

Original Research Article

## **Atherogenic Indices and Polymorphism of the FXR Gene in Obese Individuals, Overweight and Normal Weight Individuals**

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**Abstract:** Obesity is the result of interplay between genetic and environmental factors. Polymorphisms in various genes controlling appetite and metabolism predispose to obesity when sufficient calories are present. It is not known whether polymorphisms in FXR gene are associated with high lipid level among obese individuals. This study was designed to investigate and compare the lipid profile and polymorphism of the FXR gene in obese individuals, overweight and normal weight individuals. We investigated 35 obese subjects, 35 overweight subjects and 35 normal weight subjects. Plasma triglyceride, plasma cholesterol, plasma high density lipoprotein (HDL), plasma low density lipoprotein (LDL) and fasting blood glucose were determined in these subjects. These subjects were also genotyped for farnesoid receptor. Abnormal allele of FXR gene occurred more frequently in the obese category than those in overweight and normal weight category. Plasma triglyceride was significantly lower in FXR homozygous (FXR<sup>+/+</sup>) individual than FXR heterozygous (FXR<sup>+/-</sup>) individual (p<0.05). Plasma HDL was significantly higher in heterozygous subjects than homozygous subjects for FXR. Plasma cholesterol and LDL was significantly lower in FXR<sup>+/+</sup> as compared to FXR<sup>+/-</sup> (p<0.05). Genetic changes affecting FXR function have the potential to be proatherogenic and early management strategy should be encouraged to protect these group of individual from atherogenic complications.

**Keywords:** Atherogenic indices, polymorphism, FXR gene, Obese, Overweight, Normal Weight

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### **INTRODUCTION**

Obesity is a medical condition that results in accumulation of excess body fats, to the extent that it has an adverse effect on health, leading to reduced life expectancy [1]. It is defined by Body Mass Index (BMI) and further evaluated in terms of fat distribution via the waist-hip ratio and total cardiovascular risk factors. Body Mass Index (BMI) defines people as underweight when their BMI is <18.5 kg/m<sup>2</sup>, normal weight when BMI is between 18.5 kg/m<sup>2</sup> and 24.9 kg/m<sup>2</sup>, overweight (pre-obese) when their BMI is between 25 kg/m<sup>2</sup> and 30 kg/m<sup>2</sup>, and obese when it is greater than 30 kg/m<sup>2</sup> [1]. BMI provides a simple, convenient measurement of obesity, a more important aspect of obesity is the regional distribution of excess body fat [2]. Mortality and morbidity vary with the distribution of body fat, with the highest risk linked to excessive abdominal fat [3]. Central obesity is related to a number of diseases; cardiovascular disease (CVD) and non-insulin dependent diabetes mellitus (NIDDM) [4].

Obesity increases the risk of many adverse physical and mental conditions [4]. These co-morbidities

are commonly shown in metabolic syndromes, a combination of medical disorders which includes: diabetes mellitus type 2, high blood pressure, high blood cholesterol, and high triglyceride level hence the need to assess the lipid profile and fasting blood glucose in obese subjects. [5]. An estimated 1.3 billion people are obese, the world health organization predicts that by 2015 approximately 2.3 billion people will be overweight and 700 million obese [1]. Its prevalence in developed countries such as the United States is as high as 26.6% in men and 32.2% in women above age 21. In Africa, more than one third of African women and a quarter of African men are overweight and obese and the world health organization predicts a rise to 41% and 30% respectively in the next ten years. Diet plays an important role in obesity. Total calorie consumption has been found to be related to obesity. Most of these extra calories came from an increase in carbohydrate consumption rather than fat consumption [7]. The sources of these extra carbohydrates are sweetened beverages, which now account for almost 25 percent of daily calories in young adult [5]. Consumption of sweetened drinks is believed to be contributing to the rising rates of obesity [7]. As societies become increasingly reliant on energy-dense, big-portion, fast-

food meals, the association between fast-food consumption and obesity becomes more [8]. Like many other medical conditions, obesity is the result of interplay between genetic and environmental factors. Polymorphisms in various genes controlling appetite and metabolism predispose to obesity when sufficient calories are present. As of 2006 more than 41 of these sites have been linked to the development of obesity when a favorable environment is present [9].

Medical illnesses that increase obesity risks include several rare genetic syndromes as well as some congenital or acquired conditions: hypothyroidism, Cushing's syndrome, growth hormone deficiency[5].

Certain medications may cause weight gain or changes in body composition; these include insulin, sulfonyleureas, thiazolidinediones, atypical antipsychotics, antidepressants, steroids, certain anticonvulsants (phenytoin and valproate), pizotifen, and some forms of hormonal contraception. These drugs can slow the way the body burns calories, increase appetite, or cause the body to hold on to extra water, all of which can lead to weight gain [5].

