STUDY OF RELATIONSHIP BETWEEN KEY FACTORS OF ADIPOGENESIS AND OBESITY INDUCED ADIPOKINES IN SUBJECTS WITH AND WITHOUT DIABETES

Aims and Objectives:

1. To study expression level of transcription factors that are involved in adipogenesis in obese and non obese subjects with diabetes and without diabetes and their relationship with adipocyte-secreted adipokines.
2. To look for possible differences in relation to above parameters between visceral adipose tissue and subcutaneous adipose tissue.

Introduction:

Type2 diabetes mellitus(T2DM) has become widespread in India with corresponding obesity. Although the precise underlying mechanisms in the development of diabetes are as yet unknown, the initial pathophysiological event is usually insulin resistance, which involves a genetic component that is exacerbated by obesity and a sedentary lifestyle. There is significant correlation between obesity and insulin resistance in both subjects with diabetes and without diabetes (1). Obesity is characterized by increased adiposity. Adipose tissue is consisting mainly of adipocytes and stromal vascular cells which include blood cells, fibroblasts, pericytes and preadipocytes. Mature adipocytes exert endocrine function by expressing several adipokines such as Adiponectin, Leptin, Tumor necrosis factor-α (TNFα), Interleukin-6(IL-6) that regulate inflammation, metabolic functions, insulin sensitivity among other physiological functions.

Adiponectin is 29kDa protein molecule, exclusively secreted from adipocytes, that was initially called adipocyte complement related protein of 30kDa (ACRP30). It has been considered as a biomarker for insulin resistance, T2DM, metabolic syndrome and cardiovascular disease. Adiponectin is anti-diabetic and anti-atherogenic (1,2). It induces insulin sensitivity. On the other hand Leptin, TNFα, IL-6 are pro-atherogenic, pro-diabetic inflammatory cytokines (2). It has been suggested that adipocyte dysfunction leads to obesity and insulin resistance and obesity induces inflammation. Adiponectin, secreted more from visceral adipose tissue (VAT) than subcutaneous adipose tissue (SCAT) is decreased in abdominal obesity whereas Leptin is secreted abundantly from SCAT than VAT. VAT is more infiltrated with inflammatory cells and is more capable of generating TNFα and IL-6 than SCAT. Their levels are also increased in abdominal obesity (2).

Several essential transcription factors of adipogenesis regulators are required in maintenance of adipocyte functions. Peroxisome proliferator activator receptor Y(PPARY), is a master regulator of differentiation of preadipocyte into mature adipocyte. CCAAT enhancer binding protein or c/EBPα is another important transcription factor involved in adipogenesis(3). However, cells lacking C/EBPα are capable of adipogenesis, but are not insulin sensitive. It has been shown that C/EBPα maintains the level of PPARy in

developing adipocytes and also has a critical role in establishment of insulin sensitivity. Sterol regulatory element binding protein-1c(SREBP-1c), a basic helix-loop-helix transcription factor that is expressed in adipocytes and regulated during adipogenesis, is initially called ADD1 for adipocyte differentiation and determination(4). From the current works it has been suggested that SREBP is an insulin modulated transcription factor that is involved in the regulation of genes associated with cholesterol metabolism. It has been shown that inflammatory cytokines might be linked to adipogenesis. Factors such as TNF-α and IL-6 for which plasma levels are elevated in type 2 diabetic patients are also able to interact with some of the transcription factors involved in adipogenesis such as SREBP1c or PPARγ (5). Research shows that other genes typical of the differentiated state mostly. Leptin are strongly promoted by the direct actions of C/EBPα (4).So the shift in expression of transcription factors such as PPARy, c/EBPα, SREBP-1c, involved in preadipocyte differentiation and maturation of adipocytes may affect the expression as well as function(3,5).

In this study we will study the expression of adiponectin, leptin, TNFα and Il-6 in mature VAT, SCAT and serum and their relationship with adipogenic transcription factors such as PPARY, c/EBP, SREBP those are expressed in preadipocytes.

