VITAMIN D AND HSCRP LEVELS AMONG INSULIN RESISTANT PATIENTS IN A TERTIARY CARE HOSPITAL AT KOLKATA.

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Abstract

Vitamin D deficiency probably takes a part in the development of insulin resistance. Insulin resistance also markedly affects the obese population. High-sensitivity C-reactive protein is a sensitive marker of subclinical inflammation and strongly predicts increased risks of insulin resistance.

In this study, 123 obese persons were selected, and homeostatic model assessment (HOMA-IR) was measured in them. Serum Vitamin D and hsCRP were also measured in all participants. Among participants with HOMA-IR >2.7, significantly higher BMI, serum hsCRP and lower vitamin D were observed;

Keywords: Obesity, BMI, HOMA-IR, Vitamin D, hsCRP.

Introduction

In addition to serum calcium homeostasis vitamin D has proved its role in many biological functions in our body[1,2]. Vitamin D deficiency is a significant risk factor of many diseases, indicated by some authors [3,4]. Occurrence of low level of vitamin D and insulin resistance can take place simultaneously, furthermore vitamin D may have a significant part in insulin secretion as well as its deficiency can promote type 2 diabetes mellitus documented by some studies [5]. These studies also suggested several pathophysiological mechanisms of vitamin D related insulin resistance [1,5,6]

Vitamin D exerts its activity by binding with vitamin D receptors, located inside the nucleus of a cell. These receptors have been identified in some other tissues, of which skeletal muscles and adipose tissues are of special significance, since they are the main determinants of peripheral insulin uptake and sensitivity [1,2,7]. Some researchers documented the common risk factors like sedentary life style and obesity to develop vitamin D deficiency and type 2 diabetes [8]. Further, epidemiological data have linked low vitamin D levels to cardiovascular diseases and type 2 diabetes [9]. The mechanisms by which obesity and its comorbidities are related to vitamin D deficiency are not well understood.

In healthy individuals C-Reactive Proteins (CRP) is present in plasma in minimal amounts but the concentration increases 100 fold in response to injury, infection or inflammation. CRP is named so for its ability to precipitate the somatic C-polysaccharides of *Streptococcus*

pneumonie and is the first acute phase protein to be described [10].

High-sensitivity C-reactive protein (hsCRP) is a sensitive marker of subclinical inflammation and strongly predicts increased risks of T2DM [11]

In the present study, we determined the prevalence of insulin resistance, vitamin D status, and hsCRP levels in obese population. Furthermore we tried to find out the significance of these parameters in the studied population.

Materials and methods:

Study design: This hospital based, cross sectional, non interventional study was conducted during the period of November 2018 to April 2019, during the period of reduced sunlight in the Department of Biochemistry, Calcutta National Medical College, Kolkata, West Bengal, India.

Selection of case group: The study group primarily included 123 (one hundred twenty three) obese patients attending endocrinology and General Medicine Outdoor Patient Department. Obese patients were selected by calculation of BMI. This is performed by standardized protocol based on height and weight measurements. Overweight was defined as a BMI of 25-29.99 kg/m² and obesity was defined as a BMI 30 kg/m² or higher according to WHO criteria.

Exclusion criteria of case group: Known case of diabetes mellitus, parathyroid diseases, use of an anticonvulsant or systemic glucocorticoids; use of a vitamin D or calcium supplements, hepatic disease, renal disease, or malabsorptive disorder; disorder of bone or calcium

metabolism (including known vitamin deficiency); patients with cardiovascular or respiratory diseases, hypothalamic disease; or genetic disorder that predisposes to obesity (such as Prader-Willi syndrome), pregnancy.

Sample collection: The amount of blood collected in absolute fasting after 9 hours with all aseptic precautions in 2 parts was 5 ml. The first part collected in ethylene diamine tetraacetic acid (EDTA) vial for estimation of glycated haemoglobin (HbA_{1c}). The second part collected was allowed to clot, serum was separated for estimation of Fasting Plasma Glucose (FPG), insulin, vitamin D and hsCRP.

Measurements of biochemical analytes: HbA_{1c}, the index of long term glycemic control, was determined with Micromat II (Biorad) instrument based on boronate affinity chromatography [12].

FPG was analysed using reagent kit (Merck) by Glucose Oxidase method [13].

Serum insulin was determined by ELISA with monoclonal antibody based reagent (Monobind) [14].

Insulin resistance was estimated using homeostatic model assessment (HOMA-IR). The HOMA-IR was calculated as follows: HOMA-IR: (Fast Plasma Glucose (mmol/L) × Insulin (μIU/mL))/22.5 [15].

The cutoff value was set as 2.7. Subjects were classified into two groups according to HOMA-IR: the insulin resistant group as Group 1 (HOMA- IR > 2.7) and the non-resistant group (HOMA-IR < 2.7) as Group 2.

Serum 25 (OH)D concentrations were measured using a commercially available radioimmunoassay kit (Minividas

Biomerieux, France). According to the report of the Institute of Medicine, vitamin D status is categorized as: risk of deficiency < 12 ng/mL, risk of inadequacy 12-19 ng/mL, sufficiency 20-50 ng/mL [16].

Serum hsCRP

For the estimation of serum hsCRP, 2 ml of fasting, venous, non-haemolysed blood sample was withdrawn without the aid of a tourniquet, in a plain sterile bulb. The blood samples were analysed immediately. The estimation of serum hsCRP was done on XL-600 Automatic analyzer with the kit (Erba Mannheim) based on the measurement of antigen-antibody reaction by the end point method [17].

Statistical methods used: Statistical analysis was done by independent t test or one way ANOVA as appropriate, p value <0.05 was considered as significant.

