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Research Article

Role of Roof plate-specific SPONDIN3 Mutation in the Determination of Obesity Phenotypes/Fat Distribution and Susceptibility to Cardiovascular disease in Sudanese Patients in Khartoum State

Ashraf M.A. Alkinain [1]*, Kamal Eldin Ahmed Abdelsalam [2], Mutaz Ibrahim Hassan [1]

[1] Shendi University College of Medical Laboratory, Clinical Chemistry, Shendi, River Nile State, Sudan

[2] Faculty of Medical Laboratory Sciences, Seconded to Shaqra University, Saudi Arabia

ABSTRACT

Background: Obesity is a major risk factor for the development of cardiovascular disease. A growing database of clinical evidence implicates intra-abdominal adiposity as a powerful driving force for elevated cardiometabolic risk [1]. Addressing intra-abdominal adiposity should play a central role in future strategies aimed at improving cardiovascular outcomes in patients with abdominal obesity and its associated cardiometabolic risk in Sudan.

Objectives: It is to find the mutation in R-SPONDIN3gene and its association to both of fat deposition around the abdomen and susceptibility to cardiovascular disease in Sudanese patients in Khartoum State.

Material and methods: Conventional PCR was done to detect R- SPONDIN3 in 300 participants (males and females) classified into three groups. The first group will include one hundred participants with abdominal obesity, the second group will include one hundred participants already diagnosed with CVD entangled with obesity (positive control group), while the third group will include one hundred healthy lean volunteers (negative control group). Data was analyzed using SPSS Version 22 software. P value < 0.05 was considered as statistically significant.

Results: In this study, the results of Conventional PCR were significantly different in (P <0.001) in Heart group subjects as compared to healthy controls and obese group. Comparison between the different studied groups according to gene expression showed significant differences (P <0.001) mean value of gene expression in healthy group subjects was 1.0 ± 0.0, Obesity group was 2.44 ± 0.50 and heart group subjects was 4.54 ± 0.87 respectively .

Conclusion: clinically, detect R- SPONDIN3 mutation in patients with diagnosed with CVD entangled with obesity and amount of the gene expressed cleared different between obese and CVD subjects entangled with obesity.

Keywords: R-SPONDIN3 gene, abdominal Obesity, CVD.

*Address for Correspondence:

Ashraf Alkinain, Shendi University College of Medical Laboratory, Clinical Chemistry, Shendi, River Nile State, Sudan

INTRODUCTION AND LITERATURE REVIEW

Abdominal obesity, also known as central obesity, is when excessive abdominal fat around the stomach and abdomen has built up to the extent that it is likely to have a negative impact on health. There is a strong correlation between central obesity and cardiovascular disease (CVD).

CVD is now the most common cause of death.

The etiology of CVD is multifactorial and influenced by several factors in addition to obesity [2]. Most individuals that develop CVD have multiple CVD risk factors. The clustering of risk factors observed in many individuals is generally referred to as metabolic syndrome. There are several different definitions of the metabolic syndrome, but most are based on the abdominal obesity [3] .

Obesity

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. A crude population measure of obesity is the body mass index (BMI), a person's weight (in kilogram) divided by the square of his or her height (in meter). Obesity is a complex disease, with no simple relationship between fat mass and impaired health. Simply focusing on fat mass is often inadequate. Some individuals have a high quantity of fat but low metabolic risk, while others with relatively little fat are at high risk of disease development. This variation is largely dependent on two factors, size and location of adipocytes [4]. While higher cut-offs might be applicable in older people [5] . There are pros and cons in adapting BMI cut- offs depending on age and ethnicity, and the WHO has thus far recommended the

same definitions of overweight and obesity to be used globally, in all adults, and independent of age. Central fat, located around the abdomen, is associated with a greater risk of disease than peripheral fat [6]. The most commonly used measures are Waist Circumference (WC) and Waist to Hip Ratio (WHR). Several organizations have defined cut-points for abdominal obesity around one or both of these measurements, with different cut-points for men and women: WC>102 cm or WHR≥0.90 for men; WC>88 cm or WHR≥0.85 for women. More advanced measurement techniques allow for quantification of abdominal and gynoid fat masses. Abdominal fat is considered to be fats located on the abdomen, while gynoid fat is located at the lower limbs and/or around the hip area [7]. However, till yet there is no consensus on precise definitions of these fat masses, and there are therefore no specific recommendations on cut-off points. Women, compared to men, have higher percent body fat and deposit it in a different pattern, with relatively more adipose tissue in the hips and thighs. This female fat distribution, independent of total body fat, confers protection against metabolic diseases.