A sedentary lifestyle plays a significant role in development of obesity .Worldwide there has been a large shift towards less physically demanding work and currently at least 60% of the world's population gets insufficient exercise. This is primarily due to increasing use of mechanized transportation and a greater prevalence of labor-saving technology in the home [1].In children, there appears to be declines in levels of physical activity due to less walking and physical education. The World Health Organization [1],indicates that people worldwide are taking up less active recreational pursuits.

Smoking has a significant effect on an individual's weight [10].People gain weight when they stop smoking, one reason is that food often tastes and smells better. Another reason is that nicotine raises the rate at which the body burns calories so that fewer calories are burned when one stops smoking. Those who quit smoking gain an average of 4.4 kilograms (9.7 lb) for men and 5.0 kilograms (11.0 lb) for women over ten years [10].

During pregnancy, women gain weight so that the baby gets proper nourishment and develops normally. After pregnancy, some women find it hard to lose the weight [11]. This may lead to obesity, especially after a few pregnancies. In the United States the number of children a person has is related to their risk of obesity. A woman's risk increases by 7% per child, while a man's risk increases by 4% per child [12].

In the developing world, urbanization is playing a role in the increasing rate of obesity. In China overall rates of obesity are below 5%; however, in some

cities, rates of obesity are greater than 20% [1]. Malnutrition in early life is believed to play a role in the rising rates of obesity in the developing world [5].

As one gets older, one tends to lose muscle especially if less active [1]. Muscle loss can slow down the rate at which the body burns calories. Midlife weight gain in women is mainly due to aging and lifestyles. Menopause also increases weight gain [1].Key features in obesity are insulin resistance, impaired, altered lipid metabolism, and an increase in metabolically unfavorable fat depots.

Bile acid metabolism is involved in all these features [14] and bile acid composition is altered in obesity [14]. Bile acids are produced in the liver and released into bile. After a meal, these bile acids are secreted into the intestine in which they are modified by bacteria. From the intestine, 95% of bile acids are reabsorbed into the bloodstream. Interestingly, their blood levels are rapidly regulated by glucose intake. In addition to their function in the absorption of lipophilic nutrients, bile acids serve as signaling molecules [15]. The best-characterized bile acid receptor is farnesoid X receptor (FXR). It is encoded by *NR1H4* (nuclear receptor subfamily 1, group H, member 4).

The bile acid receptor (BAR), also known as farnesoid X receptor (FXR) or NR1H4 (nuclear receptor subfamily 1, group H, member 4) is a nuclear receptor that is encoded by the *NR1H4* gene in humans[16].

FXR is expressed at high levels in the liver and intestine. Chenodeoxycholic acid and other bile acids are natural ligands for FXR. Similar to other nuclear receptors, when activated, FXR translocates to the cell nucleus, forms a dimer (in this case a heterodimer with RXR) and binds to hormone response elements on DNA, which up- or down-regulates the expression of certain genes[16].

One of the primary functions of FXR activation is the suppression of cholesterol 7 alpha-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid synthesis from cholesterol. FXR does not directly bind to the CYP7A1 promoter. Rather, FXR induces expression of small heterodimer partner (SHP), which then functions to inhibit transcription of the CYP7A1 gene. In this way a negative feedback pathway is established in which synthesis of bile acids is inhibited when cellular levels are already high. In addition, FXR controls the enterohepatic circulation of bile acids by increasing the transcription of the intestinal ileal-bile acid binding protein , inhibiting the hepatic expression of bile acid transporters Na-taurocholate cotransporting polypeptide [16]. Overall, bile acid-activated FXR controls bile acid homeostasis. FXR also regulates lipoprotein metabolism. FXR-deficient mice display elevated serum cholesterol and triglyceride levels [17]. Moreover, FXR regulates genes controlling lipid

metabolism, such as apolipoprotein (apo) C-II, apo C-III, apo A-I, apo E, and phospholipid transfer protein. In addition, FXR induces peroxisome proliferator-activated receptor- expression [18]. another nuclear receptor controlling triglyceride metabolism, corroborating a potential role for FXR in triglyceride metabolism. FXR has also been found to be important in regulation of hepatic triglyceride levels [19]. Farnesoid X receptor has been shown to interact with: Peroxisome proliferator-activated receptor, gamma co activator 1 alpha [20] and Retinoid X receptor alpha. A number of ligands for FXR are known, of both natural and synthetic origin. Agonists :Cafestol [21], Chenodeoxycholic acid, Obeticholic acid, and Fexaramine.

