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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173, Agaram Road, Selaiyur, Tambaram,
Chennai - 600 073. Tamil Nadu.

Ref. No.SMS-2015-O-02

Date: 17.08.2016

TO

Mr. E. Prabhakar Reddy
Professor/Biochemistry,
BIHER.

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: Comparison of Bone Turnover Markers on Osteoporosis in Pre and Postmenopausal Women

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

Approved on: 10.08.2016

Payment details:

Voucher No.23 **Dated: 25.08.2016**

With Regards

Dean-Research

Bharath University

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

CASH / PAYMENT VOUCHER

Date 25/08/2016

V.No. 23

Debit _____ Amount

Rs. 1,00,000/-

PAID TO Dr. E. Prabhakar Reddy

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. 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

2. **Details of Co-Principal Investigator**

Name	: Dr. A. Vaithiyalingam
Designation	: Professor
Highest Qualifications	: MD
Department	: Orthopedics
E-mail	: drvaithiyalingam@gmail.com
Contact no	: 9500077553
Date of Joining	: 26.10.2009

Technical details

1. Introduction:

The word 'menopause' is derived from two Greek words, 'meno' (month) and 'paus' (to stop). Clinically, menopause is said to have occurred when menstruation has ceased for twelve months (1). Physiologically, menopause is defined as the permanent cessation of menses resulting from reduced ovarian hormone secretion that occurs naturally or is induced by surgery, chemotherapy, or radiation (2). The post-menopausal stage in women is essentially an oestrogen-deficient state (1). Both menopause and aging are associated with an accelerated loss of bone mass. Menopause occurs when the balance between bone formation and resorption is upset and resorption is excessive, resulting in a negative remodelling balance (3). Biochemical markers of bone turnover have been shown to provide valuable information for the diagnosis and monitoring of metabolic bone disease (4). They reflect the whole-body rates of bone resorption (Resorption markers) and bone formation (Formation markers). Therefore, they may provide a more representative index of the overall skeletal bone loss than would be obtained by measuring the rates of change in Bone Mineral Density (B.M.D) at specific skeletal sites (5). Osteoporosis is more common in post-menopausal women and not only gives rise to morbidity but also markedly diminishes the quality of life in this population. There is lack of information regarding the risk factors of osteoporosis in developing countries (6). The occurrence of Osteoporosis in postmenopausal women is very common problem especially in India who are exposed to many of the risk factors like Family h/o osteoporosis, history of anorexia or bulimia, prolonged amenorrhea, low calcium diet, lack of exercise, Vitamin D deficiency. But there are very few Indian studies regarding the prevalence of osteoporosis in postmenopausal women and also regarding the biochemical markers which indicate bone turnover in our setup. Osteoporosis is an important public health problem in middle-aged and older women. Until recently, the diagnosis and monitoring of treatment for osteoporosis has been confined to clinical assessment, radiography and bone densitometry. However, in recent years biochemical markers of bone formation and resorption have been developed to quantify bone turnover and remodeling, with possible applications in clinical practice. Osteoporosis is a major health and economic problem. An international consensus development conference has stated that osteoporosis is a systemic skeletal disease characterized by low bone mass and microarchitect deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. This silently progressing metabolic bone disease is widely prevalent in India, and osteoporotic fractures are a common cause of morbidity and mortality in adult Indian men and women (7). Osteoporosis has been linked to an increased fracture risk and subsequent mortality in the later life. Previous prediction models have focused on osteoporosis in postmenopausal women; however, a prediction tool for osteopenia is needed. Osteoporosis is a serious global health issue due to the rapid increase in the size of the aging population (5-6) steoporosis related fractures have been found to be associated with significant costs, increased morbidity and mortality, reduced quality of life, and loss of independence (5-6).The prevalence of osteoporosis increases with age for all sites, and by WHO definition up to It is important to

think clearly about the 2 principle determinants in adult bone health (a) Maximum attainment of Peak bone mass (PBM) in young adulthood, and (b) the rate of bone loss with advancing age. With the onset of menopause, rapid bone loss occurs which is believed to average approximately 2% to 3% over the following 5 to 10 yrs, being greatest in the early postmenopausal years (7). Life time losses may reach 30% to 40% of the peak bone mass in women and 20% to 30% in men. The pathogenesis of postmenopausal osteoporosis involves the interplay of many factors- Nutritional, Environmental, Genetic factors (8).