Visceral and central abdominal fat and waist circumference show a strong association with type 2 diabetes. Visceral fat, also known as organ fat or intra-abdominal fat, is located inside the peritoneal cavity, packed in between internal organs and torso, as opposed to subcutaneous fat. An excess of visceral fat is known as central obesity, the "pot belly" or "beer belly" effect, in which the abdomen protrudes excessively. This body type is also known as "apple shaped" as opposed to "pear shaped" in which fat is deposited on the hips and buttocks [8] .

Genetic determinants of total adiposity and distribution in women and men

Genome-wide association studies (GWAS) recently have identified genetic determinants of common polygenic obesity that interact with environmental variables in complex ways, but so far explain only a small percentage of the inter-individual variation in BMI.GWAS and meta-analyses of GWAS have also identified novel loci associated with central or peripheral fat distribution, some of which are sex-specific. Differential mRNA expression is also noted between abdominal and gluteal tissue [9]. R-Spondin 3 (RSPO3) is also called cysteine-rich and single thrombospondin domain containing-1 (CRISTIN1), protein with TSP type-1 repeat (PWTSR), is a member of the R-spondin protein family. R-spondins (RSPO) are a recently discovered secretory protein family with four members in human and mouse. Although all four RSPO proteins activate the canonical Wnt pathway, RSPO2 and RSPO3 are more potent than RSPO1, whereas RSPO4 is relatively inactive. RSPO-3 is expressed ubiquitously and expressed at higher level in placenta, small intestine, fetal thymus and lymph node. RSPO3 is the activator of the beta-catenin signaling cascade, leading to TCF-dependent gene activation. RSPO3 acts both in the canonical Wnt/beta-catenin-dependent pathway and in non-canonical Wnt signaling pathway, probably by acting as an inhibitor of ZNRF3, an important regulator of the Wnt signaling pathway. RSPO3 also acts as a ligand for frizzled FZD8 and LRP6 and may negatively regulate the TGF-beta pathway. R-SPONDIN3 play a part in controlling fat distribution [10] .

MATERIALS AND METHODS

Study Design

This was a case control study to find the mutation in R-SPONDIN3 . The study were carried out during the period from August 2016 to March 2019.

Study area

The study was conducted in Khartoum State in Ahmed Gasim hospital Cardiac Surgery and Renal Transplant Center, Alshaab Teaching Hospital and Obesity Centers

Study Group

The study was include 300 participants (males and females) classified into three groups. The first group was include one hundred participants with abdominal obesity(obese), the second group was include one hundred participants already diagnosed with CVD entangled with obesity (positive control group), while the third group was include one hundred healthy lean volunteers (negative control group).

Inclusion Criteria

Participants with abdominal obesity (obese), participants already diagnosed with CVD entangled with obesity (positive control group) and hundred lean volunteers.

Exclusion Criteria

Any history of chronic hypertension, kidney disease, liver disease, coagulation disorders. Prior informed consent was taken from all. A detailed history was taken from all subjects that include age, history of hypertension, , renal disease, hepatic dysfunction or any other acute or chronic illness. Details of drug intake was also noted. Blood pressure recording along with a detailed physical examination was done.

Ethical considerations: This case control study was approved by the research committee – College of Medical Laboratory Sciences –Shendi University. Informed consent was obtained from each participant before taking the samples.

A simple random sample were taken, seven milliliters venous blood samples were withdrawn from fasting participants and was divided into 2 tubes under aseptic conditions using sterile evacuated tubes from each subject as follows:

Three milliliters venous blood was put into serum separator gel (SSG) tube for performing lipid profile.