Data collection and processing for statistical analysis:

Statistical analysis was aimed

- To assess the significance of difference between the mean values of Fasting plasma glucose (FPG), HbA_{1c}, Serum insulin and vitamin D, HOMA-IR, BMI, hsCRP.
- To find out any correlations between HOMA-IR and vitamin D status in group 1 (HOMA-IR>2.7).

Results: one hundred and twenty two obese subjects were participated in the study. Group 1 included sixty seven subjects (37 females and 30 males) and group 2 included fifty five subjects (30 females and 25 males) the mean age was 45±11.26 yrs, on the other hand in group 2, mean age was 44±10.32 yrs.

Table 1:

	FPG (mg/dl)	HbA _{1c} %	Insulin(μIU/ml)	BMI	Vitamin D(ng/mL)	HOMA-IR	hsCRP	
HOMA-IR<2.7 N=55 Group 2	Mean-95.70 SD-6.40 SEM-0.86	Mean-5.19 SD-0.21 SEM-0.028	Mean-7.20 SD-0.32 SEM-0.043	Mean-27.93 SD-0.66 SEM-0.089	Mean-17.34 SD-0.86 SEM-0.116	Mean-1.69 SD-0.15 SEM-0.020	Mean -7.899667 SD-1.099299 SEM-0.012	SD-
HOMA-IR>2.7 N=67 Group 1	Mean-104.31 SD-8.54 SEM-1.04	Mean-5.44 SD-0.39 SEM-0.047	Mean-16.58 SD-2.08 SEM-0.254	Mean-31.6 SD-1.49 SEM-0.182	Mean-9.26 SD-2.37 SEM-0.289	Mean-4.26 SD-0.71 SEM-0.086	Mean-8.458649 SD-0.88101 SEM-0.234	SD -
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0023	

Table 1 show that there is a significant higher BMI value and lower 25(OH) D in group 1 in comparison to group 2. The mean FPG, insulin, HbA_{1c}, and hsCRP levels were also significantly higher in group 1 when compared to group 2.

Discussion: The most significant finding in this study is that vitamin D levels became lower with increasing insulin resistance. The opposite trend was found with hsCRP levels.

Some studies documented the evidences of low 25(OH)D in obese subjects [6,15]. This fact may be explained by the

storage of vitamin D in adipose tissue because of its high lipid solubility and decreased exposure to obese subjects to sunlight [6,8,18]. Some studies also noticed the improvement of metabolism of glucose, calcium and vitamin D with increased physical activity and decreased body weight. Some authors conducted researches to find out the association between hypovitaminosis D and insulin resistance. However, ethnicity, dietary intake, physical activity and obesity are potential confounders that may affect the relationship between vitamin D and type 2 diabetes mellitus [19]. Vitamin D, obesity and insulin

resistance were selected as a common parameters by some researchers [16,20,21]. It is still an issue whether low 25(OH)D concentrations directly affects the pathogenesis of insulin resistance or BMI also contributes. Kabadi et al [22] have shown that the combined effects of vitamin D deficiency and obesity cause an increased risk of insulin resistance. Some other studies didn't find any association between HOMA-IR and 25(OH)D levels after adjusting BMI [23]. Some authors highlighted that 25(OH)D and insulin resistance are probably depends on bodyweight [24]. Obesity is definitely a risk to develop insulin resistance [25], furthermore increased fat accumulation is associated with decreased serum 25(OH)D concentrations [21,26].

In our study, insulin resistance is associated with the low 25 (OH) D levels. The significantly increased levels of FPG, HbA1c and insulin in the group 1 may indicate a long-term muscle insulin resistance. Furthermore, higher prevalence of hypovitaminosis D and restricted physical activity in obese subjects suggested the significant role of maintenance of a normal vitamin D concentrations and an modification of life style in prevention of obesity and diabetes. We have selected the study population who does not differ in ethnicity, dietary intake, physical activity and obesity to minimize the confounding factors.

Dong et al. observed that 25(OH)D levels were inversely related to BMI, waist circumference, total fat mass, and percentage of fat mass in a group of adolescents [27].

Several possibilities have been emphasized to explain the lower 25(OH)D levels observed in obesity, including decreased sun exposure due to sedentary lifestyle, poor diet, and decreased bioavailability of vitamin D(3) from cutaneous and dietary sources because of its deposition in body fat compartments [28]. Vitamin D receptors and vitamin D-binding proteins are known to exist in pancreatic tissue, furthermore calcium plays an essential role in β -cell insulin secretion Thus insulin resistance plays a significant role in hypovitaminosis of 25(OH)D [29,30]. Mechanism links between vitamin D and insulin sensitivity have been proposed by some authors. A vitamin D response element in the insulin receptor gene promoter might affect insulin receptor protein expression [31]. Furthermore, 1,25-dihydroxyvitamin D₃, the active form of vitamin D, induces increased transcription and protein expression of the insulin receptors [32].

Previous studies suggest that inflammation may promote insulin resistance through the production of proinflammatory cytokines such as interleukin-1 β , interacting with adipose tissue-specific macrophages and activation of innate immune system [33]

Therefore, CRP may adversely affect insulin sensitivity through direct action on the liver. Additionally, tumor necrosis factor alpha (TNF- α) and IL-6, which are pro-

inflammatory cytokines secreted by adipose tissue, can stimulate CRP production in the liver[34]

Conclusion: The focus on treatment of insulin resistance and intensification of physical activity in obese subjects will encompass the normalization of vitamin D levels. CRP levels will help in the assessment of insulin resistance. On the other end, future studies are needed to determine the clinical sequelae of lower 25(OH)D levels in obese subjects, the amount and duration of treatment necessary to replenish 25(OH)D in them, and whether treatment with vitamin D can improve primary clinical endpoints such as insulin resistance.

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