Bharath
INSTITUTE OF HIGHER EDUCATION AND RESEARCH
(Declared as Deemed-to-be University under section 3 of UGC Act, 1956)
(Vide Notification No. F.9-5/2000 - U.3, Ministry of Human Resource Development, Govt. of India, dated 4th July 2002)
Accredited by NAAC NATIONAL ASSESSMENT AND ACCREDITATION COUNCIL
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Chennai - 600 073. Tamil Nadu.

Ref. No.SMS-2015-O-09 Date: 17.02.2017

TO

Ms. T. Mohanalakshmi
Associate Professor/Microbiology
BIHER

BHARATH INSTITUTE OF HIGHER EDUCATION & RESEARCH (Declared as Deemed to be University u/s 3 of UGC Act 1956) 17.02.2017 OFFICE OF THE DEAN-RESEARCH Selaiyur, Chennai-600 073. INDIA

Thro: Concern Head of the Department

Greetings!!!

We are happy to announce that the Research Advisory Committee has approved your proposal for Seed Money Scheme-2015 which was presented by you. You are requested to complete the proposal and send the progress report to the Dean Research in the prescribed time period.

Title of the Project: Triglycerides fasting or non-fasting? Current knowledge in diagnostic values

Seed Money Amount: Rs.1, 00,000/- (Rupees One Lakh Only)

Approved on: 15.02.2017

Payment details:

Voucher No.30 **Dated: 24.03.2017**

With Regards

Dean-Research

Bharath University

SELAIYUR, CHENNAI - 600 073, TAMIL NADU, INDIA.

CASH / PAYMENT VOUCHER

Date 24/03/2017

V.No. 30

Debit _____ Amount

Rs. 1,00,000/-

PAID TO Dr. T. MohanaLakshmi

RUPEES One Lakh only

TOWARDS Seed Money Scheme-2015

BHARATH UNIVERSITY CHENNAI 600 073. INDIA.

Authorised by **Finance Manager** **Cashier/Accountant** **Payee's Signature**

PROPOSAL SUBMISSION

1. **Details of Principal Investigator**

Name	: Dr. T. Mohanalakshmi
Designation	: Associate Professor
Highest Qualifications	: Ph.D.
Department	: Microbiology
E-mail	: drpebyreddy@yahoo.com
Contact no	: 9849616163
Date of Joining	: 10.06.2014

2. **Details of Principal Investigator**

Name	: Dr. E. Prabhakar Reddy
Designation	: Professor
Highest Qualifications	: Ph.D.
Department	: Biochemistry
E-mail	: drpebyreddy@gmail.com
Contact no	: 9159186879
Date of Joining	: 21.10.2009