Four milliliters venous blood was put into a sterile ethylene di-amine tetra-acetic acid (EDTA) tube.

Quality controls and managements: Blood was collected with care and adequate safety precautions to ensure test results were reliable. Quality Assurance (QA) and standard Operating System was followed for all biological and clinical tests to achieve validity and reliability of test results.

Methods of BMI estimation:

It calculates a value indicative of the fat content of the body by dividing the weight by the square of height.

$$BMI = \frac{mass(kg)}{(height(m))^2}$$

BMI Categories:

Categories	BMI
Underweight	Less than 18.5
Normal weight	18.5 – 24.9
Overweight	25 – 29.9
Obese	30 or higher

Statistical Analysis Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.(Armonk, NY: IB was M Corp)Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level. Chi-square test was used for categorical variables, to compare between different groups. Student t-test.was used for normally distributed quantitative variables, to compare between two studied groups. F-test (ANOVA) was used for normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) (LSD) for pairwise comparisons. Pearson coefficient to correlate between two normally distributed quantitative variables. Mann Whitney test was used for abnormally distributed quantitative variables, to compare between two studied groups. Kruskal Wallis test was used for abnormally distributed quantitative variables, to compare between more than two studied groups, and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons.

RESULTS

Conventional PCR was done to all of the three groups (control, obesity and heart group). In this study, the results of Conventional PCR were significantly different in (P <0.001) in Heart group subjects as compared to healthy controls and obese group (Table 1).

Comparison between the different studied groups according to gene expression showed significant differences (P <0.001) mean value of gene expression in healthy group subjects was 1.0 ± 0.0, Obesity group was 2.44 ± 0.50 and heart group subjects was 4.54 ± 0.87 respectively (Table 2) (Figure 1).

Correlation between gene expression and age in heart and obesity group showed weak positive correlation with the r value of age in heart group was 0.034 and obesity group was 0.007 respectively (Table 3).

Correlation between gene expression and BMI in heart and obesity group showed weak Negative correlation with the r value of age in heart group was -0.259 and obesity group was 0.078 respectively (Table 3).

Correlation between gene expression and WHR in heart and obesity group showed weak Negative correlation with the r value of age in heart group was -0.064 and obesity was -0.145 respectively (Table 3).

Comparison between gene expression and fat phenotype among the heart group showed significant association (P <0.001) mean value of gene expression among heart group subjects was 5.0 ± 1.05 Visceral fat and 4.15 ± 0.36 Subcutaneous fat respectively and Comparison between gene expression and fat phenotype among the obesity group showed insignificant association (P = 0.663) mean value of gene expression among obesity group subjects was 2.45 ± 0.51 Visceral fat , 2.53 ± 0.51 Gluteal Fat and 4.15 ± 0.36 Subcutaneous fat respectively (Table 4) Figure (2) .

Comparison between gene expression and sex among the heart group showed insignificant association (P = 0.926) mean value of gene expression among heart group subjects was 4.53 ± 0.91 male and 4.55 ± 0.81 female respectively and respectively and Comparison between gene expression and sex among the obesity group showed insignificant association (P = 0.154) mean value of gene expression among obesity group subjects was 2.52 ± 0.51 male and 2.38 ± 0.49 female respectively (Table 5).

Comparison between the heart and obesity groups according to fat phenotypes showed significant differences (P <0.001) number 46 and percent 46 % Visceral fat, number 0 and percent 0 % Gluteal Fat and number 54 and percent 54 % Subcutaneous fat respectively among the heart group and number 31 and percent 31 % Visceral fat, number 17 and percent 17 % Gluteal Fat and number 52 and percent 52 % Subcutaneous fat respectively among the obesity group respectively (Table 6).

Comparison between the different studied groups according to sex showed significant differences (P = 0.035) number 54 and percent 54 % male and number 46 and percent 46 % female among control group, number 60 and percent 60 % male and number 40 and percent 40 % female among heart group and number 42 and percent 42 % male and number 58 and percent 58 % female among obesity group respectively (Table 7).

