedentary life style, there has been an extensive increase in the number of overweight and obese individuals all over the world. If left unchecked, this number may increase further. Unfortunately unhealthy life style adoption has also led to increased obesity prevalence in children as well, irrespective of geographical locations. NAFLD is mostly seen as clinically silent, and its impact has most likely been underestimated. Most findings on physical examination are also normal. If symptoms are present, it is minimal and nonspecific, such as fatigue and right upper quadrant discomfort [7].

Rapid weight loss and poor eating habits also may lead to NAFLD. Diagnosis includes exclusion of other liver diseases like excessive alcohol consumption must be excluded. History and physical examination should be performed along with biochemical analysis: including blood profile, serological test is conducted by examining blood serum, and imaging test includes: ultrasonography, computed tomography, and magnetic resonance imaging [8]. Presently there is no drug specific treatment present for NAFLD, therefore treatment strategies based on improving risk factors of the disease including calorie restriction, physical activities and the presence of antioxidants in the diet are recommended[9].

Non-alcoholic fatty liver disease is prevailing at a high rate in Western countries than Asian countries. The estimated overall prevalence is 15 to 40% in Western countries while 9-40% in Asian countries [10]. Based on differences in dietary habits and life style, NAFLD prevalence has been described as 10–39% in various populations including North America, Japan, Northern and Southern Europe, South America, Australia, and in the Middle East[11] .While no authentic data available from Pakistan. Therefore, the main aim of the present study is design study on NAFLD in local Pakistani population in order to investigate the prevalence of nonalcoholic fatty liver disease in local Pakistani population and to identify the risk markers associated with nonalcoholic fatty liver disease.

EXPERIMENTAL SECTION

Materials and Methods

Present study had been approved by Institutional Review Board and Ethics committee for the use of human subjects, PMAS Arid Agriculture University Rawalpindi.

Present study is population based epidemiology to find risk markers and prevalence rate of fatty liver in local population. For data collection, regular visits to general OPDs (Out Patient Departments) and gastroenterology/diabetic clinics of five local hospitals in Rawalpindi and Islamabad were made. The data comprised of two major sections; Physical parameters and Biochemical profile based on standard criteria set for the confirmation of fatty liver disease. Since metabolic syndrome (MS) is also a strong predictor of fatty liver, so MS susceptibility in interviewed subjects was also explored according to International Diabetes Federation (IDF) criteria.

For MS diagnosis, all patients and subjects having three or more of the following criteria were selected confirmed: increased BMI plus any two of the following four factors: High triglyceride levels >150 mg/dl; Low HDL-cholesterol < 40 mg/dl (men) and < 50mg/dl (women); High arterial pressure ≥ 130/85mmHg; High fasting glucose ≥ 100mg/dl.Instead of waist circumference, we have used BMI as a diagnostic component as BMI ≥27 is considered obese. The subjects were also asked to provide any history of hypertension, diabetes mellitus, dyslipidemia, myocardial infarction, stroke, as well as alcohol consumption and smoking. Questionnaire was designed for data collection and an informed consent for biological data (Blood sampling). The hospitals visited were; Pakistan Institute of Medical Sciences **(**PIMS**),** Rawalpindi General Hospital (RGH), Holly Family, Combined Military Hospital (CMH) etc. Subjects were called for a regular interview and the information gathered via questionnaires was fed into computer based Excel sheets as well as a hard copy was maintained for records. A total of 15 hundred

subjects, reporting to the selected hospital's OPDs, were interviewed but 1366 patients were included in the study as 134 subjects were excluded after confirmation of their being in upper age limit.

The fatty liver diagnosis is established by ultrasonography followed by the exclusion of the secondary causes of hepatic steatosis: Alcohol intake of 30 g/day or more for males and 20g/day or more for females, Wilson disease, intestinal bypass surgery, glutenicentheropathy, ingestion of drugs known to produce hepatic steatosis including methotrexate, tamoxifen, amiodarone, nucleoside analogues, a positive serology for hepatitis B or C virus, and a history of another known liver disease. Subjects above the age of 60 years and under 25 years and those diagnosed with Hepatitis were also excluded for correct analysis of fatty liver disease.

