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Leptin (OB GENE) Expression In Cardiovascular Diseases

Biochemistry

KEYWORDS : OB gene, Leptin, Cardiovascular disease, Blood Pressure and Body Mass index

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ABSTRACT

Aim: The aims of this study were to assess the ability of Leptin (OB gene) Expression in Cardiovascular Disease. Cardiovascular disease (CVD) is the leading cause of death and disability in developed nations and is increasing rapidly in the developing world. By the year 2020, it is estimated that CVD will surpass infectious diseases as the world's leading cause of death and disability. Leptin is known to exert a major influence over the central regulation of food intake and energy expenditure via cerebral leptin receptors. Design/Methods: Serum leptin was estimated in 300 subjects who included 150 non – CVD subjects and 150 CVD patients. Age varied between 35 to 75 yrs. Age, sex, height, weight body mass index (BMI) and waist to hip ratio, blood pressure (BP), and family history were recorded for each subject Results: The amino acid sequence homology for OB –R from various species is analyzed and conserved sequences are differentiated from the rest of the sequences by using different colors. Conclusions: The tests are to be used for monitoring the level of OB Gene expression in cardiovascular disease

I. Introduction

The leptin receptor complex is composed of a single subunit belonging to the class I cytokine receptor family and exists as preformed complex with the extra cellular part of the receptor containing several structural domains[1]. The structure of the OB – R is characterized into signal peptide, cytokine receptor homology (CRH) domain which is formed by two subdomains that have a fibronectin type III (FN3 III) fold, immunoglobulin (Ig-like) fold, transmembrane region and intracellular region that contains box I and box II motifs. The box-1 and box-2 motifs are known to be critically involved in OB – R activation[2] but the mechanisms underlying ligand induced receptor activation are still poorly understood. Therefore attempts are made to understand the basic structural, functional properties of OB – R from different species such as human, monkey, cattle, pig, dog, mouse, rat, chick, turkey, bat, sheep, goat, horse, camel, Asiatic elephant, frog, zebra fish and madaka fish through detailed study of OB – R amino acid sequences from the available data. The individual amino acid frequencies of leptin receptor of above species were computed using protparm tool (www.expasy.org/tools/protparm.html). Hydropathy plot for leptin receptor of all species were determined by using Kyte – Doolittle software. The secondary structure of leptin receptor for all the listed species was predicted using SOPMA software and phylogenetic tree is computed for leptin receptor from different species using Clustal W software[3].

This will enhance our understanding of leptin receptor structure, function and evolutionary relationships and also for evolving further strategies for effective and targeted treatment of leptin resistance associated cardio vascular diseases.

II. MATERIALS AND METHODS

IIa. SUBJECTS

Serum leptin was estimated in 300 subjects who included 150 non – CVD subjects and 150 CVD patients. Age varied between 35 to 75 yrs. Age, sex, height, weight body mass index (BMI) and waist to hip ratio, blood pressure (BP), and family history were recorded for each subject

IIb.Target population:-

The study was conducted during the period of December 2015 to August 2016 in department of Biochemistry, Ponnayah Ramajayam institute of Medical Science, Kanchipuram District, Tamil Nadu. India.

IIc. Sample size:-

300 samples were tested. Among that 150 normal subjects and 150 cases were cardiovascular disease patients.

IId. Methodology and data collection

Collection of serum samples

Serum samples were collected from the patients admitted in Medicine department of Ponnayah Ramajayam institute of Medical Science, Kanchipuram District, Tamil Nadu. India. 5ml of venous blood will be collected from the patients.

All assays were performed within 48hrs of separation of serum.

The collected samples were stored air tight at 2^0C – 8^0C in the refrigerator.

III. Biochemical analysis

Serum Leptin was done by Elisa method and OB gene expression was done by RT-PCR method.

IIIa. Quantification of Human Ob-Rb mRNA:

Gene expression of Ob-Rb were analyzed using RT-PCR technique. The sequence of the primers used were as follows:

Ob-Rb : Sense : 5′-ATAGTTCAGTCACCAAGTGC -3′

Antisense :5′-GTCCTGGAGAACTCTGATGTCC-3′

2µg RNA was reverse transcribed using MuLV cDNA synthesis kit (Medox Biotechnologies India Pvt. Ltd). 2µL of cDNA was then amplified using PCR. PCR reaction mixture contained PCR master mix (Ampliqon) and primers specific for Ob-Rb. Amplification cycles were 2 min at 95°C for initial denaturation, 30 sec at 95°C for denaturation, 30 sec at 68°C for annealing, and 30 sec at 72°C for extension and 10 min at 72°C for final extension during 35 cycles for Ob-Rb; 2 min at 94°C for initial denaturation, 30 sec at 94°C for denaturation, 30 sec at 54°C for annealing, and 30 sec at 72°C for extension and 8 min at 72°C for final extension during 35 cycles for Ob-Rb. Finally, Ob-Rb yielded PCR products of 513 bp, 399 bp and 340bp respectively and the

amplification of the GAPDH yielded a product of 206 bp. The products thus obtained were resolved on a 2% agarose gel stained with EtBr and the bands were quantitated.