Comparison between the different studied groups according to age showed significant differences (P <0.001) mean value of age in healthy group subjects 40.70 ± 4.81, Obesity group was 43.73 ± 8.85 and heart group subjects was 50.61 ± 8.65 respectively. Comparison between the control and heart groups according to age showed significant differences (P_1<0.001). Comparison between the control and obesity groups according to age showed significant differences (P_2 = 0.015). Comparison between the heart and obesity groups according to age showed significant differences (P_3 = <0.001*) (Table 7).

Comparison between the different studied groups according to BMI showed significant differences (P <0.001) mean value of BMI in healthy group subjects 40.70 ± 4.81, Obesity group was 43.73 ± 8.85 and heart group subjects was 50.61 ± 8.65 respectively . Comparison between the control and heart groups according to BMI showed significant differences (P_1<0.001). Comparison between the control and obesity groups according to BMI showed significant differences (P_2 = 0.015). Comparison between the heart and obesity groups according to BMI showed insignificant differences (P_3=0.131) (Table 7).

Comparison between the different studied groups according to WHR showed significant differences (P <0.001) mean value of WHR in healthy group subjects 40.70 ± 4.81, Obesity group was 43.73 ± 8.85 and heart group subjects was 50.61 ± 8.65 respectively . Comparison between the control and heart groups according to WHR showed significant differences (P_1<0.001). Comparison between the control and obesity groups according to WHR showed significant differences (P_2 = 0.015). Comparison between the heart and obesity groups according to WHR showed insignificant differences (P_3=0.316) (Table 7).

Table (1): Comparison between the different studied groups according to conventional PCR

Conventional PCR	Control group (n = 100)		Heart Group (n = 100)		Obesity group (n = 100)		χ^2	P
	No.	%	No.	%	No.	%		
Negative for mutation	100	100.0	81	81.0	100	100.0	40.569*	<0.001*
Positive for mutation	0	0.0	19	19.0	0	0.0		

Table (2): Comparison between the different studied groups according to gene expression

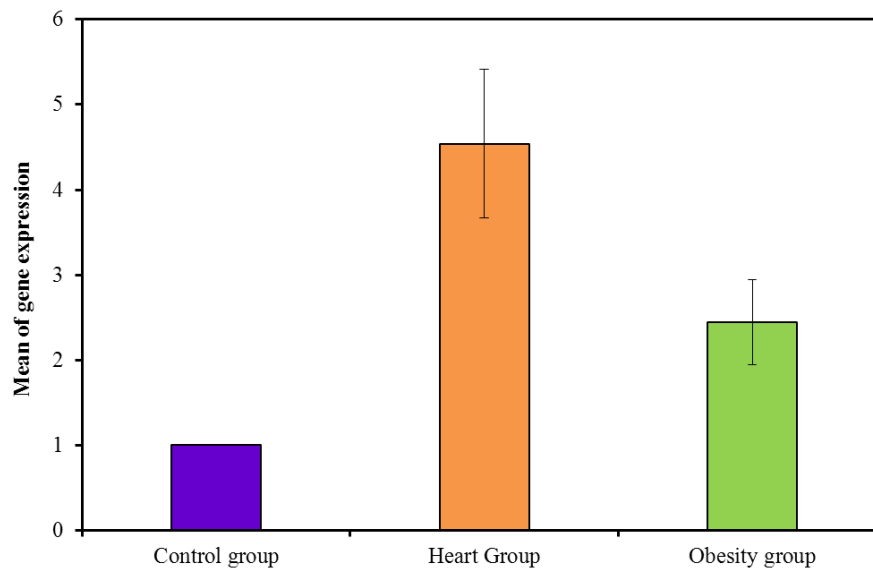| Gene expression	Control group (n = 100)	Heart Group (n = 100)	Obesity group (n = 100)	F	P
Mean ± SD.	1.0 ± 0.0	4.54 ± 0.87	2.44 ± 0.50	946.172*	<0.001*
Sig. bet. Grps	p_1<0.001*,p_2<0.001*,p_3<0.001*				

