Very low HDL cholesterol: The GSH Experience

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TO BE SUBMITTED TO THE UNIVERSITY OF CAPE TOWN

In fulfilment of the requirements for the degree

Master of Medicine (MMed) in Medicine

Faculty of Health Sciences

UNIVERSITY OF CAPE TOWN

Date of Submission: 13 December 2021

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DECLARATION

I, Kurai Ngarivume, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Signature: Signed by candidate

Date: 13.12.2021

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Acronyms and abbreviations

ART   : Antiretroviral therapy

HIV   : Human immunodeficiency virus

HDL-C   : High density lipoprotein cholesterol

LDL-C   : Low density lipoprotein cholesterol

TG   : Triglycerides

GSH   : Groote Schuur Hospital

TG   : Triglycerides

CHD   : Coronary heart disease

CV   : Cardiovascular

CVD   : Cardiovascular disease

BMI   : Body mass index

WC   : Waist circumference

WHR   : Waist hip ratio

SD   : Standard deviation

IQR   : Interquartile range

WHO   : World Health Organisation

ASCVD   : Atherosclerotic cardiovascular disease

DM   : Diabetes Mellitus

Chapter 1

INTRODUCTION

Purpose of study

Analyse and describe patients with very low high density lipoprotein cholesterol (HDL-C) attending a specialist lipid clinic at Groote Schuur Hospital (GSH) in Cape Town, South Africa.

Background

Dyslipidaemia is a growing health problem in developing countries. This is largely secondary to changes associated with economic development such as urbanisation. The follow on effects of this have seen a consequent decrease in physical activity and changes in dietary patterns which now includes the increased intake of highly processed foods [1]. Dyslipidaemia is a crucial causal factor in the pathogenesis of atherosclerosis. Although apo-B containing lipoproteins are the major targets of therapy, in epidemiological studies low HDL-C is a strong predictor of cardiovascular risk. Importantly, the adverse cardiovascular outcomes associated with low levels of HDL-C are independent of level of LDL cholesterol [2].

The Emerging Risk Factors Collaboration Study showed that the strong inverse association between coronary artery disease risk and HDL-C levels remained even after adjusting for other lipid and non-lipid risk factors. In the Emerging Risk Factors Collaboration Study, individual records from 302 430 people without vascular disease at baseline from 68 long-term prospective studies, mostly from Europe and North America were combined and analysed. The study found that concentrations of HDL-C and non-HDL-C were each strongly associated— in opposite directions—with CHD risk in an approximately log-linear manner [3]. The protective role of HDL-C is thought to be mainly due to the role it plays in reverse cholesterol transport mobilizing and removing excess cholesterol from peripheral tissues to the liver where it is metabolised [4]. An independent inverse association between HDL cholesterol efflux capacity and incident cardiovascular events has been shown both in the Dallas Heart Study and in the European Prospective Investigation of Cancer-Norfolk study [5, 6]. Despite these findings, the relationship between HDL efflux capacity and measured HDL-C is complex and not linear. In fact, HDL-C levels only account for approximately one-third of the variance in the cholesterol efflux capacity of HDL-C. HDL-C has multiple other properties including anti-atherogenic, anti-oxidative, anti-proliferative, anti-thrombotic, anti-apoptotic, anti-infective, and anti-inflammatory properties [7]. Therefore, maintaining optimal HDL-C functionality would be clinically relevant. Low and very low HDL-C generally is predictive of increased cardiovascular risk. However, not all patients with low HDL-C are at high cardiovascular risk as some ApoA1 mutations, such as ApoA1 Milano, that are associated with low HDL-C have even been found to be cardioprotective illustrating that the relationship between HDL-C levels and cardiovascular risk is complex and not always linear [8]. To date, there are no studies to show that pharmacological manipulation of HDL-C reduces morbidity or mortality from atherosclerotic cardiovascular disease (ASCVD).In fact, the AIM-HIGH and HSP2-THRIVE studies failed to show benefit despite raising HDL-C, as one of several lipid effects expected with addition of niacin therapy [37,38]. This has been attributed to the

importance of HDL-C particle composition and function rather than the quantity of HDL-C alone.

The aetiology of very low HDL-C is multifactorial and may be artefactual (for example assay interference from paraproteinemia), primary genetic, or secondary to many factors including hypertriglyceridemia, drugs, and malignancies [9]. The clinical characteristics of patients with very low HDL-C are varied and affected by co-existence of other comorbidities such as diabetes and hypertension. It is important therefore to study these characteristics as some of them are modifiable if well managed.

There is little data on the epidemiology of lipid disorders in South Africa. The available studies mainly focus on total cholesterol and LDL-cholesterol and none has specifically studied extremely low HDL-C. The worldwide literature on patients with extremely low levels of HDL-C is also limited to a few studies mainly from Europe and Asia.