The farnesoid X receptor (FXR; gene symbol NR1H4) is a nuclear receptor that functions as the main sensor of intracellular bile acid levels. The human NR1H4 gene is located on chromosome 12 and is composed of 11 exons and 10 introns [22]. The translation initiation codon of the NR1H4 gene lies at the 3' end of exon 3, whereas exons 1 and 2, together with the 5' region of exon 3, contain the 5' untranslated region (5'-UTR). Multiple FXR isoforms can be generated via alternative promoter usage and alternative splicing, and these isoforms may have differential transactivation abilities on specific target promoters. There are limited number of studies that describes how fxr gene mutation or polymorphism correlates with altered lipid and glucose metabolism in humans.

#### STATEMENT OF PROBLEM

Obesity is the result of interplay between genetic and environmental factors. Polymorphisms in various genes controlling appetite and metabolism predispose to obesity when sufficient calories are present. It is not known whether polymorphisms in FXR gene is associated with high lipid level among obese individuals, so the present study is designed to investigate and compare the lipid profile and polymorphism of the FXR gene in obese individuals, overweight and normal weight individuals .

#### AIM OF STUDY

This study is designed to investigate and compare the atherogenic indices and polymorphism of the FXR gene in obese individuals, overweight and normal weight individuals.

#### OBJECTIVES

- To determine the allelic frequency of Farnesoid X receptor gene polymorphism in obese, overweight and normal weight individual.
- To determine the atherogenic indices in obese, overweight and normal weight individuals.

#### HYPOTHESIS

- There is no relationship between farnesoid X receptor gene polymorphism and atherogenic indices in obese category

- Abnormal allele of FXR gene does not occur more frequently in the obese category

#### MATERIALS AND METHOD

##### Study design

A case control study:

The study was approved by the Federal Medical Centre, Owo Ethical Committee (Ref: No. FMC/OWO/380/VOL.XXIV/01)

##### Duration of research

Three months (June 2014 to September 2014)

##### Materials

- Needle and syringe; was used for withdrawing blood from the suitable venipuncture site.
- Tourniquet; was used to increase the distension of vein when a blood sample is being collected.
- Swab; was used to clean the vein puncture site and allow for free flow of blood.
- Plain specimen container; was used to collect the plasma that was spun for lipid profiles estimation.
- EDTA bottle; was used to collect the blood withdrawn from the vein puncture site for lipid profile estimation.
- Scale; was used to take the subjects weights.
- Tape rule was used to take the subject waist circumference

##### Study area

The study was conducted in Owo, Owo local government. Owo is a town in Ondo State, situated in the Southern-Western Nigeria, latitude 7.19620 and longitude 5.586810 at an elevation/altitude of meters. It is at the southern edge of the Yoruba hills, and at the intersection of roads from Akure, Kabba and Benin City. The ethnic compositions of Ondo State are largely from the Yoruba subgroups of the Akoko, Akure, Ikale, Ilaje, Ondo, and Owo. Ijaw minority (such as Apoi and Arogbo) populations inhabits the coastal areas; while the Ife-speaking people of Oke-Igbo constitute another minority.

Ondo State is predominantly an agricultural State with over 60% of its labour force deriving their income from farming. Agriculture (including fishing) constitutes the main occupation of the people of the state. Indeed, Ondo state is the leading cocoa producing state in Nigeria. Other agricultural products include yams, cassava and palm produce. The State is richly blessed with varied and favourable ecological and climatological conditions with vegetation ranging from mangrove swamps of the southern coastal riverine areas through the rainforest of the midlands to the derived Savannah in the Northern part of the State. Thus the State can support the cultivation of a large variety of crops. The average annual rainfall is about 1300mm and average atmospheric temperature of 30°C which support

the high breed of mosquitoes. Study protocol was approved by ethical board committee of Federal Medical Center Owo, Ondo State, Nigeria.

#### Sample size

Sample size was determined by the formula

$$N = 4pq/l^2$$

Where, N is sample size,

Q is 1-p, and l is permissible error (5% of p)

P is prevalence of obesity among individuals in Ondo state = 1.5 % = 0.015 (Mustapha and Sanusi, 2013)

$$\text{Sample size} = 4pq/l^2$$

$$P = 1.5$$

$$q = 1 - p, 1 - 1.5 = 0.5$$

l is constant

$$N = 4 \times 1.5 \times 0.5 / 1^2 = 3$$

$$N = 3$$

The prevalence of overweight individuals in Nigeria from previous study is **7.9%** (Mustapha and Sanusi, 2013)

$$\text{Sample size (N)} = 4pq/l^2$$

$$P = 7.9\% \text{ or}$$

$$q = 1 - p, 1 - 7.9\% = 6.9$$

l is constant

$$N = 4 \times 7.9 \times 6.9 / 1^2 =$$

$$N = 218$$

The total number of 221 subjects will participate in this project. But For convenience, a total of 105 samples comprising of 35 obese individuals, 35 overweight individuals and 35 normal weights as the controls were used in this study.