Figure (1): Comparison between the different studied groups according to gene expression

Table (3): Correlation between Gene expression and different parameters in each group

	Gene expression			
	Heart Group		Obesity group	
	r	p	r	p
Age (years)	0.034	0.739	0.007	0.948
BMI (kg/m2)	-0.259	0.009*	0.078	0.441
WHR	-0.064	0.525	-0.145	0.150

Table (4): Relation between Fat phenotypes and gene expression in each group

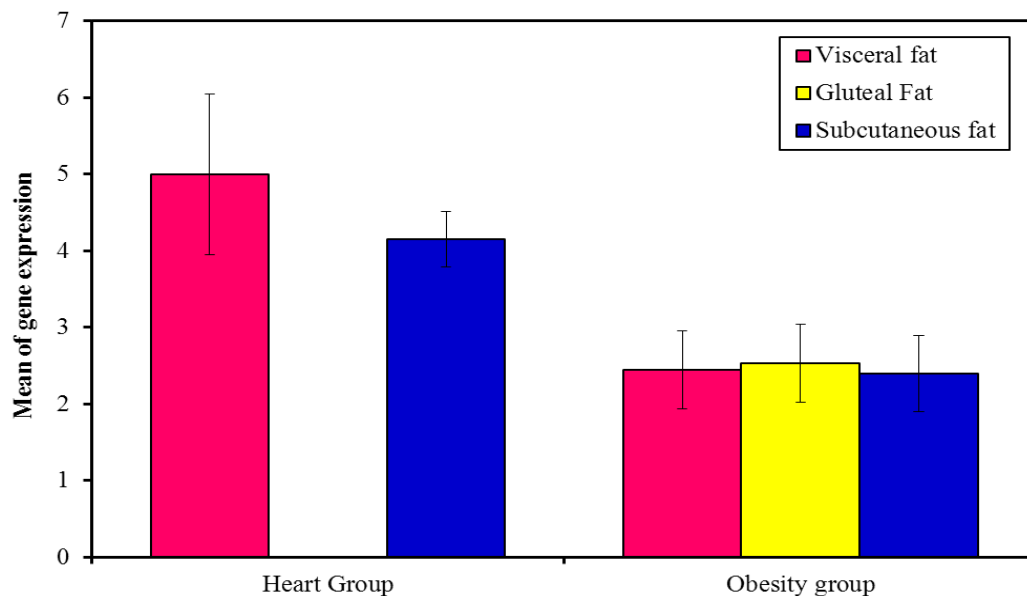| Gene expression	Fat phenotypes			Test of sig.	p
	Visceral fat	Gluteal Fat	Subcutaneous fat		
Heart Group	(n= 46)	(n= 0)	(n= 54)		
Mean ± SD.	5.0 ± 1.05	-	4.15 ± 0.36	t=5.229*	<0.001*
Obesity group	(n= 31)	(n= 17)	(n= 52)		
Mean ± SD.	2.45 ± 0.51	2.53 ± 0.51	2.40 ± 0.50	F=0.413	0.663

Figure (2): Relation between Fat phenotypes and gene expression in each group

Table (5): Relation between sex and gene expression in each group

Gene expression	Sex		t	p
	Male	**Female**		
Heart Group	**(n = 60)**	**(n = 40)**	0.093	0.926
Mean ± SD.	4.53 ± 0.91	4.55 ± 0.81		
Obesity group	**(n = 42)**	**(n = 58)**	1.437	0.154
Mean ± SD.	2.52 ± 0.51	2.38 ± 0.49		

Table (6): Comparison between the different studied groups according to fat phenotypes

Fat phenotypes	Heart Group (n = 100)		Obesity group (n = 100)		χ^2	P
	No.	**%**	**No.**	**%**		
Visceral fat	46	46.0	31	31.0		
Gluteal Fat	0	0.0	17	17.0	19.960*	<0.001*
Subcutaneous fat	54	54.0	52	52.0		

Table (7): Comparison between the different studied groups according to demographics data

