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"STUDY OF SERUM HIGH SENSITIVE C-REACTIVE PROTEIN IN PREDIABETES"

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***ABSTRACT**--Prediabetes is an early stage of hyperglycemia , which progresses to diabetes mellitus , which further increases risk of developing complications. High sensitive C- reactive protein (hsCRP) is an acute phase protein released in inflammation. HsCRP is found in association with diabetes and cardiovascular risk factors.To study high sensitive serum C - reactive protein in prediabetes.The patients who are diagnosed as prediabetes as per the WHO criteria as impaired fasting blood glucose and/or impaired glucose tolerance test by glucose oxidase method will be taken for study. Detail history and examination will be done. BMI, hip circumference, waist circumference, waist hip ratio and blood pressure will be measured for every individual case and control.The results of the study is expected to be an increase in hsCRP levels in the population with prediabetes. Suggest elevated hsCRP levels in cases with prediabetes.*

***KEY WORDS**--HsCRP , Prediabetes.*

I. INTRODUCTION

The association of high sensitivity C-reactive protein (hsCRP) with prediabetes in the Indian population has been observed in a few studies. The occurence of prediabetes and diabetes is increasing over years. As given in the International Diabetes Federation in 2017, an estimated 451 million people had Diabetes Mellitus in the world. By 2045, this is estimated to be 693 million. There were 72.9 million cases of diabetes mellitus in India in 2017 as per international diabetes federation diabetes atlas. (1)

Anjana RM et al in 2011 showed that, in India, prevalence of prediabetes and diabetes in 2011 was 77.2 and 62.4 million respectively.(2)

The prevalence of prediabetes considering impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) were 8.3 in Tamilnadu, 12.8 in Maharashtra, 8.1 in Jharkhand and 14.6 in Chandigarh. (2)

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Many studies implied that important risk factors for prediabetes were age, familial diabetes mellitus, abdominal obesity, high blood pressure and socioeconomic strata. (2) Furthermore, the risk for development of Diabetes Mellitus (DM) type 2 , was higher in prediabetics that is around 7.6% , while it was 0.6% in normoglycemics. (3 Prediabetes or impaired fasting glucose (IFG) refers to a state in which the fasting blood glucose is increased above normal value , but is not that high as to call it as diabetes mellitus.(6)

Prediabetics typically have no signs and symptoms as such, but we have to suspect prediabetics with the following (6)

Diabetes is a non communicable disease which often is associated with a family history. The common symptoms are polydipsia, polyuria, polyphagia , changes in body weight excluding obvious reasons . Other flu like symptoms such as weakness and malaise may be present. Patient may also present with symptoms like blurred vision , tingling or loss of sensations in extremities , delayed healing of wounds , recurrent infections .(6)

High sensitive C- reactive protein :- C-Reactive Protein (CRP) is a substitute of the proinflammatory interleukin IL-6. (8) HsCRP is a protein that belongs to the pentraxin group of proteins. It is synthesised by the liver.(9,10) Endothelial cells of blood vessels , smooth muscle cells and even adipose tissue are some of the other places where hsCRP is synthesised. (7,8) Tillett and Francis discovered hsCRP in the year 1930. (11) CRP was named so as it could precipitate the somatic C –polysaccharide of Streptococcus pneumonia.(9) It should be kept in mind that hsCRP has no relation with C-peptide or protein C . It is known to activate the complement system and it binds to Fc receptors. (8) A rise in hsCRP indicates inflammation and vice versa low to normal hsCRP excludes infection or inflammation. (8)

According to several recent studies CRP is known to be a marker of inflammation in many cardiovascular diseases. It is coming up as a separate new marker for risk in cardiovascular diseases. (14) Furthermore, serum hsCRP levels were also found to be elevated in patients with impaired glucose tolerance (IGT) (16) or diabetes. (16)

The hsCRP is a highly sensitive form of CRP. The association of hsCRP has been shown with cardiovascular disease. HsCRP is produced in the liver by proinflammatory cytokines such as tumour necrosis factor alpha(TNF-a) and interleukin-6 (IL-6)which are produced by visceral adipose cells . Elevation of fasting plasma glucose (FPG) is found in association with elevated concentrations of hsCRP.