#### Study population

A total number of 105 individuals participated in this study .Their age bracket was 18-50 years. A written consent form was signed by each participant after full explanation of the procedure of the study. All participants had the right to withdraw at any time during the study without any explanation. They were told that all the data were confidential and were only for research purposes.

#### Data collection procedure

Data collection took place in two steps. The first step was to fill out the questionnaire and the second step involved the use of anthropometric measurements for the determination of Body Mass Index (BMI). The questionnaire included socio-demographic data, present, past and family history of any medical condition, data about physical activity and dietary habits. At the end of the session, anthropometric measurements were taken. Height was measured using a meter rule in standing position without footwear and weight also measured with minimum clothes using a weighing scale; both were used to derive BMI. Waist circumference measurements were determined using a tape rule at the narrowest part of the waist. Blood pressure and pulse

rate were taken simultaneously using a sphygmomanometer.

#### Sample collection

All participants were asked not to eat after 10pm, the evening before test. The following day, 5mls venous blood sample was collected from each participant for laboratory analysis. For venous glucose determination, blood was collected into sodium fluoride tubes and for lipid profile estimation, in lithium heparin. Blood specimen was also collected into an EDTA (ethylene diaminetetraacetic acid) tubes or bottles for genetic analysis.

#### Processing of specimen

100ul of blood was collected from sample in the EDTA tubes for genetic analysis. Serum was then separated from red cells by centrifuging the blood samples at 1500rpm for 15mins. The serum was then stored in refrigerator at 2 - 4°C prior to analysis

## METHODS

### Anthropometrics Measurement

**Weight:** Weight of each participant was determined with each of them wearing minimal clothing in kilograms (Kg).

**Height:** Height was measured in meter using an appropriate ruler with the participants standing erect, bare-footed and looking straight ahead.

**Waist Circumference (WC):** was measured in inches with a flexible but non elastic measuring tape. Waist circumference was measured at level of the natural waist.

**Body mass index (BMI):** was calculated by dividing the body weight (in kilograms) by the height (in meters squared). Body mass index was calculated as follows:

$$= \frac{\text{Weight (kg)}}{\text{Height (m)}^2} = \text{kg/m}^2$$

Body mass index

Participants were grouped as;

Normal weight: BMI is between 18.5 kg/m<sup>2</sup> - 24.9 kg/m<sup>2</sup>

Overweight: BMI is between 25 kg/m<sup>2</sup> – 29.9 kg/m<sup>2</sup>

Obese: BMI is greater than 30 kg/m<sup>2</sup>

### Method of DNA extraction

The blood samples used for this investigation was collected, identified authenticated and properly mixed and was kept at 4<sup>o</sup>c together with the plasma which was separated while the extracted DNA was kept at room temperature. All this was done at the very day of sample collection.

### Extraction of DNA

- Using an eppendoff tube, three hundred microliter of red cell lysis was added to one hundred

microliter of the whole blood cell and mixed thoroughly.

- The set up was left on the bench for thirty minutes and then spun at 5000rpm for five minutes.
- It was shaken to decant the mixture.
- One hundred microliter of cell lysis was added together with one hundred and fifty microliter of protein precipitate. It was left at room temperature for fifteen minutes.
- The mixture was spun 5000rpm for 5minutes.
- The supernatant of the centrifuged mixture was taken into another eppendoff tube containing two hundred and fifty microliter of isopropanol.
- A strand of DNA was seen and centrifuged down to decant the supernatant in order to add 70% of alcohol.

### Polymerase Chain Reaction (PCR)

- 0.5ul of 1.25uM of each of the three primers was added into a 0.2 ml thin walled PCR tube containing 12.5ul of 2x dream Taq green master mix
- The mixture was made up to 24ul with nuclease free water
- 1ul of template DNA was then added to the mixture

The primers used in this amplification process are

- COMMON FORWARD PRIMER –  
5' – GTTGTAGTGGTACCCAGAGGCCCTG -3'
- COMMON REVERSE PRIMER –  
5' -TATGCTAACAGAACACGCGGCAGGC – 3'
- MUTANT FXR F  
5' –  
GTTGTAAGTGGTAACCCAGAAGGCCCTG-3'
- MUTANT FXR R  
5' – GGGTGGGATTAGATAAATGCCTGCTCT  
– 3'

- The 0.2 ml thin walled PCR tubes was covered with their respective caps and then placed into the thermal cycler. The lid was firmly fixed on the thermal cycler in order to start the program
- The PCR was conducted under the cycling conditions below.
  - 95°C - 2min
  - 95°C - 30sec
  - 65°C - 30sec
  - 72°C - 1min
  - 72°C - 7min
  - 4°C - forever.