Technical details

1. Introduction:

Basically fasting state is essential for triglycerides estimation because as mentioned above it remains high for several hours after meal and the Friedewald equation, used for calculation of LDL cholesterol (LDL cholesterol= total cholesterol – HDL cholesterol - [triglycerides/5]), uses fasting triglycerides value. If non-fasting triglycerides value is used in this equation the LDL cholesterol, the primary target of lipid lowering therapy, will be underestimated. However, this problem can be overcome to some extent by using direct LDL cholesterol estimation as this can be done in non-fasting specimen. As a fast-food meal consisting of e.g. a burger, a shake, and fries might be considered a fat tolerance test, in areas where fast-food consumption is especially high patients may be advised to avoid high-fat, fast-food meals on the day of lipid profile testing. Also, as LDL cholesterol is often calculated by the Friedewald equation, which includes the triglyceride concentration, calculated LDL cholesterol has been thought to be affected substantially by food intake; however, directly measured and calculated LDL cholesterol values are similar using both fasting and non-fasting lipid profiles (Tanno et al., 2010; Mora et al., 2009). Fasting Triglyceride and non-fasting Triglyceride levels If this Friedewald equation is employed, there may be some underestimation of LDL cholesterol when chylomicrons are present, which may even be circumvented if a modification of this equation is used (Hegele et al., 2014). Lipid-lowering trials have used fasting lipid measurements and, in order to follow evidence-based practice, fasting blood sampling has often been the standard in everyday risk assessment. There is evidence that the non-fasting condition may marginally lower plasma LDL cholesterol concentrations owing to liberal intake of fluids, and therefore lead to a potential minor misclassification of cardiovascular risk, as well as to error in initiating or altering lipid-lowering medication; although not all studies agree, this risk is small and may chiefly apply to diabetic subjects (Martin et al., 2013; Watts et al., 2011; Langsted et al., 2011). While a non-fasting sample is sufficient to diagnose an isolated hypercholesterolemia, such as familial hypercholesterolemia, or elevated Lp (a), it can possibly confuse the dist inction between familial hypercholesterolemia and genetic forms of high triglycerides. Since non-fasting may therefore impair the accuracy in diagnosing some forms of hyperlipidaemia, we recommend that laboratories should also offer measurement of fasting triglycerides according to clinical context and indications, as in the case of very high non-fasting triglyceride concentration. Life-threatening or extremely abnormal test results deserve special attention and reactions of the clinical biochemical laboratory. In this regard, the following extreme hyperlipidaemias should be noted: triglycerides.10 mmol/L (880 mg/dl) because of risk of acute pancreatitis, (Klop, 2011; Sidhu et al., 2012) LDL cholesterol .5 mmol/L (190 mg/dl) in adult's or.4 mmol/L (155 mg/dL) in children and particularly (Sidhu et al., 2012). mmol/L (500 mg/dl) because of suspicious h e t e r ozygous and homozygous familial hypercholesterolaemia,(Steiner et al., 2011; Lund et al., 2011; Wyler von ballmoos et al., 2015) respectively, and Lp (a).150 mg/dL (99th percentile) for very high risk of myocardial infarction and aortic valve stenosis (Nordestgaard et al., 2013; Cuchel et al., 2014; Wiegman et al., 2015).

It is also important to refer patients with very low concentrations of LDL cholesterol, apolipoprotein B, HDL cholesterol, or apolipoproteinA1 to a specialist lipid clinic for further evaluation of a major monogenic disorder of lipid metabolism Each country, state, and/or province in individual countries should adopt strategies for implementing routine use of non-fasting rather than fasting lipid profiles as well as flagging of abnormal values based on desirable concentration. At present, the majority of guidelines recommend a fasting serum lipid test. This recommendation is based on achieving consistency between patients and over multiple tests by ensuring a relatively standardized metabolic state. It is also because the majority of research has been performed using fasting lipids; therefore it was assumed that making comparisons and analyzing risk would be less precise if using non-fasting tests. Triglyceride storage and circulation in blood is kept under control by several enzymes, which are regulated by many genes and they reflect the nutritional needs of organism including fasting and non-fasting status (Nordestgaard et al., 2010; Thanassoulis et al., 2013). In addition, triglycerides are closely associated with many other lipid factors. They are positively associated with atherogenic LDL cholesterol, presence of small LDL particles, remnant lipoproteins, apolipoprotein C-III (Apo C-III) and negatively with athero protective HDL cholesterol and apolipoprotein C-II (Apo C-II). This implicates that reliable assessment of the real and independent role of circulating triglycerides in the process of atherosclerosis and other diseases in epidemiological and clinical studies is difficult and that robustness of their association with cardiovascular disease depends more on the extent of statistical standardization for other risk and lipid factors than on their real biological role. Factors affecting Triglyceride levels and related risk Triglyceride levels are increased for six to eight hours after a standard meal (Kamstrup et al., 2014; White et al., 2010). If a patient has consumed a very high fat content meal prior to testing, or if they have slow lipid particle clearance after food (post-pandrial dyslipidaemia), triglyceride levels could be increased more than the estimated 0.3 mmol/l variance, and misrepresent clinical significance. Measuring non-fasting triglyceride levels may provide additional information for determining cardiovascular risk. Peak nonfasting triglyceride levels, four hours after a meal, are reported to be a strong predictor of cardiovascular events and insulin resistance, and risk equations may be developed based on these levels in the future (Kamstrup et al., 2014; White et al., 2010).