IV. Results:

Table:1. Relation between waist – hip ratio and Leptin status

Particulars	Normal waist-hip ratio	Large waist-hip ratio	Over large waist-hip ratio	P valve
Number of participants	57	27	16	NS
Percentage of study population	57%	27%	16%	NS
Leptin	7.67±0.75	15.42±2.10	23.63±2.50	0.000*a,b

a-Comparison between normal and large waist-hip ratio

b-Comparison between normal and over large waist-hip ratio

P< 0.001 is considered significant

NS- Not significant

NA- Not applicable

The observed results in Table 1 clearly reveals that 57% (n=57) of the participants were found to have leptin level between 4.53-10.73 ng/ml (Normal) and 27% (n=27) of the participants were found to have leptin level <18.05 ng/ml (Large Waist hip ratio) and also16% (n=16) of the participants have leptin level <20ng/ml (over large waist hip ratio). The observed mean leptin level of normal waist hip ratio, large waist hip ratio and over large waist hip ratio students were 7.67±0.75, 15.42±2.10 and 23.63±2.50 respectively.

Leptin Receptor (OB-R) Gene sequences

Amino acid sequences of leptin receptor (OB – R) from different species are collected from NCBI data base and their accession numbers of are listed in Table .1.The conserved sequences in these were identified using Clustal w 2 software.

Table .2. List of the accession numbers, name of the species and the No. of Amino acids of Ob-Rb

S. No	Accession No	Species Name	No. of amino acids
1	AAB09673	*Homo sapiens (*human*)*	1166
2	AAF34683	*Macaca mulatta* (monkey)	1162
3	NP_001012285	*Bos Taurus* (cattle)	1165
4	NP_001019758	*Sus scrofa* (pig)	1165
5	NP_001019805	*Canis familiaris* (dog)	1166
6	AAC52705	*Mus musculus* (mouse)	1162
7	AAB06616	*Rattus norvegicus* (rat)	1162
8	NP_989654	*Gallus gallus* (chick)	1148
9	AAG40323	*Meleagris gallopavo* (turkey)	1147
10	AAU47264	*Myotis lucifugus* (bat)	1153
11	NP_001009763	*Ovis aries* (sheep)	895
12	AAW31714	*Capra hircus* (goat)	114
13	AAD31284	*Equus caballus* (horse)	57
14	AA091912	*Camelus dromedaries* (camel)	117
15	AAD00769	*Elephas maximus* (a.elephant)	57
16	ABD63000	*Xenopus tropicalis* (frog)	1151
17	NP_001106847	*Danio rerio* (zebrafish)	1091
18	ABC86922	*Oryzias melastigma* (madakafish)	1109

Multiple sequence alignments are central to protein classification and analysis and it becomes possible to identify the sequence conservation patterns that are indicative of active site, secondary structure types, homologies etc., Expanding on the basic facilities of simple gap insertion and deletion offered in the Clustal W programme, it provides amongst other functions and homology scores etc.

V. Discussion:

The observed results clearly reveals that 57% (n=57) of the participants were found to have leptin level between 4.53-10.73 ng/ml (Normal) and 27% (n=27) of the participants were found to have leptin level <18.05 ng/ml (Large Waist hip ratio) and also16% (n=16) of the participants have leptin level <20ng/ml (over large waist hip ratio). The observed mean leptin levels of normal waist hip ratio, large waist hip ratio and over large waist hip ratio students were 7.67±0.75, 15.42±2.10 and 23.63±2.50 respectively.

Abdominal fat has several characteristics that distinguish it from lower body fat and it is an important modulator of the relationship between BF% and CVD[4]. Several correlative and predictive studies have shown that increased abdominal fat increases one's risk for several complications of obesity including insulin resistance, metabolic syndrome, type 2 diabetes mellitus and ultimately, CVD.

In addition to body composition and lipid profile, various adipose tissue hormones have recently been shown to impact CVD risk. Leptin and adiponectin are influenced by body fat status, with associations to CVD[5].

Leptin exerts its biological effects *via* activation of single transmembrane cell surface receptors leading to activation of downstream signaling pathways[6]. The leptin receptor (OBR) is a member of the cytokine receptor family, with high homology to the gpl30 signalling component of the IL-6 receptor[7]. The receptor contains a single transmembrane domain, but depending on the length of the intracellular domain, can exist as either a short isoform (OBRa, OBRc, OBRd and OBRf) or long isoform (OBRb) [8]. A soluble receptor also exists, OBRe, with no transmembrane or intracellular domains, and is found circulating in plasma. Leptin stimulation of OBR results in activation of various signalling pathways including JAK-STAT, MAPK pathways[9].

VI. Conclusion:

We conclude that leptin receptor structure, function and evolutionary relationships and also for evolving further strategies for effective and targeted treatment of leptin resistance associated disease like obesity and cardiovascular.

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