PHYSICAL DATA COLLECTION

The physical data criteria used for patient recruitment (incorporated in a questionnaire form) was as follows; Age (25-60), gender, level of physical activity, dietary habits, history of present/past illness, medication history, anti-diabetes and anti-hypertension treatments, alcohol intake, smoking habits, family history etc. For Body Mass Index (BMI) measurements, anthropometric data was recorded which included height and weight using standard metric scales via weighing balance and measuring tapes along with waist circumference, if permission granted, in some patients. Body weight was determined with subjects wearing light clothes and no shoes, using an electronic balance. Height was determined using a wall mounted, measuring tape with subjects in standing position and feet together.

The BMI was calculated using the formula. WC was measured with a tape measure at the narrowest part of the torso between the lowest rib and the level of the iliac crests at end expiration while standing BMI was calculated by formulas; Weight (Kg)/Height ($m^{2)}$. The blood pressure readings were recorded using digital BP measuring apparatus. In selected cases, abdominal ultrasonography parameters were also checked out for correct diagnosis of fatty liver [12]. Real-time ultrasonography (US) records of subjects were taken of the upper abdominal organs through two experienced physicians using a scanner equipped with a 3.5 mmHz transducer (Siemens Adama, German).

BIOCHEMICAL DATA COLLECTION

For the correct diagnosis of NAFLD, standard biochemical tests and assays are routinely performed using commercially available kits as well as recorded from available reports. Biochemical tests results, if available for patients, recorded were; Serum triglyceride, HDL-cholesterol, LDL-cholesterol, total cholesterol, fasting glucose and liver enzymes (***ALT and AST).***

RESULTS AND DISCUSSION

Table 1:Characteristics of the study population

Parameter	Mean ± SEM
Age	41.69±0.26
Weight	66.15±0.36
Height	1.65±0.002
BMI	24.37±0.13
B.P (systolic)	125.81±0.40
B.P. (diastolic)	84.74±0.30
BSF	102.063±1.27
Cholesterol	172.66±1.34
TG	143.05±2.35
HDL	45.65±0.34
LDL	105.14±0.91
ALT	29.13±0.39

Table 2:Age Wise Prevalence of Elevated Fatty liver (FL) Risk Factors inthe Study Subjects

Sample Parameters	Age Group A (20 to 40 years)	Age Group B (41 to 60 years)	Overall (20 to 60)
n	624	742	1366
Percentage with High TG levels	19.39	40.43	30.8
Percentage Low HDL Levels	21.31	38.14	30.45
Percentage With high cholesterol	13.3	24.8	19
Percentage With high LDL	11.53	22.51	17.49
Percentage high Systolic BP	13.30	27.49	21
Percentage with high diastolic BP	33.33	53.7	44.5
Percentage with BSF > 100	18.26	33.29	26.4
Percentage with BSF > 120	9.61	22.24	15.96
Percentage with high BMI >27	14.9	29.65	22.9

Table 3:Percentage Prevalence of Biochemical Risk Factors in US Confirmed FL Subjects

Biochemical Risk Markers	Males (%)	Females (%)	Total (%)
BMI	45	66.5	55.9
BP(SYS)	43.3	34.8	40
BP(DIA)	61.9	55	59.9
BSF	54.8	60	58
T.CHOLESTEROL	45	41.5	44.5
HDL	63	44	41
LDL	33	39	37
ALT	50	50	100

Table 4:Percentage Prevalence of Fatty Liver Disease among Two Age Groups

Gender	Age Group A 20-40 Years old	Age Group B 40-60 Years old
Males	24	78
Females	34	65
Total	26	73

QUESTIONARE FORM

Population History: Performa

	Date of data collection (dd/mm/yy)	☐☐ ☐☐ ☐☐☐☐
	Name of Participant	
	Full Address	Phone Number **Mobile:** **Home:**
	Age (dd/mm/yy) **Age Limit: 20-60 years**	☐☐ ☐☐ ☐☐☐☐
	Gender	MaleFemale ☐ ☐
	Available Clinical data; check levels of	Fasting plasma glucose_____ mg/dL Total cholestrol _____ mg/dL HDL cholestrol _____ mg/dL LDL cholestrol _____ mg/dL ALT _____ mg/dL Fasting TGs _____ mg/dL C-reactive protein _____ +/-
	Height	☐☐ cm ☐☐
	Weight	☐☐ Kg ☐☐
	Waist circumference	☐☐ cm ☐☐
	Hip circumference	☐☐ cm ☐☐

Performa for Blood Pressure Measurements

Blood Pressure Recordings	No. of Recordings	Systolic (mmHg)	Diastolic (mmHg)

	1st		
	2nd		
	3rd		

The current study includes data collection from about fifteen hundred subjects (fatty liver patients) visiting OPDs (Out patient Departments) of selected local hospitals in Rawalpindi and Islamabad to check prevalence of fatty liver (FL). Inclusion criteria were focused on subjects, whom were experiencing one or more internationally defined risk factors of FL disease like obesity, diabetes, metabolic syndrome and diagnosed negative viral liver infections i.e. hepatitis B and hepatitis C. In addition, we also focused to check fatty liver prevalence in adult subjects. Therefore subject exceeding the age of 60 years were considered elderly and excluded from the analysis as a number of health problems do arise only because of old age.