This retrospective descriptive study reviewed and analysed patients with very low HDL-C (defined as less than 0.6 mmol/L) seen at a tertiary level lipid referral clinic. The detailed data review and analysis will shed light on the characteristics of patients with very low HDL-C in the local population and may lead to a better understanding of this group of patients and serve as a base for further studies into these patients. Studying patients with extreme phenotypes may ultimately lead to a better understanding of an entire population.

Chapter 2

METHODOLOGY

Study setting

All data used in the study was collected from records from a specialist lipid clinic at Groote Schuur Hospital, a university teaching and tertiary hospital in Cape Town, South Africa. The study was approved by the University Of Cape Town Faculty Of Health Sciences Human Research Ethics Committee. The patients who were seen at the specialist clinic were referred by primary and secondary health care centres from within the province and included those who were referred from different departments from within Groote Schuur Hospital including the cardiology unit.

 The referral criteria to the Groote Schuur Lipid Clinic are:

- Total cholesterol levels persisting above 7.5mmol/L or below 2.5 mmol/L (after dietary management).
- Low density lipoprotein levels (LDL-C) above 5mmol/L or below 1.5 mmol/L.
- High density lipoprotein (HDL-C) cholesterol concentration above 2.5mmol/L or below 0.6mmol/L or below 0.8mmol/L if the triglyceride concentration is below 2.5mmol/L.
- Hypertriglyceridemia of more than 5 mmol/L.
- Xanthomata of the skin or tendons irrespective of lipid levels, with the exception of xanthelasma.
- Premature atherosclerosis.
- Statin intolerance once hypothyroidism and other disorders have been excluded.
- Unusual metabolic defects that involve lipid metabolism or promote atherosclerosis, including homocystinuria.

Detailed standardised histories and examinations were performed at the initial consultation and blood tests were taken which served as baseline data for the study. On subsequent visits, review of the patient symptoms was carried out and follow up bloods were taken. The patients would exit the specialist lipid clinic either by being discharged, voluntary non-attendance or through death.

Study design

A retrospective record review and data analysis involving 128 patients was carried out. These patients were those who were followed up at the specialist lipid clinic at Groote Schuur Hospital. All patients' records meeting our inclusion criteria were reviewed. There was no age or time frame restrictions for patients to be eligible for this study.

Data collection

Eligible patients were identified by searching the GSH Lipid Clinic database that recorded data on first presentation for all patients. A computer list was then generated for all the patients with HDL-C of less than or equal to 0.6mmol/L. From this list, patients' records were then retrieved from the records room. There were few files which were missing from the records. Figure 1 below illustrates how the data was collected.

Ethics and Permissions

The Human Research Ethics Committee of University of Cape Town gave permission to extract data from the electronic database as well as to access patient records. The patients' names and contact details were not recorded anywhere during the study. This was therefore an anonymous study and t the HREC thus waived the need for individual participant consent.

FIGURE 1: FLOW CHART ILLUSTRATING DATA SET OF PATIENTS WITH VERY LOW HDL-C CHOSEN FOR ANALYSIS
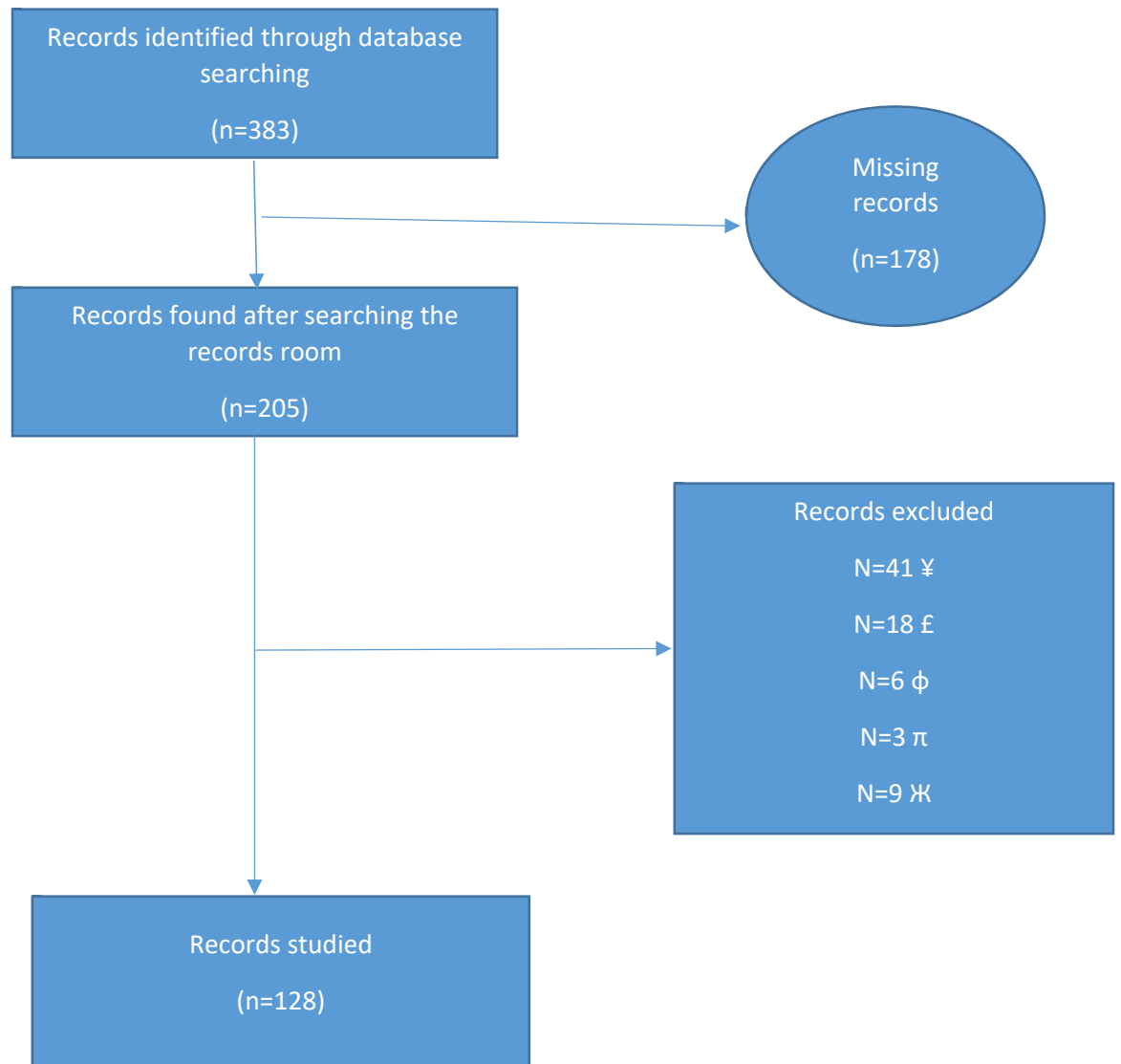
KEY