Total alkaline phosphatase activity in serum has been used commonly as a biochemical marker of osteoblast function, but lacks specificity because of the contribution of activity derived from the liver, in particular. Human alkaline phosphatases (ALP) are a group of enzymes of similar specificity coded for by at least four different gene loci that catalyse the hydrolysis of phosphate esters at an alkaline pH (9-11). The gene for tissue non-specific ALP encodes the isoenzyrnes expressed in liver, bone and kidney. In healthy individuals about half the activity of alkaline phosphatase in serum is derived from bone and the remainder from liver. The isoforms differ only in the degree of sialylation and glycosylation, reflected in differences in electrophoretic mobility, heat stability and precipitation by lectin. Methods to separate and quantify bone ALP in the presence of liver ALP, based on these properties, have not had sufficient specificity or sensitivity to be useful clinically. In the USA, the prevalence of osteoporosis in postmenopausal women aged 50 years has been found to be 15.8% in non-Hispanic whites, 7.7% in non-Hispanic blacks, and 20.4% in Mexican Americans (12). Among postmenopausal women, the average bone mineral density (BMD) was found to be highest among African Americans, followed by among Hispanics, native Americans, and Asians (13). In Taiwan, the prevalence of osteoporosis among women aged 40 years has been estimated at 10.1% and 7.5% based on the BMD of the spine and femoral neck, respectively (14).

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

Wright NC, Looker AC, Saag KG, Curtis JR, Delzell ES, Randall S, et al. The recent prevalence of osteoporosis and low bone mass in the United States based on bone mineral density at the femoral neck or lumbar spine. J Bone Miner Res 2014; 29:2520e6.

Despite the availability of diagnostic tools and treatment protocols, osteoporosis remains underdiagnosed and undertreated (15). Women reach peak bone mass between the ages of 20 years and 30 years. Then, BMD decreases gradually and continues to decline rapidly after menopause (16). Therefore, predicting the risk of osteoporosis at an earlier age (e.g., premenopausal) or a preclinical phase (i.e., osteopenia) is crucial for early prevention of osteoporosis. Osteoporosis is more common in post-menopausal women and not only gives rise to morbidity but also markedly diminishes the quality of life in this population. There is lack of information regarding the risk factors of osteoporosis in developing countries (17-18). Serum alkaline phosphatase (ALP) is the most commonly used biomarker of bone formation. ALP is a ubiquitous enzyme that plays an important role in osteoid formation and bone mineralization.

The serum ALP pool consists of several dimeric isoforms that originate from various tissues, such as the liver, bone, intestine, spleen, kidney, and placenta (19-21).

2.1. International Status:

Thus, the aim of the present study is to evaluate the risk of accelerated bone mass loss by assessing bone markers, such as alkaline phosphatase (ALP) and serum calcium, in post-menopausal women. Therefore, a tool that includes easily accessible factors may be a plausible approach to predict the risk of osteopenia. Some biomarkers (e.g., blood or urine markers) can easily be obtained during routine health checkups and improve the sensitivity in predicting osteoporosis at the preclinical phase (i.e., osteopenia). However, biomarkers are rarely included in the currently available prediction tools. Therefore, this study aimed to develop a simple and accurate prescreening tool for identifying premenopausal and early postmenopausal women (aged 40-55 years) with a high risk of osteopenia via the incorporation of biomarkers. Thus, the aim of the present study is carried out to assess the clinical utility of biochemical markers of bone turnover, such as Total Calcium, Ionized calcium, Alkaline phosphatase in pre- and post-menopausal women and to evaluate the risk of accelerated bone mass loss by assessing bone markers, such as alkaline phosphatase (ALP) and serum calcium, in post-menopausal women.

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

We performed a cross-sectional study of 200 pre- and post-menopausal women, at Department of Orthopaedics, Sree Balaji Medical College and Hospital, chennai. The Institutional Ethical Committee approved the study and Informed consent was obtained from each participant in the study. The study group consisted of 100Postmenopausal women in the age group of 46-65 years and 100 Premenopausal women in the age group of 25-45 years. All the participants were non smokers, non-alcoholic and ambulatory. The women were neither pregnant nor on oral contraceptive pills. None of the postmenopausal women had suffered any fracture in the previous 1 year nor were they on Hormone replacement therapy or any other medication that might affect bone turnover. Based on time since menopause, 100 postmenopausal women were

categorized into 2 groups. 30 women were in their early postmenopausal period (<5years) and remaining 70 women were in their late postmenopausal period (>5years). Height and Weight of all the participants were noted and Body mass index (BMI) was calculated using the formula = Weight (Kg) / Height2 (m).