### Gel Electrophoresis

A total of 15 uL of PCR product was loaded in each well, with 100bp ladder occupying the first well. The gel was allowed to run for 30 minutes at 120V from negative to positive electrode. After the period, the gel was placed on the UV transilluminator and observed for the bands of DNA. The negative control lane showed no visible bands. The picture of visible bands on the gel

was finally taken and compared with respect to the ladder.

### Glucose Estimation

Using Glucose Oxidase Peroxidase Method [22] and reagent kit of RANDOX

#### Principle

Glucose oxidase catalyses the oxidation of glucose to produce hydrogen peroxide and gluconic acid. The hydrogen peroxide, in the presence of enzyme peroxidase is broken down and the oxygen given off reacts with 4-aminophenazone and phenol to give a pink colour.

Reference range for adult;

Fasting blood glucose level → 3.3-5.6 mmol/l or 60-100mg/dl

### Serum Triglycerides Estimation

Using Acetylacetone Method [22] and reagent kit of RANDOX

#### Principle

- Triglycerides are hydrolyzed by the enzyme lipase to produce glycerol and fatty acids.
- The enzyme glycerol kinase acts on glycerol in the presence of ATP to form glycerol-3-phosphate and ADP.
- Glycerol-3-phosphate is oxidized by glycerol phosphate oxidase to dihydroxyacetone and hydrogen peroxide
- Oxygen is released from hydrogen peroxide in the presence of peroxidase, which oxidizes p-chlorophenolchromogen to form coloured compound.

Reference range; 80-150 mg/dl or 0.9-1.7mmol/l

### Estimation Of Total Serum Cholesterol

Using Enzymatic Method[22] and reagent kit of RANDOX

#### Principle

- Cholesterol esters is hydrolyzed in the presence of cholesterol ester hydrolase to give fatty acids and cholesterol
- The total cholesterol is then oxidized in the presence of cholesterol oxidase to produce hydrogen peroxide and chol-4-en-3-one
- The hydrogen peroxide produced is then reduced in the presence of peroxidase to water and oxygen
- The produced oxygen is made to react with 4-aminophenazone in the presence of phenol to give a red colour complex

Reference ranges;

150-250 mg/dl or 3.88-6.2mmol/l

### Estimation Of Serum High Density Lipoprotein

Using Watson Method Or Precipitation Method [22] and reagent kit of RANDOX

**Principle**

Chylomicrons, very low density lipoprotein (VLDL), low density lipoprotein (LDL) are precipitated by phosphotungstic acid in the presence of magnesium ions leaving HDL in the solution. After centrifugation, high density lipoproteins are in the supernatant. HDL content of supernatant is measured by an enzymatic method

Reference ranges;

Men: 30-60 mg/dl. Or 0.8-1.5

Women: 40-70 mg/dl. Or 1-1.8

**Estimation Of Serum Low Density Lipoprotein**

Using The Friedewald Formular; [23]

LDL = TOTAL Cholesterol – HDL – TG/2.2, were all concentrations are given in mmol/l or LDL = Total Cholesterol – HDL –TG/5, if all concentrations are given in mg/dl

Note; TG/2.2 or TG/5 represents an estimation of the very low density lipoprotein (VLDL)

**Reference range;**

< 100 mg/dl – Optimal

100-129 mg/dl – Near optimal

130 – 159 mg/dl – Borderline high

160 – 189 mg/dl – High

> 190 mg/dl – very high

**RESULTS**

**Table 1: Comparing frequency of subjects with one copy of wild type allele (heterozygous individuals) and those with two copies of the wild type allele (homozygous individuals) among obese, overweight and normal weight individuals**

CATEGORY	FXR+/+	FXR+/-	P VALUE
OBESE N (%)	9 (37.5%)	15 (62.5%)	P = 0.0422
OVERWEIGHT N (%)	19 (61.3%)	12 (38.7%)	
NORMALWEIGHT N (%)	16 (48.5%)	17 (51.5%)	

An overall prevalence rate of 62.5% of FXR+/- was observed among obese category while 38.7% and 51.5% of FXR+/- was noted among overweight and

normal weight subjects respectively. FXR+/- was a significant (p < 0.05) risk factor for an increase weight gain

**Table 2: Comparing concentrations of Triglyceride, Total Cholesterol, Low Density Lipoprotein, High Density Lipoprotein and Fasting Blood Sugar of between populations that is homozygous and heterozygous for FXR gene**