Light to moderate drinking, e.g. one to three standard drinks per day for males or one to two standard drinks per day for females, has very little acute effect on triglyceride levels (Patel et al., 2004).However, excessive alcohol intake may cause an increase in triglyceride levels immediately following intake and after fasting (Patel et al., 2004).When alcohol consumption is accompanied by a meal containing fat it has a significant additive effect on the resultant triglyceride increase (Patel et al., 2004).

2. Review of status of Research and Development in the subject

Sundvall J, Laatikainen T, Hakala S, Leiviska J, Alfthan G. Systematic error of serum triglyceride measurements during three decades and the effect of fasting on serum triglycerides in population studies. Clin Chim Acta. 2008; 397:55–59.

Recently, we derived correction factors whereby insufficiently fasted/postprandial serum Tg values can be converted into "corrected" fasting values [15]. In view of the potential error resulting from using non-fasting serum Tg values in the calculation of LDL-C and consequently in the classification of subjects into the metabolic syndrome, we transformed non-fasting data to fasting data using our recently published factors [15].

2.1. International Status:

Serum lipid profile is measured for cardiovascular risk prediction and has now become almost a routine test. The test includes four basic parameters: total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. It is usually done in fasting blood specimen. Fasting refers to 12–14 h overnight complete dietary restriction with the exception of water and medication. This may hold true due to two main reasons: post prandial triglycerides remain elevated for several hours, most reference values for serum lipids are established on fasting blood specimen. NCEP and European guidelines also recommend doing lipid profile in fasting blood specimen for assessment of cardiovascular risk. However, these guidelines allow total and HDL cholesterol in the non-fasting specimen as these lipids are not much different in fasting and non-fasting specimens. In addition, non-HDL cholesterol (total cholesterol − HDL cholesterol), a secondary target of therapy in adult treatment panel III, may also be used in the non-fasting state.

2.2. National Status:

NIL

3. Progress/ achievement so far, if any

a). Reference papers was collected.
b). Literature survey was studied.
c). Materials and methods were designed.

4. Work plan

4.1 Methodology

The National FINRISK 2007 Study (FR07) is the eight consecutive population-based risk factor survey in Finland. The surveys have been carried out with 5-year intervals since 1972 and the sample sizes have varied from 6500 to 13 500 men and women, depending on the survey year. The total sample size of the FR07 study was 9957 persons in the age range of 25-74 years. It was a random sample, drawn from the population register and stratified according to sex, 10-year age group and five geographical areas. Of the 9957 invited persons, 6247 (62.7%) took part in both the health examination and blood sampling [3]. During the year 2007 the same subjects participated in health examinations twice. In January-April, subjects were requested to fast for at least 4 hours before phlebotomy. The participants of the

surveys were asked the time in full hours since their last meal by a study nurse. For the present analysis (non-fasting FR07, visit 1) eligible were those who reported having fasted ≥2 to ≤8 hours (1979 men) and ≥2 to ≤7 hours (2303 women) based on our earlier work on effects of fasting on Tg values [15]. In the substudy carried out in May-June the same participants were requested to fast for 10 hours (true-fasting FR07, visit 2).

In addition to the two groups described above, we identified a separate group drawn from the FR07 sample comprising 552 subjects who fulfilled the following fasting criteria: ≥8 hours for men and ≥7 hours for women in visit 1 and who had also participated in the true-fasting study, i.e., visit 2. These subjects are referred to as the Reference Group and they are not included in either of the above parent population samples. Individuals with serum Tg values >10 mmol/L were excluded from all analyses. In addition to reporting the results on all men and women, we defined categories of healthy men (n = 824) and women (n = 994) as those who had a BMI ≤35; reported an alcohol consumption below the 90th percentile in self-reported questionnaire; had no diagnosed cardiovascular disease (CVD), diabetes or cancer; had no medication for hypercholesterolemia; and had normal blood pressure. The results are also presented for subgroups with severe obesity BMI>35 (men n = 101, and women n = 183). Subjects were classified into a high LDL-C group by using the cut-off value ≥3.00 mmol/L calculated by the Friedewald formula [16]. Subjects were classified as having the metabolic syndrome according to three different definitions [9-11].