All data were collected using designed questionnaire and consent forms (Questionnaire form). The physical data consisted of anthropometric measurements (weight, height, waist circumference), blood pressure (systolic and diastolic) and to some extent dietary habits also recorded. Only BMI criteria were used to differentiate between over-weight and lean subjects. For BMI calculation, height and weight measurements were taken and their ratio was measured from overall body weight (kg) divided by height (m^2).

The biochemical test values collected were; BSF, Total cholesterol, HDL, LDL, Triglycerides, and liver enzyme ALT. Additionally, abdominal ultrasound records of fatty liver patients, confirmed by a hospital's gastroenterologist, were also checked. The abdominal ultrasound is considered a sensitive technique for the confirmation of extra fat deposits in a patient's liver. Details of FL risk factor and their contribution towards disease prevalence in present study are provided below.

Risk Factors for Fatty Liver Disease Diagnosis

Fatty liver (FL) disease (excess accumulation of fats in hepatocytes) has been significantly linked with factors that are already involved in MS. Thus the subjects affected by MS risk factors are also screened out to for the prevalence of FL. Therefore, in present study the IDF criteria were used to screen FL patients from those normally reporting all symptoms and signs of MS. According to IDF, metabolic syndrome (MS) is diagnosed in patients who have central obesity (waist circumference >94 cm in men and >80 cm in women) along with two or more of the following abnormalities; fasting blood glucose (≥100 mg/dL) and a patient being on use of glucose lowering agents, hypertriglyceridemia (Serum TGs ≥150 mg/dL), low HDL cholesterol (<40 mg/dL in men and <50 mg/dL in women), high blood pressure (≥130 mmHg systolic or ≥ 85 mmHg diastolic and/or one being on regular use of blood pressure lowering agents). In existing study all other parameters that may have been the cause of developing fatty liver disease were also recorded.

In present study, all patients with confirmed FL diagnosis did fulfill the criteria IDF. The average age of the whole study sample was 41.69 years, (42.29 years in men and 41years in women) as shown in Table 1. In overall records 34.52 % men and 35.34 % women were found to have MS, thus with an overall prevalence of 34.92 % for MS. Thus in MS diagnosed patients, 10.8% were also diagnosed positive for FL whereas only 3.8% prevalence was found among normal subjects. Out of them (14.7%) subjects were the FL on the whole was found as shown in Table 2.

Subjects having FL were found to be affected by one or more biochemical risk factors[13]. FL was found in both males and females with an average age of 42 and 41 years old respectively. The following overall risk parameters were found: obesity (22.9%), high glucose including impaired fasting glucose level and diabetes mellitus (26.4%), hypertension: systolic (21%) and diastolic (44.5%), hypertriglyceridemia (30.8%), low HDL-C (30.4%) and LDL cholesterol (17.49%). The average BMI of the study sample was 24.37 kg/m^2. Based on these results an overall prevalence of 34.92% was found for MS having ≥3 components based on modified IDF criteria as shown in Table 2. The age wise distribution FL prevalence was found to be higher in 41–60 age groups 73% than in 20–40 age groups and 26% respectively. A high prevalence rate with increasing age may be due high rate of over-weight/obesity and altered glucose regulation with advancing age. Though not recorded in this study, association of increased prevalence of insulin resistance in older people may also be related. In our study population, the MS associated risk factors found strongly linked in US confirmed FL subjects are; obesity (55.9%), high glucose (including impaired fasting glucose level and diabetes mellitus) (58%), hyper- tension systolic (40%) and diastolic (59.9%), Total Cholesterol (44.5%),hypertriglyceridemia (60%), low HDL-C (41%), high LDL (37%) and raised ALT level. So it could be concluded that the chances of FL has higher in older age as shown in Table 5 [13].