ANALYTE	FXR+/- MEAN±SD	FXR+/+ MEAN±SD	p value
Triglyceride (mmol/l)	1.55±0.24	1.47±0.21	p < 0.05
Cholesterol (mmol/l)	5.19±1.07	4.11±0.93	p < 0.05
LDL (mmol/l)	2.00±0.77	1.57±0.52	p < 0.05
HDL (mmol/l)	2.42±0.57	2.06±1.6	p > 0.05
FBS (mmol/l)	4.87±1.04	4.75±0.99	p > 0.05

Table 2 show that there was statistically significant increase in mean values of plasma triglycerides, total cholesterol and low density lipoprotein, (p<0.05), but no statistically significant difference in mean value of High Density Lipoprotein

and Fasting Blood Sugar (p>0.05) when population with one copy of wild type allele (heterozygous) was compared with group with two copies of wild type (homozygous) of FXR gene.

**Table 3: Comparing concentrations of Triglyceride, Total Cholesterol, Low Density Lipoprotein, High Density Lipoprotein and Fasting Blood Sugar in obese, overweight and normal categories in heterozygous population (FXR<sup>+/-</sup>)**

PARAMETERS	OBESE MEAN±SD	OVERWEIGHT MEAN±SD	NORMALWEIGHT MEAN±SD
Triglyceride (mmol/l)	1.73±0.17**	1.61±0.19**	1.33±0.15
Cholesterol (mmol/l)	5.65±1.05*	4.87±1.13	5.01±0.97
LDL (mmol/l)	2.41±0.92*	1.66±0.68	1.88±0.51
HDL (mmol/l)	2.33±0.50	2.39±0.66	2.53±0.58
FBS (mmol/l)	4.96±0.86	4.57±0.90	4.99±1.28

\* = P< 0.05 (Normal weight Vs Overweight)

\*\*= P< 0.01 (Normal weight Vs Obese)

Table 3 statistically, (p < 0.05) the table above show a significance increase in the mean values of triglyceride among the overweight and obese

individuals when both were compared with the normal weight group. A statistical significance increase (p < 0.05) was also seen in the mean value of total

cholesterol and low density lipoprotein in obese group when compared with the normal category. However no statistically significant difference was seen in mean

value of High Density Lipoprotein and Fasting Blood Sugar when the normal weight was compared with the overweight and the obese individuals. ( $p>0.05$ )

**Table 4: Comparing concentrations of Triglyceride, Total Cholesterol, Low Density Lipoprotein, High Density Lipoprotein and Fasting Blood Sugar in obese, overweight and normal categories in heterozygous population (FXR<sup>+/+</sup>)**

PARAMETERS	OBESE MEAN±SD	OVERWEIGHT MEAN±SD	NORMALWEIGHT MEAN±SD
Triglyceride (mmol/l)	1.71±0.12**	1.49±0.18**	1.32±0.14
Cholesterol (mmol/l)	4.10±0.61	4.24±1.07	3.95±0.91
LDL (mmol/l)	1.74±0.46	1.57±0.56	1.47±0.52
HDL (mmol/l)	1.57±0.31	1.91±0.55	2.51±2.63
FBS (mmol/l)	4.87±0.76	4.82±1.14	4.59±0.94

Table 4 show that there was statistically significant increase in mean value of triglyceride ( $p<0.01$ ), but no statistically significant difference in mean value of Total Cholesterol, High Density

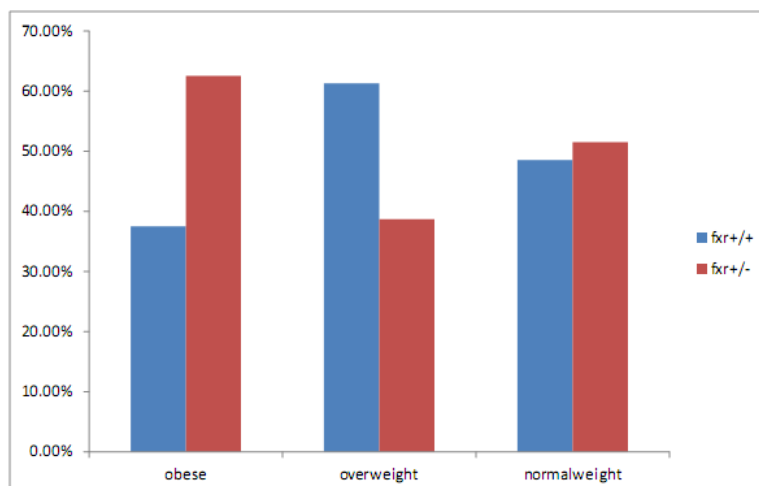
Lipoprotein, low density lipoprotein and Fasting Blood Sugar ( $p>0.05$ ) when the normal was compared with obese and overweight individuals in population with two copies of wild type allele of FXR gene