Assymptomatic individuals with high hsCRP levels , are several times more prone to have coronary artery disease.(10)Determining high hsCRP levels can help to prevent future risk of grave cardiovascular diseases such as myocardial infarction or even sudden cardiac death in apparently healthy looking population . (10)

Control of both hsCRP and LDL cholesterol have been associated with decreased progression of atherosclerosis and improved clinical scenario.(11) Use of statins prophylactically in patients with more than normal range hsCRP levels , even in a patient with normal lipid profile has been known to significantly reduces risk of cardiac related death.(12)

As there are few studies on this topic, we intend to study hsCRP in pre-diabetes in Central Indian rural population.

II. OBJECTIVES

To correlate levels of hsCRP with cardiovascular risk factors such as age ,gender ,BMI, waist-hip ratio , hypertension , lipid profile in prediabetes

Study design:

Observational Case control study

Setting:

This study will be carried out in Acharya Vinoba Bhave Rural Hospital (AVBRH) a tertiary care hospital attached to Jawaharlal Nehru Medical College (JNMC) , Sawangi, Wardha, Maharashtra from September 2018 to August 2020.

Cases will be prediabetics as per WHO criteria.

Controls will be age and sex match asymptomatic individuals.

Participants:

Cases will be prediabetics as per WHO criteria.

Inclusion criteria :- All patients coming to IPD and OPD in the age group of 18 and above diagnosed and fulfilling WHO criteria

WHO criteria of prediabetes stated by Buysschaert & Bergman in 2009 are :

- Fasting serum glucose level between 110-125 mg/dl. and/or
- Two hour plasma glucose levels after 75gm OGTT is between 140mg/dl to 199mg/dl. (4)

Exclusion criteria :- Patients with burns, any injury ,infections such as pneumonia or tuberculosis ,myocardial infarction , collagen vascular diseases such as lupus ,vasculitis or rheumatoid arthritis ,inflammatory bowel disease and certain malignant tumours especially of the breast ,lung and gastrointestinal tract , acute pancreatitis ,post surgery ,leukemia , hormone replacement therapy , obese individuals , metabolic syndrome or any tissue injury. These are conditions where hscCRP may be deranged. Also exclude patients taking medications such as statins , niacins and fibrates where hs-CRP is decreased. (15)

Controls:-

Normal healthy individuals with age and sex match will be controls.

Variables :

All study subjects will be explained the study procedure and nature of the evaluation to be done. Each subject will be evaluated by WHO criteria for prediabetes. The BMI , WHR , waist circumference , hip circumference , blood pressure , lipid profile and hsCRP will be evaluated.

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III. METHODS

❖ The patients who are diagnosed as prediabetes as per the WHO criteria as impaired fasting blood glucose and/or impaired glucose tolerance test by glucose oxidase method will be taken for study.

❖ Patient will undergo detailed history and clinical examination and will be subjected to following

❖ **BMI** :

BMI or Quetelet index is defined as weight in kgs divided by square of body height in metres and is expressed in units of $kg/m^{2.}$

WHO: Categories of Body Mass Index (BMI) for Asia-Pacific Region (16)

CATEGORY	BMI RANGE (kg/m^2)
Underweight	<18.5
Normal	18.5 - 22.9
Overweight	23 - 24.9
Obese	>25

Waist Circumference (WC) : According to WHO , WC is measured as the circumference at the middle point between the lower part of the last intercoastal rib that is palpable and the upper border of the iliac crest (17)

Hip Circumference (HC) : It is measured as the circumference at the most widespread part of the buttocks. (17)

WAIST/HIP RATIO ***(WHR) :*** It is measured by dividing the waist circumferenc by hip circumference.

World Health Organisaton cut off points (17)

INDICATOR	CUT-OFF POINTS	RISK OF METABOLIC COMPLICATIONS
WC	>94 cm[M] , >80cm[F]	INCREASED
HC	>102cm[M] , >88cm[F]	SUBSTANTIALLY INCREASED
WHR	>0.90[M] , >0.85[F]	SUBSTANTIALLY INCREASED

BLOOD PRESSURE -We will measure blood pressure by auscultatory method using the stethoscope and sphygmomanometer.

AHA Guidelines For Hypertension (November 13 ,2017) (18)

Normal : < 120/80 mmHg ;

Elevated: Systolic BP 120-129 mmHG and diastolic BP < 80mmHG

Stage 1 : Systolic BP 130-139 mmHg or diastolic BP 80-90 mmHG ;

Stage 2; Systolic BP atleast 140 or diastolic BP atleast 90 mmHg ;

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Hypertensive crisis: Systolic >180 mmHG and/or diastolic BP >120 mmHG ,with patients needing immediate changes in medication excluding other problems , or urgent hospitalisation if there are signs of any organ damage.