Research methods and procedures The activities and sub-activities related to the objectives proposed in the study are shown below. Detailed methodologies have also been discussed

Recruitment of subjects - Obese diabetic, obese non diabetic, Non-obese diabetic and Non-obese non-diabetic or Control

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1. **Collecting Clinical history of subjects**
2. **Physical examination- Measurement of height, weight, BMI, Waist circumference, Body fat Percent, total body fat, Visceral fat**

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1. **Blood collection**
2. **Serum isolation and biochemical assay- Blood glucose level(FBS, PPBS), HbA1c, Lipid profile, TNFα, IL-6, Leptin, Adiponectin,**

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Collection of Visceral adipose tissue (VAT) and subcutaneous adipose tissue (SCAT) from the recruited subjects

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Isolation of preadipocytes and adipocytes by Type 1 Collagenase digestion of both VAT and SCAT and directly used for RNA isolation

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1. **Analysis of PPARy, c/EBPα, SREBP -1c mRNA expression from preadipocytes**
2. **Analysis of TNFα, leptin, IL-6 and adiponectin mRNA from adipocytes**

Statistical analysis of correlation between mRNA expression in mature adipocytes and preadipocytes from both VAT and SCAT in each group

Patients recruitment and collection of VAT, SCAT and blood

Obese and non-obese subjects of both diabetic and non diabetic group, undergoing abdominal surgical procedure on their own merit will be recruited from Dept. of Surgery, Institute of Post-Graduate Medical Education and Research and Seth Sukhlal Karnani Memorial Hospital (IPGMER and SSKM), Kolkata,India.

Inclusion criteria:

- Subjects with BMI ≥25kg/m2 will be considered as obese,
- Subjects with HbA1c >5.6%will be considered as diabetic.
- Subjects with BMI ≤ 24.9 kg/m2, and HbA1c with ≤ 5.6% will be considered as non diabetic and non obese or control.

Exclusion criteria:

- Subjects having cardiovascular disorder, lung disease, kidney disease, Cancer will be excluded.
- Subjects taking medicines like TZD will be excluded from our study

The study will be performed under the regulation of Institutional Ethical Clearance vide memo no .IPGMER/IEC/2018/049 Dt-20.01.2018

Isolation of preadipocytes and adipocytes from VAT and SCAT

White adipose tissues (VAT and SCAT) will be obtained from recruited patients who underwent abdominal surgery in SSKM hospital after obtaining their informed consents. Approximately 1g of adipose tissues will be digested by Sterile ***0.075-0.1% collagenase type IA*** according to standard method to obtain preadipocytes and mature adipocytes. RNA will be isolated from these cells using RNA Kit. RNA sample concentration will be measured Spectrophotometrically in 260nm wavelength.

Real-Time Reverse Transcription-Polymerase Chain Reaction

RNA for Adiponectin, Leptin, TNFα and IL-6 from adipocytes and RNA for PPARy, c/EBP and SREBP obtained from preadipocytes will be converted into cDNA and those cDNA will be amplified and quantified by RT-PCR. β-acting gene will be employed as house-keeping gene.

Primers for correspondind mRNA

GENE	PRIMER
Adiponectin	**forward: 5'-AAGGAGATCCAGGTCTTATTGG-3', reverse: 5'-ACCTTCAGCCCCGGGTAC-3'**
Leptin	**forward: 5'-TTTGGCCCTATCTTTTCTATGTCC-3' reverse: 5'-TGGAGGAGACTGACTGCGTG-3'**
TNFα	**forward5'-GCCACCACGCTCTTCTG-3′ reverse 5′-GGTGTGGGTGAGGAGCA-3′;**
IL-6	**Forward: 5′-ACAACCACGGCCTTCCCTACTT-3′ Reverse: 5′-CACGATTTCCCAGAGAACATGTG-3′;**
PPARy	**forward:5′-CCAGAGTCTGCTGATCTGCG-3′ reverse:5′-GCCACCTCTTTGCTCTGCTC-3′**
c/EBP-α	**Forword: 5'-GTTAGCCATGTGGTAGGAGACA-3' Reverse: 5'-CCCAGCCGTTAGTGAAGAGT-3'**
SREBP1	**Forword:5'-GGTGAGTGGCGGAACCAT-3' Reverse:5'-GCCGGTTGATAGGCAGCTT-3'**
β-acting gene	**Forward: 5'-CCCTGTATGCCTCTGGTC-3' Reverse: 5'-GTCTTTACGGATGTCAACG-3'**

Serum Assay

Serum Adiponectin, leptin, TNFα and IL-6 will be analysed by commercially available standard ELISA kit.