DEMOGRAPHIC CHARACTERISTICS

The overall prevalence of FL disease in study population was 14.7%. The distribution of FLD in two age groups was 26 % in 20-40 years old age group and 73% in 40 -60 years age group. Table 5 shows that males and females both were at low risk of FL in age group of 20-40, i.e. 24% males and 34% females. Similarly, MS seemed to be more

prevalence in both groups 40-60 years old such as 78% and 65% respectively as shown in Table 4. Total sample size of the present study was further grouped into four categories; Normal males (NM) 460, Diseased males (DM) 242, Normal female (NF) MS. 430 and disease females (DF) 235 comparatively. Fatty liver diagnosed subjects were 202 out of 1366, 54 were in non MS and 148 in MS. From the MS patients US confirmed FL male and female subjects were 85 and 63 respectively. FL was found to be more distributed in males as compared to female, 55.94% and 43.84% respectively [14]. The number of fatty liver confirmed patients found in non MS subjects were i.e. 3.7%, so a low prevalence of FL tends to exist in non MS subjects as compared to MS patients as shown in Table 2. Thus factors other than MS risk markers could also be responsible for causing fat deposits in liver. Thus we could conclude that MS risk factors are the major cause of FL in our study population.

ANTHROPOMETRIC PROFILE

The anthropometric measurements were obtained at the time the patients were being checked in to the clinic. The body mass index (BMI) is a proxy for human body fat based on an individual's weight and height. The cut off value for BMI were based on accepted criteria and obesity was labeled if the BMI $\geq$ 27 kg/m^2 because Asians have greater body fat at a given BMI. Our results showed a mean BMI of 25.68 in males and 26.95 in females with a 95% confidence interval (CI) in both males and females (25.27–26.09) and (26.3–27.6) respectively (fig 2). In both genders, prevalence of overweight BMI ($\geq$23kg/m^2) shows linear increase with age and was found to be more in males than females. The overall prevalence of BMI ($\geq$23kg/m^2) was high in 41-60 years of age group (29-65%) than 20-40 age groups (14.9%). BMI raised in FL subjects 45% in males and 66.6% in females. So BMI was extra risk factor for developing FL. BMI distribution among males and female normal and diseased subjects shown in Table 2 and Table 5).

HYPERTENSION PROFILING OF TOTAL STUDIED POPULATION

Our results showed a mean systolic blood pressure of 125.81(SD =14.94) with 125.53 (SD =14.6) in males and 126.113 (SD =15.28) in females. Mean diastolic blood pressure is found to be 84.7 (SD =11.4) with 84.4 (SD =11.25) in males and 84.1 (SD =11.6) in females. Both in males and females, the incidence of hypertension shows linear increase with age. Out of the total 1366 study subjects, 21% and 44.5% and out of 202 fatty liver subjects 40% and 59.9 % whereas out of the 477 metabolic syndrome patients, 49.5% and 80.5% showed higher than normal values of systolic and diastolic blood pressure respectively as shown in Table 2. which clearly demonstrate that hypertension is an important predictor of fatty liver disease that was due to Increased consumption of saturated fats, (high salt diets, carbonated drinks, juices and lower consumption of fruits, vegetables and legumesas well as decreased levels of physical activity are among the most important factors leading to higher ratio of hypertensive subjects in our population [15].

BIOCHEMICAL ANALYSIS

Biochemical analyses were performed on following risk parameters internationally identified for the diagnosis of fatty liver disease and its risk factors.

Blood Sugar Fasting Patterns in Total Studied Population

The overall percentage of IFG (impaired fasting glucose), and established diabetes in the study subjects has been found to be 26.4% and 15.96% respectively. Impaired fasting glucose levels have been seen in 22.1% of the males in the sample population and 30.9% females. In FL patients it was raised by 58%. It was found to be raised 54.8% in males and 60% in females FL confirmed patients. The present study shows that diabetes is more prevalent overall in females (33.3%) as compared to males (13.3%) as shown in Table 4. Also with advancing age, the incidence of impaired fasting glucose levels as well as diabetes increases and found to be less percentage in 20–40 age group as compared to 41–60 age group that was 9.6% and 22.24% respectively. Diabetes was the strong indicator or risk factor involved in increasing FL disease. As previously established, diets high in sugar, salt and fried items consumption is quite high in the local population.