Investigations -Fasting Blood sugar and 2 Hour OGTT by glucose oxidase method.

-Serum total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) and very low density lipoprotein (VLDL) is to be done by autoanalyser radoxdytona

MEASUREMENT OF ***HS-CRP:*** 1ml of venous blood is collected in a plain vacutainer and centrifuged and hsCRP is measured by spectro photometric method by ELISA kit by XEMA Company**.** A solid-phase ultra-sensitive enzyme immunoassay will be done for the quantitative analysis of serum high sensitive C-reactive protein. The test is based on two-site sandwich enzyme immunoassay principle.Specimens may be stored for upto 48 hours at +2 to 8 degree celcius before testing.The specimens must be frozen at minus 20 degrees or lower if needed to be stored. The reagents should be at room temperature before use. All the pipetting of calibrators and samples must be done within 3 minutes.

Results will be calculated by the mean absorbance values (OD450) for each pair of calibrators and samples will be plotted on a graph and the corresponding concentration of hs-CRP will be calculated in unknown samples from the calibration curve.

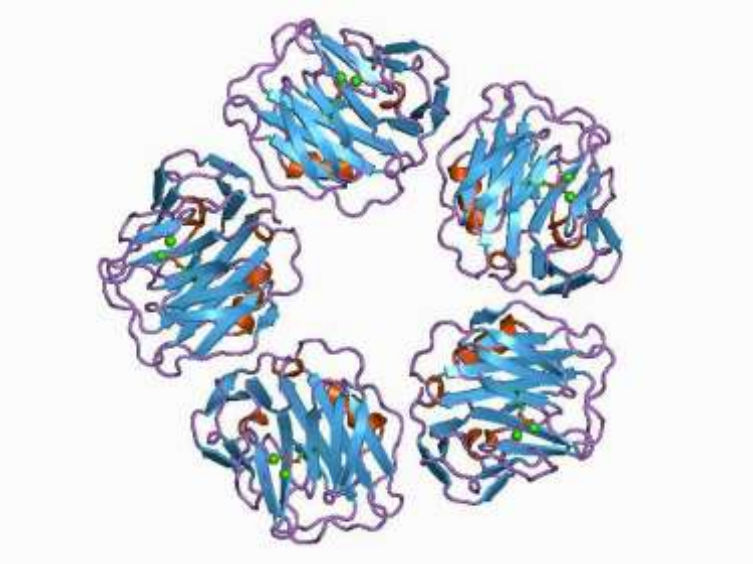

Figure 1: An image depicting C-reactive protein (pentraxin family related protein)

IV. EXPECTED OUTCOMES/RESULTS

A few suggest that a rise in hsCRP levels is a adverse factor that can cause diabetes in the future. Ryauichi Kawamoto et al (2011) suggested that serum hsCRP levels rise with the rise in FPG(Fasting plasma glucose), in both genders .(4) C.Sabanayagam et al (2011) concluded that data collected from two large Asian cohorts showed

that elevated hsCRP levels were associated with prediabetes. (5) Participants of older age and those with risk factors were those with prediabetes. Population with prediabetes had increased diastolic blood pressure and higher hsCRP levels. The people with prediabetes had decreased HDL cholesterol while increased total cholesterol and a BMI >25kg/msq. High hsCRP levels are found to be in association with people with prediabetes .

V. DISCUSSION

In a study by Sabanyagam et al it was found that there was strong association between hscrp and prediabetes. The association was found to be indifferent of several risk factors such as smoking , BMI and total cholesterol . It was found to be stronger in women with BMI less than 25kg/msq. HsCRP <1 mg/l was kept as the reference range. The odds ratio (95% confidence interval) of prediabetes in people with hsCRP 1-3 mg/l and >3 mg/l was 1.31 (0.99-1.74) and 2.17 (1.61-2.92), p (trend) < 0.0001 in one population group where fasting plasma glucose was used as a measurement of prediabetes. In another population group where HbA1C was used as measurement of diabetes , the hscrp levels were 1.23 (1.00-1.52) and 1.31 (1.06-1.64), p (trend) = 0.02 . This , thus concluded that hsCRP was higher than normal in the population with prediabetes (18). A number of related studies in this region were explored for additional information relevant to geographic context (19-35). Some articles related to other related non-communicable entities(36-58) and sociocultural aspects (59-84) were reviewed.

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