**Table 5: Comparing concentrations of Triglyceride, Total Cholesterol, Low Density Lipoprotein, High Density Lipoprotein and Fasting Blood Sugar of both populations that is homozygous and heterozygous for FXR gene among the obese group**

ANALYTE	FXR <sup>+/-</sup> MEAN±SD	FXR <sup>+/+</sup> MEAN±SD P VALUE
TG (mmol/l)	1.73±0.17	1.71±0.12 P > 0.05
TC (mmol/l)	5.65±1.05	4.10±0.61 P < 0.05
LDL (mmol/l)	2.41±0.92	1.74±0.46 P < 0.05
HDL (mmol/l)	2.33±0.50	1.57±0.31 P < 0.05
FBS (mmol/l)	4.96±0.86	4.87±0.76 P > 0.05

Table above show that there was statistically significant increase in mean value of Total Cholesterol, High Density Lipoprotein and low density lipoprotein ( $p<0.05$ ) among the heterozygous individuals compared with the homozygous individuals in the obese category,

but no statistically significant difference in mean value of Triglyceride, and Fasting Blood Sugar ( $p>0.05$ ) when the homozygous was compared with the heterozygous group in the obese category.



**Fig-1: A Graph showing the percentage frequency of FXR mutant alleles among each category**

Abnormal allele of FXR gene occurred more frequently in the obese category than those in overweight and normal weight category.

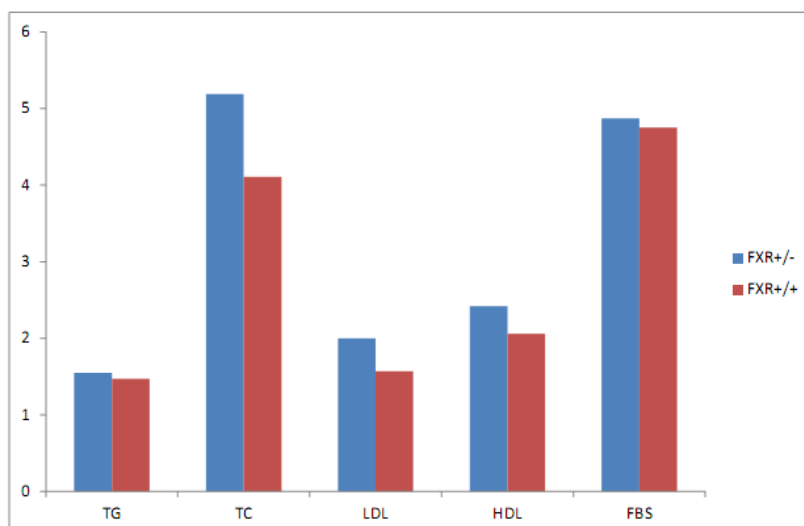


Fig-2: A graph showing the concentrations of each parameter in homozygous and heterozygous individuals

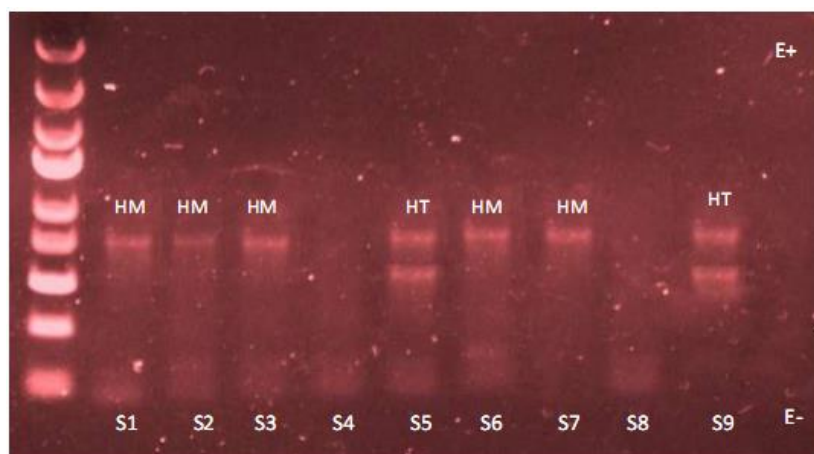


Fig-3: DNA bands of farnesoid X receptor gene in Polymerase chain reaction amplification

HM = Homozygous for FXR (FXR<sup>+/+</sup>)  
 HT = heterozygous for FXR (FXR<sup>+/-</sup>)  
 E+ = positive electrode (Anode)  
 E- = negative electrode (Cathode)

## DISCUSSION

The nuclear receptor FXR is a metabolic regulator that control bile acid (BA) & lipid as well as glucose & energy metabolism [14]. FXR can exert direct transcriptional control or indirectly influence metabolic pathways by modulating the homeostasis of BA which control metabolism also independent of FXR [14]. The characteristics of FXR deficient (FXR<sup>-/-</sup>) mice; dyslipidemia, transient hypoglycemia upon fasting peripheral insulin resistance and reduce adipose tissue mass [16] [24]. reflects the importance of FXR for the maintenance of metabolic homeostasis. Yet the impact of FXR polymorphism in humans on obesity has not been assessed.