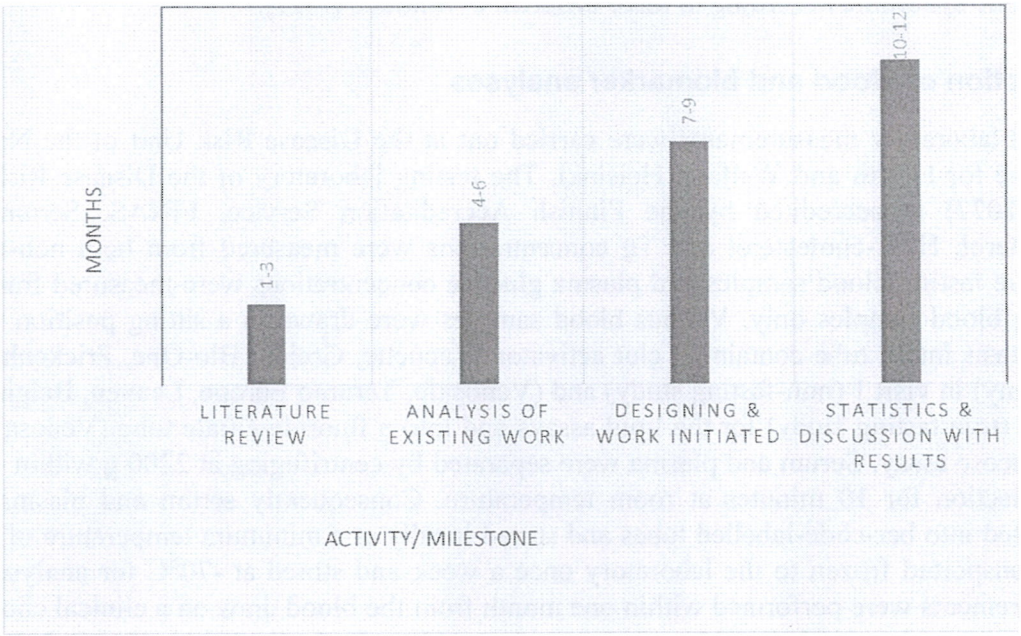
Collection of blood and biomarker analyses

All laboratory measurements were carried out at the Disease Risk Unit of the National Institute for Health and Welfare, Helsinki. The testing laboratory of the Disease Risk Unit (No. T077) is accredited by the Finnish Accreditation Service, FINAS. Serum total cholesterol, HDL-cholesterol and Tg concentrations were measured from both non-fasting and true fasting blood samples and plasma glucose concentrations were measured from true fasting blood samples only. Venous blood samples were drawn in a sitting position with a light stasis into a tube containing clot activator (Vacuette, Greiner Bio-One, Frickenhausen, Germany) in visit 1 (non-fasting study) and (Venosafe, Terumo Europe, Leuven, Belgium) in visit 2 (true fasting study) for the lipid assays and into a fluoride-citrate tube (Venosafe) for the glucose assay. Serum and plasma were separated by centrifuging at 2200 g within 1 hour of collection for 10 minutes at room temperature. Consequently serum and plasma were aliquoted into bar-code-labelled tubes and stored locally at a minimum temperature of -20°C and transported frozen to the laboratory once a week and stored at -70°C for analyses. All measurements were performed within one month from the blood draw on a clinical chemistry analyzer, Architect c8000 (Abbott Laboratories, Abbott Park, IL, USA). Total cholesterol, HDL-cholesterol, Tg and glucose concentrations were determined enzymatically using commercial reagents from Abbott Laboratories.

For standardising the measurements, the laboratory has taken part in the Lipid Standardization Program organised by the Centers for Disease Control and Prevention (CDC), Atlanta, USA and External Quality Assessment Schemes organised by Labquality, Helsinki, Finland. During the course of the study comprising 6 months in 2007, the precision between series expressed as coefficient of variation (CV_a) was less than 1.5% for all analytes except for HDL-cholesterol whose mean CV_a was 2.3%. The mean (SD) systematic errors (bias) were 0.8% (0.5%) for total cholesterol, -0.6% (1.4%) for HDL-cholesterol, -1.1% (1.2%) for Tg and 0.0% (2.7%) for glucose.