	Control group (n = 100)		Heart Group (n = 100)		Obesity group (n = 100)		Test of Sig.	p
	No.	**%**	**No.**	**%**	**No.**	**%**		
Sex								
Male	54	54.0	60	60.0	42	42.0	$\chi^2=$	0.035*
Female	46	46.0	40	40.0	58	58.0	6.731*	
Age (years)								
Mean ± SD.	40.70 ± 4.81		50.61 ± 8.65		43.73 ± 8.85		F= 43.884*	<0.001*
Sig. bet. Grps	$p_1<0.001^*, p_2=0.015^*, p_3<0.001^*$							
BMI (kg/m²)								
Mean ± SD.	21.06 ± 1.41		41.01 ± 7.17		39.44 ± 6.75		F= 373.477*	<0.001*
Sig. bet. Grps	$p_1<0.001^*, p_2<0.001^*, p_3=0.131$							
WHR								
Mean ± SD.	0.84 ± 0.06		1.05 ± 0.16		1.07 ± 0.14		F= 106.401*	<0.001*
Sig. bet. Grps	$p_1<0.001^*, p_2<0.001^*, p_3=0.316$							

DISCUSSION

Body fat distribution is a heritable trait that independently predicts type 2 diabetes and cardiovascular risk. Genome-wide association studies (GWAS) meta-analyses have identified sexually dimorphic associations, with greater effect in women, between loci within RSPO3 (e.g. rs9491696) and BMI-adjusted waist-to-hip ratio (WHR). RSPO3 is a LGR4 receptor ligand and a Wnt/β-catenin signaling agonist.

The aim of study was to investigate the possible correlation between mutation in R-SPONDIN3 gene, abdominal obesity and susceptibility to cardiovascular disease.

The study was include 300 participants (156 males and 144 females) classified into three groups. The first group was include one hundred participants with abdominal obesity (obese), the second group was include one hundred participants already diagnosed with CVD entangled with obesity (Heart Group as positive control group), while the third group was include one hundred healthy lean volunteers (negative control group).All the participants their age group between 27 to 63 years old . BMI and WHR were taken for all subjects too. For detection the mutation in RSPO3 gene, Conventional PCR was done for control, obesity and heart subjects respectively and followed by Real Time PCR for all of them .The same of the three group underwent for lipid profile. For measurement of fat distribution In clinical practice, waist circumference (WC) and WHR were used used to determine regional FD. Computerized tomography (CT) and whole body MRI scan were used for evaluating the adipose tissue which were considered as gold standard for that .To measure the visceral and subcutaneous abdominal areas in total abdominal area), a CT or MRI scan was taken at the level of L4–L5 or the umbilicus. The ratio of visceral to subcutaneous adipose tissue has been shown to be strongly correlated with RSPO3 gene in obese subjects and heart subjects respectively Abdominal sagittal diameter derived from CT or MRI images has also been used to determine abdominal FD . The CT and MRI were applied in 200 volunteers,

The conventional PCR was done to all of the three groups which showed clear variation in the mean value of conventional PCR (P <0.001) in Heart group subjects as compared to healthy controls and obese group.

Of the 200 cases (obesity &heart) 19 (19%) were positive for mutation in RSPO3 gene all of them from heart group and the rest of heart group 81 (81%) were negative for mutation as well as obesity group all 0 (0 %) positive for mutation (Table 4 -3). This result was leads us to do real time PCR to quantify the level of gene expressed. Comparison between the different studied groups according to gene expression showed clear variation in the mean value of gene expression with (P <0.001) in healthy group subjects was 1.0 ± 0.0 ,Obesity group was 2.44 ± 0.50 and heart group subjects was 4.54 ± 0.87 respectively (Table 2) (Figure 1). This result was agreed with the finding of finding of N.Y. Loh[11] .

In Correlation between gene expression and age in heart and obesity group showed weak positive correlation with the r value of age in heart group was 0.034 and obesity group was 0.007 respectively (Table 3). This result was agreed with finding of Dorit Schleinitz[12].who was carried   study on heart and obesity group respectively.