Lipid Profile of Total Studied Population

In the entire sample, 30.8% subjects had high serum triglycerides, 30.45% had low HDL cholesterol and 19% had high total cholesterol and 17.49 % with high LDL cholesterol as compared to the normal reported figures. In FL patients this risk factors was increased; 60% subjects had high serum triglycerides, 41% had low HDL cholesterol and 44.5% had high total cholesterol and 37 % with high LDL cholesterol. Mean lipid concentrations were cholesterol, 172.66 mg/dl; triglycerides, 143.05 mg/dl; HDL cholesterol, 45.65 mg/dl; and LDL cholesterol, 105.14 mg/dl as shown in Table 2. Lipid profile was an important indicator for FL prevalence after diabetes and obesity. Total Cholesterol was found to be raised 45% in males and 41.5 % in females, TG in males 63.7% and in females 53.9, HDL in males 63% and in females 44% and LDL 33% and 39% were found in males and females. This showed high levels of TG and Total cholesterol had important role in developing FL along with low level of HDL as

shown in Table 4. With advancing age, the incidence of lipid abnormalities increases and so could be the risk of developing FL.

Fatty Liver and Raised AlanineAminotransferase (ALT)

Table 2 shows that High ALT level also related with occurrence of FL disease it found to be raised more in subjects with having MS (29.6%) as compare to non MS (11%). ALT serum concentration level was found to be high in FL diagnosed subjects that was 100%. ALT levels were found higher in males 21.4% then in female 14.3%.

OVERALL PREVALENCE OF FATTY LIVER DISEASE

To study prevalence of fatty liver disease, the study sample, (*n* =1366), was collected from local Pakistani population reporting for medical complications to hospitals in Rawalpindi and Islamabad. Out of 1366 subjects, 701 were male and 665 were female as shown in Table 2. Based on the criteria mentioned elsewhere, the physical and biochemical profiles for all subjects were recorded. The subjects diagnosed for FL based on biochemical parameters were also confirmed by their Ultrasonographic records from respective gastroenterology department of relevant hospitals. The complete data was statistically analyzed as already elaborated in materials methods section. Based on analysis an overall prevalence of FL was found to be 14.7% among 1366 subjects. The alarmingly high rate of FL among study population and the identified risk factors do indicate that MS seems to be the major cause of liver fats. Since over weight and obesity along with raised lipids and glucose were also prevalent among samples, so it further confirms that the root cause of liver problems seems to be MS.

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REFERENCES

[1] J. Ludwig, T. Viggian& M.C. GilldOttB, *Clin. Proc*,**1980**, 55, 434-438.
[2]A. Wieckowska, A. McCullough, A. E. Feldstein, *Hepatol*,**2007**, 46, 582-9.
[3]A. Feldstein, E. Canbay& A.P. Angulo, *Gastroenterol*, **2003**,125, 437-43.
[4]S. Bellentania, G. Bedognia, L. Migliolia& C. Tiribellib, *Eur. J. of Gastroenterol. &HepatoL*, **2004**, 16, 1087-1093.
[5] O.W. Hamer, DAG. Aguirre, J.E. Casola, M. Lavine, Woenckhaus, & C.B. Sirlin, Fatty liver: imaging patterns and pitfalls, *Radio. Graphics*, **2006**, 26, 1637-1653.
[6] K. G. Alberti, P. Zimmet, J. Shaw, *Diabet. Med*, **2006**, 23, 469-480.
[7] S. Ramesh, A.J. Sanyal, Semin*Liver Dis*, **2004**, 24, 399-413.
[8] P. L. Brian, M. Zobair&Younossi, *Ann. of Hepatol*, **2009**, 8(1), S51-S59.
[9] J.D. Browning, L. S. Szczepaniak, R. Dobbins, P. Nuremberg, J.D. Horton & J.C. Cohen, *Hepatol*,**2004**, 40, 1387-95.
[10] G.C. Farrell, C.Z. Larter, *Hepatol*,**2006**, 43, S99-S112.
[11]B.P. Mulhall, J.P. Ong & Z.M. Younossi, *J GastroenterolHepatol*,**2002**, 17(11), 113-643.
[12] J.E. Lavine, J.B. Schwimmer, *Clin. Liver Dis*, **2004**, 8(3), 549-558.
[13]C.M. Radu, D. Grigorescu, M. Crisan, D. Lupsor, Constantin & L. Dina, *J.Gastrointestin. Liver Dis*, **2008**, 3(17), 255-260.
[14]Y. Zhou, Y. Li, Y. Nie, J. Ma, L. Lu, S. Shi, M. Chen & P. Hu, *World J Gastroenterol*, **2007**, 13(47), 6419-6424.
[15]R. SinghBeegom, RB, *Intl. J. Cardiol*, **1997**,58(1), 63-70.