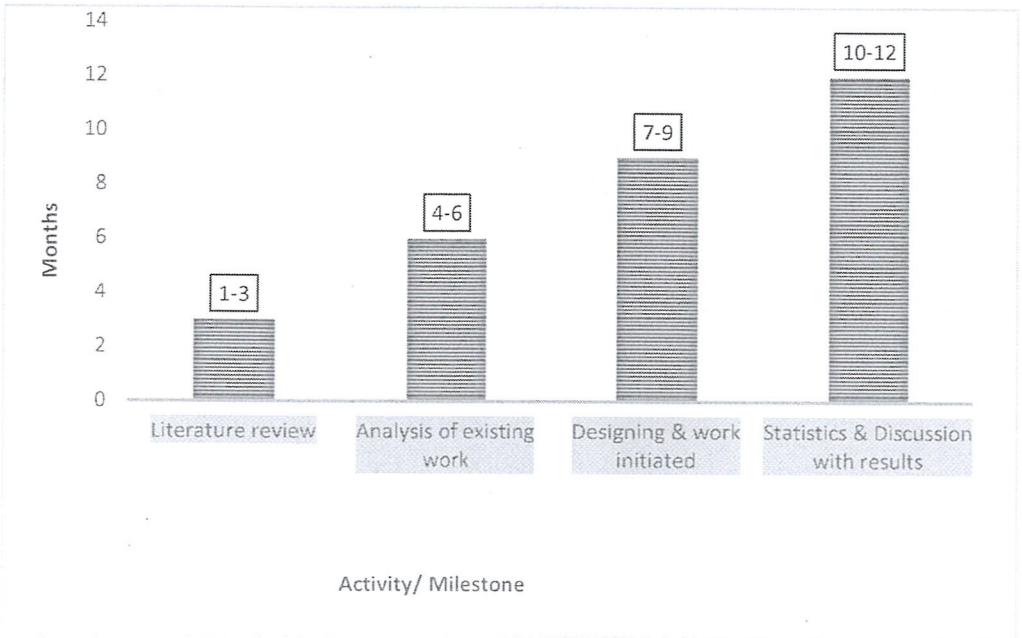
5 ml of random blood sample was collected in a plain bulb from each participant. Serum was separated immediately by centrifuging at 2000rpm for 15min and analysed for Total calcium, Ionised Calcium and Alkaline phoshatase. Random sample of urine was collected at the same time in a clean plastic bulb and analysed for Hydroxyproline and Creatinine immediately.

Statistical Analysis:

The data were presented as Mean ± SD. Statistical analysis were done by using Microsoft Excel and SPSS for windows version 11.5 (SPSS, Inc., Chicago). P value <0.05 was considered statistically significant.

4.2 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

Bone turnover markers, in particular those associated with bone resorption rate, have potential for clinical use in many applications related to skeletal disorders. Guidelines for their use are gradually becoming available. Novel, more specific markers as well as improvement and standardization of measurement techniques will enhance reliability and facilitate the use of bone turnover markers in practice. Therefore, an increase in bone turnover accelerates bone mass reduction in postmenopausal women, whereas a decrease in bone turnover is associated with the preservation of bone mass. In normal post-menopausal women, an increase in bone turnover accelerates bone mass reduction. The present study reveals that serum calcium levels are significantly reduced in post-menopausal women, whereas serum ALP levels are significantly increased. In addition, a significant negative correlation was observed between serum calcium and ALP levels in the experimental group. Measurement of bone turnover through urinary hydroxyproline, Alkaline phosphatase and Calcium could form a tool available to assist health care professionals to predict fracture risk. Early prediction of osteoporosis has important public health and clinical implications, as osteoporosis usually has no symptoms until the occurrence of fractures. The results from this study suggest that simple, easy, common biochemical markers can still be used to assess the bone turnover in postmenopausal women and hence their risk of developing osteoporosis and fractures. conclude that bone ALP is useful in monitoring alendronate treatment of patients with osteoporosis. It has better clinical utility for following both treatment groups and monitoring individual patients. The biochemical markers of bone turnover provide dynamic measures of bone remodeling and thus potentially useful in predicting the course of changes in bone mass. The inclusion of serum total alkaline phosphatase level in the model, which is easy to obtain from routine health checkups, significantly enhanced the sensitivity for detecting osteopenia in women aged 40 to55 years.

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.	Kalpana Thalava1, ***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.**	Total Antioxidant Status and Oxidative Stress in Diabetes Mellitus and Metabolic Syndrome	International Journal of Pharmaceutical Sciences Review	40(1)	271-277	2016

	Prabhakar Reddy		and Research			
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. Seshadri Reddy** Assistant Professor, Dept of Biochemistry AIIMS Deoghar **Mobile No**: 8106145001 **E-mail id:** lifeschemistry@live.com	2. **Dr. Manne Munikumar** Data Manager (Bioinformatics) Clinical Division, ICMR-National Institute of Nutrition Jamai-Osmania (Post) Hyderabad-500007, Telangana **Mobile No**: 9492373997 **E-mail id:** mannemk@gmail.com

CERTIFICATE FROM THE INVESTIGATOR

Project Title: Comparison of Bone Turnover Markers on Osteoporosis in Pre and Postmenopausal Women

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 involves 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: 11.07.2016
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. E. Prabhakar Reddy
Name of the Co-Principal Investigator	Dr. A. Vaithiyalingam
Name of the Department	Biochemistry
Title of project	Comparison of Bone Turnover Markers on Osteoporosis in Pre and Postmenopausal Women
Recommendation of the evaluation committee (Recommended/Revision/Not Recommended)	Recommended
Financial allocation recommended	Rs. 1,00,000/-

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. P. Jayakumar)