Correlation between gene expression and BMI in heart and obesity group showed weak Negative correlation with the r value of age in heart group was -0.259 and obesity group was 0.078 respectively (Table 3). This result was agreed with finding of Michael M [13] .

Correlation between gene expression and WHR in heart and obesity group showed weak Negative correlation with the r value of age in heart group was -0.064 and obesity was -0.145 respectively (Table 3). This result was agreed with Rajiv G, [14].

Comparison between gene expression and fat phenotype among the heart group showed significant association (P <0.001) mean value of gene expression among heart group subjects was 5.0 ± 1.05   Visceral fat and   4.15 ± 0.36 Subcutaneous fat respectively and Comparison between gene expression and fat phenotype among the obesity group showed insignificant association (P = 0.663) mean value of gene expression among obesity group subjects was 2.45 ± 0.51   Visceral fat , 2.53 ± 0.51 Gluteal Fat and 4.15 ± 0.36 Subcutaneous fat respectively (Table 4) Figure (2) . This result was agreed with finding of Kalypso Karastergiou[15] .but study the obesity was diagnosed according to the Japanese obesity criteria by using CT & MRI Technologies .

In the Comparison between among heart group according to sex and gene expression showed insignificant differences (P = 0.926) mean value of gene expression among heart group subject was 4.53 ± 0.91 male and 4.55 ± 0.81 female. Comparison between among obesity group according to sex and gene expression showed insignificant differences (P = 0.154) mean value of gene expression among heart group subject was 2.52 ± 0.51 male and 2.38 ± 0.49 female (Table 5) .This result was agreed with finding of Atzmon, G [16].

In the Comparison between the heart and obesity groups according to fat phenotypes showed significant differences (P <0.001) number 46 and percent 46 %   Visceral fat, number 0 and percent 0 % Gluteal Fat and number 54 and percent 54 % Subcutaneous fat   respectively among the heart group and number 31 and percent 31 % Visceral fat, number 17 and percent 17 % Gluteal Fat and number 52 and percent 52 % Subcutaneous fat respectively among the obesity group respectively (Table 6). This result was agreed with finding of Ian J. Neeland[17]and Tobin M. Abraham[18].

In the Comparison between the different studied groups according to age showed significant differences (P <0.001) mean value of age in healthy group subjects 40.70 ± 4.81, Obesity group was 43.73 ± 8.85 and heart group subjects was 50.61 ± 8.65 respectively. Comparison between the control and heart groups according to age showed significant differences (P$_1$<0.001). Comparison between the control and obesity groups according to age showed significant differences (P$_2$ = 0.015). Comparison between the heart and obesity groups according to age showed significant differences (P$_3$ = <0.001*) (Table7). This result was agreed with finding of Kalypso [15].

In the Comparison between the different studied groups according to BMI showed significant differences (P <0.001) mean value of BMI in healthy group subjects 40.70 ± 4.81, Obesity group was 43.73 ± 8.85 and heart group subjects was 50.61 ± 8.65 respectively. Comparison between the control and heart groups according to BMI showed significant differences (P$_1$<0.001). Comparison between the control and obesity groups according to BMI showed significant differences (P$_2$ = 0.015). Comparison between the heart and obesity groups according to BMI showed insignificant differences (P$_3$=0.131)(Table 7) .

In the Comparison between the different studied groups according to WHR showed significant differences (P <0.001) mean value of WHR in healthy group subjects 40.70 ± 4.81, Obesity group was 43.73 ± 8.85 and heart group subjects was 50.61 ± 8.65 respectively. Comparison between the control and heart groups according to WHR showed

significant differences (P_1<0.001). Comparison between the control and obesity groups according to WHR showed significant differences (P_2 = 0.015). Comparison between the heart and obesity groups according to WHR showed insignificant differences (P_3=0.316) (Table 7) . This result was agreed with finding of Adamska M [19] who concluded the obese and hearts subjects having higher WHR when compared with healthy subjects.

CONCLUSION

The amount of the R- SPONDIN3 gene expressed cleared different between obese and CVD subjects entangled with obesity.

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