In this study it was observed that an overall prevalence rate of 62.5% of FXR<sup>+/-</sup> was observed among obese category, while 38.7% and 51.5% of FXR<sup>+/-</sup> was noted among overweight and normal weight subjects respectively. This indicates that (FXR<sup>+/-</sup>) could

be associated with increase weight gain among obese individuals when compared to those in overweight and normal weight category. Cariou *et al.*, [24] reported that FXR homozygous mice FXR<sup>+/+</sup> experienced an increased adipose tissue mass with larger adipocytes. Also, FXR activation in vitro stimulated adipocytes differentiation by promoting peroxisome proliferation activated receptor (PPAR)- $\gamma$  activity [25].

The discrepancies in these observed effects might be attributed to different study models (mice, rats, humans & cell lines). This controversy can also be due to the compensating effect of the wild type allele among the heterozygous individuals.

Watanabe and colleague [26], reported that FXR activation in mice models reduce plasma triglycerides. He attributed this to an enhanced serum triglyceride clearance and also the repression of sterol regulatory element binding protein (SREBP)-1C. A



dissimilar result was observed in this current study which reports that there was a significantly lower mean plasma triglyceride value among the homozygous individual than the heterozygous individuals. Also a significantly lower mean plasma triglyceride value was observed in normal weight group when compared to those in obese group among the heterozygous individuals and in heterozygous category. Unfortunately, there was no significant difference in the plasma triglyceride value between the homozygous individuals and the heterozygous individuals among the obese category. This may be due to an increase intake of junk food in their daily dietary menu as seen in the structured questionnaire.

Traditionally, groups around the world have ascertained that there is a strong correlation between obesity and increase plasma triglyceride. According to one camp, it was reported that the validity of BMI as a measure of obesity was supported by its association with obesity-related morbidities such as hypertension, dysglycemia (impaired fasting glycemia, impaired glucose tolerance and type II diabetes mellitus) and dyslipidemia (hyperlipemia, hypercholesterolemia, hypertriglyceridemia and low high-density lipoprotein [HDL]-cholesterol) [27].

Interestingly, it was observed in this current study that there was a significant decrease in the mean value of serum total cholesterol and low density lipoprotein in homozygous individuals than the heterozygous individuals, when normal weight group was compared with obese group among the heterozygous individual and also when homozygous group was compared with *fxr*<sup>+/-</sup> in the obese category. Thus, indicating that heterozygous individuals may be at higher risk of developing atherosclerotic complications if both indices are not monitored. However no significant difference was seen in mean values of these two parameters when obese category was compared with normal weight in heterozygous population.

There was a significant high mean value of plasma high density lipoprotein (HDL) in the heterozygous individual than the results obtained in the homozygous individuals among the obese category. In vivo kinetic study indicated that elevated plasma HDL cholesterol levels observed in *FXR*<sup>-/-</sup> mice could be due to reduced selective uptake of HDL-cholesteryl esters by the liver, potentially linked to a decreased expression of the scavenger receptor B1 rather than to enhance its production [28]; however the reason for this discrepancy remains unclear. Kinetic studies in humans are clearly required to investigate further the relationship between *FXR* deficiency and plasma HDL formation and its function.

Although, this study was unable to show relationship between *FXR* gene polymorphism and

plasma insulin concentration, which in mice models has been proven to increase insulin resistance [29], however it is of interest to note that there was no significant difference in mean plasma glucose value between the homozygous individuals and the heterozygous individuals in the obese category. This indicates that *FXR* gene deficiency is not associated with plasma glucose level. Intriguingly, *FXR*<sup>-/-</sup> mice were reported transiently hypoglycemic when fasted [24] and exhibited delayed intestinal glucose absorption [30].

## CONCLUSION

Taken together, the results indicates that *FXR* could be a critical regulator of normal cholesterol metabolism and that genetic changes affecting *FXR* function have the potential to be proatherogenic if not diagnosed.

## RECOMMENDATION

A large cohort of sera from obese population needs to be examined to draw a better conclusion regarding the allelic frequency of polymorphic *FXR* gene and its relationship with obesity in the population at large.

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