The Scientific research process

1 What did this study explore?

This study aimed to assess circulatory levels of IL-18 and determine whether the presence of IL-18 promoter polymorphism influences metabolic syndrome phenotypes

2 How did the authors perform all experiments?

The experimental details are provided in the methods section of the manuscript. Briefly, the eight and height of all the subjects were measured in kilograms and meters respectively, using a weight scale with a built-in Stadiometer (ZT-120 Health Scale, Nanjing Everich China). Waist circumference and hip circumference was measured using the WHO protocol. Subjects were asked to stand in an erect posture wearing light clothing. BMI was calculated by dividing weight by height squared (kg/m2). While body fat percentage was measured using Diagnostic Scale BG55 (Beurer Germany) through bioelectrical impedance matching/analysis. 6 ml of blood was collected from the study participants after an overnight fast of 12 hours. Fasting plasma glucose and Lipid profile were measured using commercially available kits as per the vendor's instruction (Merck, France). LDL-cholesterol levels were calculated using the Friedewald equation. Fasting insulin, IL-18 and TNFα levels were measured using an ELISA kit (DIA source Immuno Assay S.A., Belgium). Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) index [fasting insulin (units per milliliter) x fasting glucose milligram/deciliter)/405] [19], and insulin sensitivity was calculated by (QUICKI) [1/[log(fasting insulin) + log(fasting glucose)]) . DNA extraction was performed using commercially available Qiagen DNA extraction kit (Cat. #51185, Valencia, CA USA). Genotyping of IL-18 –607 C/A polymorphism was performed by using tetra-primer amplification refractory mutation system (TARMS-PCR) using the GoTaq® Hot Start Green Master Mix (Cat. # M5122, Promega Corporation, USA) as per the manufacturer's instructions with the following cycling conditions for PCR: 1 cycle for 5 min at 95 °C for initial denaturation followed by 35 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s followed by a final extension of 10 min at 72 °C. PCR products were electrophoresed in 2% agarose gel. Genotyping

quality control was performed in 10% of the samples by duplicate checking (rate of concordance in duplicates was >99 %). Tetra arms primers used for amplifying IL-18 -607 (C/A) were as follows: Control Band [Outer Forward: CCTACAATGTTACAACACTTAAAAT; Outer Reverse: ATAAGCCCTAAATATATGTATCCTTA;] (product size 440 bp); A allele [Inner Forward: GATACCATCATTAGAATTTTGTG (product size 278bp)] and C allele [Reverse inner GCAGAAAGTGTAAAAATTATCAA (product Size 208bp)]. The study was approved by the institutional ethical review board (3597-BBS-ERC-15), and all subjects gave a written and informed consent.

3 How did the authors process all experimental data?

A descriptive statistical analysis of continuous variables was performed using SPSS (version 21; SPSS Inc., Chicago, IL, USA). Data on continuous variables were calculated as mean ±standard deviation (SD), whereas data on categorical variables was presented as frequencies and percentages. Statistical comparisons were computed using a student t-test, one-way analysis of variance (ANOVA) and Pearson's $\chi 2$ test of independence. Pearson's correlation (r) were used to determine the correlation between serum IL-18 levels and lipid profile, fasting blood glucose, insulin and body fat parameters. Hardy–Weinberg equilibrium (HWE) was calculated for IL-18 SNP. Significance and effect size of minor allele with study parameters were determined under an additive model of inheritance. In all statistical analysis performed p-values <0.05 were considered significant.

4 How did the authors deal with the pre-study hypothesis?

The pre study hypothesis was that IL-18 levels are increased in metabolic syndrome. Further the polymorphism is associated with MetS. The data observed was in line with the hypothesis.

5 What are the novel findings of this study?

The novel findings of this study are the IL-18 gene polymorphisms which influence the expression of IL-18 levels. We found that subjects with AA genotype had a higher Body fat, Insulin resistance, TNF alpha and IL-18 levels when compared with subjects with AC (heterozygous) or CC (wild type) genotypes. This preliminary data suggests that IL-18

polymorphism affects IL-18 levels that might cause low grade inflammation. All these increase the susceptibility to develop MetS. Further studies are required to validate our findings.

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