Experimental Work done in 1st year of study

- Subject selection

We have recruited 9 control or non obese non diabetic subjects and 7 non diabetic obese subjects undergoing abdominal surgical procedure on their own merit from Dept. of Surgery, Institute of Post-Graduate Medical Education and Research and Seth Sukhlal Karnani Memorial Hospital (IPGMER and SSKM), Kolkata, India. All subjects gave informed consent before participating in study. Subjects were categorized in control and non- diabetic obese on the basis of BMI and HbA1c% criteria. Subjects with BMI≤24.9 Kg/m2, and HbA1c% with ≤ 5.6were considered as control. Subjects with BMI ≥25 kg/m2 and HbA1c% with ≤ 5.6 were considered as non-diabetic obese. Subjects having cardiovascular disorder, lung disease, kidney disease, Cancer, Inflammatory bowel disease, autoimmune disorder will be excluded. Subjects taking medicines like TZD, Insulin have been excluded from our study.

- Anthropometric measurement:
  Height, Waist circumference, Hip circumference, neck circumference body weight were taken by stadiometer measuring tape and weighing machine respectively. Body fat percentage, total body fat mass visceral fat mass were analysed through Dual X-ray Absorbmetry.

- Serum Assay

Blood lipids, glucose, and HbA1c% were measured using standard clinical assays. Blood samples were taken in clot vial, EDTA vial; after 1 hr vials were centrifuged in 3500 rpm for 15 min. Separated serum then collected and aliqouted in different vials to avoid repeated freeze-thaw cycle and stored them in -20°C. Serum Adiponectin, leptin, TNFα and were analyzed by commercially available standard ELISA kit.

- Results:

There are significant differences in anthropometric parameters- BMI, waist circumference, hip circumference, waist height ratio, body fat percentage, body fat mass between two group. In serum analysis the result shows that there is no significant difference in lipid profile between these two group (results given in Table.1)The serum level of Adiponectin and TNF alpha are significantly different between these two group. But serum level of leptin does not show any significant difference.(results given in Table.2)

Table.1
Anthropometric parameter

Parameters	**Control (n=9)**	**Obese Non Diabetic (n=7)**	**P value**
BMI(kg/m2)	21.78±3.03	28.95±0.65	P<0.0001
Waist circumference(cm)	80.61±10.62	96.33±0.81	0.0034
Hip Circumference(cm)	92.72±7.74	108±3.47	0.0004
Neck Circumference (cm)	32.477±4.06	32.98±0.89	0.075
Waist :Height	0.511±0.05	0.631±0.014	P<0.0001
Waist :Hip	0.881±0.07	0.895±0.03	0.32
Body fat %	30.52±5.1	46.15±1.33	P<0.0001
Body fat mass (kg)	17.22±5.7	30.75±1.55	P<0.0001
Visceral fat mass (gm)	0.679±0.27	0.79±0.24	0.0265

Table2
Biochemical parameter

Parameters	**Control (n=9)**	**Obese Non Diabetic (n=7)**	**P value**
Cholesterol (mg/dl)	192±49.62	218.16±55.2	0.84
HDL(mg/dl)	38.71±5.8	35.5±8.71	0.328
VLDL(mg/dl)	26.33±5.83	26±4.19	0.435
LDL(mg/dl)	129.2±40	156.66±48.05	0.256
TG(mg/dl)	190.33±65.77	200.8±93	0.958
TNF alpha (pg/ml)	30.85±3.4	161.2±37.11	P<0.0001
Adiponectin (ng/ml)	84.77±41.2	30.2±12.34	0.01
Leptin (ng/ml)	18.26±14.27	68.91±65.72	0.02

Conclusion

In view of relatively small number of subjects so far recruited and not being matched by sex, it would be unfair to compare either the body fat%, body fat distribution and biochemical parameters between these two groups.

Future plan of the study

Analysis of serum level of cytokines and their expression along with above mentioned transcription factors in visceral and subcutaneous fat of all four groups will be performed next.

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