4.2 Time Schedule of activities giving milestones through BAR diagram. (Maximum of 1/2 pages)

S. No	Activity/ mile stolen	1st Year			
1	Literature review	**1-3 month**			
2	Analysis of existing work	-	**4-6 month**		
3	Designing & work initiated	-	-	**7-9 month**	
4	Statistics & Discussion with results	-	-	-	**10-12 month**



4.3 Expected outcome within the time period of See Money Scheme

There are two inherent sources of variability in cholesterol and triglycerides measurements: biological and analytical, Biological variation is <5% for cholesterol, LDL cholesterol, and HDL cholesterol and 20 to 30% for triglycerides, Considerable variation can occur from one assay to another between clinical laboratories, for patient care, it is important to know if the LDL is calculated or is measured directly. In order to compare results from different laboratories, it is important to know which assay method is utilized. If patient is non-fasting, a direct LDL test is recommended. Sudden changes in lipid values may indicate a change in diet, medications, or onset of a new disease state. We have shown that there are a number of clinical scenarios in which fasting lipids offer valuable clinical information, but that in others, non fasting lipids will suffice. To assess the initial risk of CVD in an untreated patient, fasting or non fasting total cholesterol and HDL-C levels provide all that is needed. Understanding a patient's metabolic burden can provide a useful baseline for lifestyle counseling. Although a diagnosis of metabolic syndrome requires a tally of metabolic risk factors measured in the fasting state, it can be approximated for practical purposes by non fasting results. Among those with a non fasting triglyceride level 200 mg/dl, a follow-up fasting lipid panel should be performed. Those who present with secondary causes of hyperlipidemia (due to diet, drugs, diseases, or disorders of metabolism) should have a fasting lipid panel performed. Indeed, it may be important for those about to initiate therapy with estrogenic hormones, steroids, retinoic acid, or certain anti neoplastic agents to understand their propensity for severe hypertriglyceridemia and subsequent risk of pancreatitis.

5. Suggested Plan of action stating the name of funding agency where the project will be communicated for financial support within the time period of project.

Nil

6. Bibliography: Nil

Nil

7. List of Projects submitted/implemented by the Investigators (Separate for Pi and Co-PI)

7.1 Details of Projects submitted to various funding agencies:

S.No	Title	Cost in Lakhs	Month of Submission	Role as PI/Co-PI	Agency	Status
1	NA	NA	NA	NA	NA	NA

7.2 Details of Projects under implementation

Sl. No.	Title	Cost in lakhs	Duration	Role as PI/ Co-PI	Agency
1	NA NA	NA	NA	NA	NA

7.3 Details of Projects completed during the last 5 years

Sl. No.	Title	Cost in lakhs	Duration	Role as PI/ Co-PI	Agency
1	NA	NA NA	NA	NA	NA

8. List of publications published by the Investigators, if any:

a) Principal Investigator

S. No	Author names	Title of paper	Name of Journal	Vol (Issue)	Page No.	Year
1.	**T Mohana Lakshmi1*,** BS Ravi Kiran2, P Jayakumar3, R Srikumar4, E Prabhakar Reddy5.	High Sensitive C – Reactive Protein in Hypertension and Metabolic Syndrome.	Research Journal of Pharmaceutical, Biological and Chemical Sciences	7(6)`	2017-2021	2016
2.	**T Mohana Lakshmi 1,** Chidambaram2 , A Vaithialingam3 , and E Prabhakar Reddy4 *	Advantages of Stem Cell Research: Role of Medical therapy in India.	Research Journal of Pharmaceutical, Biological and Chemical Sciences	5(3)	96-99	2014

3.	E Prabhakar Reddy , **T.Mohana Lakshmi** , Shankar Manohar Pawar	Antioxidants Status in Haemodialysis Patients	Int J Biol Med Res.	3(1)	1466-1468	2012

b). Co-Principal Investigator

S. No	Author names	Title of paper	Name of Journal	Vol (Issue)	Page No.	Year
1.	Kalpana Thalaval, ***E Prabhakar Reddy2**, and A Vaithilingam3.	HCG and CA-125 Levels In Pregnancy And Abortion Patients.	Research Journal of Pharmaceutical, Biological and Chemical Sciences	8(2)	2745-2749	2017
2.	1B. Sai Ravi Kiran*, 2T. Mohana Lakshmi, 3R. Srikumar, **4E. Prabhakar Reddy**	Total Antioxidant Status and Oxidative Stress in Diabetes Mellitus and Metabolic Syndrome	International Journal of Pharmaceutical Sciences Review and Research	40(1)	271-277	2016
3.	V Kowsalya, R Vijayakumar, R Chidambaram, R Srikumar, **E Prabhakar Reddy,** S Latha, I Gayathri Fathima, C Kishor Kumar	A study on knowledge, attitude and practice regarding voluntary blood donation among medical students in Puducherry, India.	Pakistan Journal of Biological Sciences	16(9)	439-442	2013

9. Budget

SI. No	Head	Amount (Rs.)
1	BP Apparatus, Stethoscopes, Body weight weighing machine, SPSS version 16 Chicago, IL, USA, ECG machine	50,000/-
2	Consumables (gels bottles, cotton, sprit, testing charges, tools, etc.)	25,000/-
3	Travel support for the purpose of research work.	10,000/-
4	Contingency	10,000/-
5	Others consumables	5,000/-
	Total	**1,00,000/-**

*In case of any joint proposal for purchasing a same equipment, each of the associated PLs is also required to give separate budget (without any clubbing) to avoid any ambiguity, if all the associated projects are not awarded by committee.

10. Name of at least two subject experts from the Institute and one from the outside Institute with their contact details:

1. **Dr. Praveen Kumar. V** Associate Professor in Microbiology, Chalmedha Anand Rao Institute of Medical Sciences, Karimnagar, Telanagana **Mobile No**: 8332063265 **E-mail id:** vpraveenkumar4@gmail.com	2. **Dr. Patta Appa Rao** Professor in Microbiology NRI Medical College, Vishakapattinam **Mobile No**: 9848766293 **E-mail id:** pattaapparao@yahoo.com

CERTIFICATE FROM THE INVESTIGATOR

Project Title: Triglycerides fasting or non-fasting? Current knowledge in diagnostic values

It is certified that

1. I do hereby agree to submit a complete proposal for financial support to the external funding agency within the time period of SMS-2015.
2. I undertake that spare time on equipment procured in the project will be made available to other users.
3. I agree to submit a certificate from Institutional Biosafety Committee, if the project involves the utilization of genetically engineered organisms. I also declare that while conducting experiments, the Biosafety Guidelines of Department of Biotechnology, Department of Health Research, GOI would be followed in to.
4. I agree to submit ethical clearance certificate from the concerned ethical committee, if the project involved field trails/experiments/exchange of specimens, human & animal materials etc.
5. I agree to abide by the terms and conditions of SMS-2015, BIHER, and Chennai.

Name and signature of
Principal Investigator

Name and signature of
Co-Principal Investigator

Date: 25.01.2017
Place: Pondicherry

Forwarded by Head of the Department

Signature of the Head

DEAN
SRI LAKSHMI NARAYANA INSTITUTE OF MEDICAL SCIENCES
OSUDU, AGARAM VILLAGE,
KOODAPAKKAM POST,
PUDUCHERRY - 605 502

PROJECT EVALUATION FORMAT

Recommendation sheet

Name of the Principal Investigator	Dr. T. Mohanalakshmi
Name of the Co-Principal Investigator	Dr. E. Prabharkar Reddy
Name of the Department	Microbiology
Title of project	Triglycerides fasting or non-fasting? Current knowledge in diagnostic values
Recommendation of the evaluation committee (Recommended/Revision/Not Recommended)	Recommended
Financial allocation recommended	Rs. 100000/-

SI. No.	Head	Amount
1	BP Apparatus, Stethoscopes, Body weight weighing machine, SPSS version 16 Chicago, IL, USA, ECG machine	50,000/-
2	Consumables- Gel bottles, cotton, sprit, testing charges, tools, etc.	25,000/-
3	Travel support for the purpose of research work.	10,000/-
4	Contingency	10,000/-
5	Others consumables	5,000/-
	Total	**1,00,000/-**

Name and Signature of the Research Advisory Committee members with date.

Internal Quality Assurance Cell IQAC Pondicherry-02 SLIMS

(Dr. G. Jayalakshmi)