¥   **=** Patients who presented to the clinic only once

£   **=** Patients who had only one follow-up visit

φ   **=** Patients who had many clinic visits but only one or two HDLC readings in the folder

π   = Patients whose results were erroneously entered in the database as HDL-C below 0.6mmol/L

Ж   = Patients with missing admission forms

Inclusion criteria

All patients with HDL-C less than or equal to 0.6mmol/L at initial presentation.

Exclusion criteria

The exclusion criteria included:

- Patients who had presented to the clinic only once. Including these patients would have made it impossible to follow up changes in HDL-C with change in management after enrolment at the specialist lipid clinic.
- Patients who had only one follow-up visit were also excluded for the same reason including those who had many clinic visits but only one or two HDL-C readings in the folder. The study aim was to examine HDL-C trends between the initial and subsequent visits and analyse the magnitude of change between best and worst results. With one follow up it would not be possible to select the best and worst HDL-C results.
- Patients whose results were erroneously entered into the database and where HDL-C was not actually below or equal to 0.6mmol/L on the first clinic visit.
- Patients whose admission forms were unavailable because demographic and anthropometric information was important and was only available on the admission forms.

Data entry

The required data was extracted and entered into a Redcap database. These data included demographics, background medical illnesses like hypertension, diabetes mellitus, myocardial infarction, stroke, and HIV status. Ethnicity was extracted from records where available. Ethnicity was no longer captured in hospital records following the democratic transition in South Africa and as a result, most records had no ethnicity documented.

Data on concurrent medication was collected. Social habits including alcohol intake, smoking and self-reported activity levels was documented. Family history of dyslipidaemia and cardiovascular disease was collected. Anthropometric measurements and lipid values were documented.

Statistical analysis

We exported the collected data from Redcap database into Microsoft Excel and then further exported it to Statistica (Version 13.5.0.17 ©1984-2018 TIBCO Software Inc.). This enabled us to generate descriptive statistics for data analysis. Most results are presented as mean (standard deviation) for normally distributed data but as median (interquartile range) for data that was not normally distributed. Correlations between HDL-C and outcome variables such as cardiovascular disease were analysed. The data also allowed the study of different variables within the cohort, these will be presented in the results section.

Chapter 3

<center>**RESULTS**</center>

GENERAL CHARACTERISTICS OF THE PATIENTS

    a) Gender and ethnicity

A total of 205 patient records were screened. Only 128 patient records were found to be eligible for the study. The study cohort was made up of 78 (60.9%) males and 50(39.1%) females (Table 3a). The ethnic makeup of the cohort was 42.19% Caucasian, 22.66% Mixed Ancestry and 0.78% as African. Ethnicity was not recorded in 34.38% of patients.

Table 3a: Gender and ethnicity in the study cohort

Variable	Statistic	Males N=78	Females N=50	All N=128
African	N (%)	1(0.78)	0(0.0)	1(0.78)
Caucasian	N (%)	30(23.44)	24(18.75)	54(42.19)
Mixed Race	N (%)	14(10.94)	15(11.72)	29(22.66)
Missing	N (%)	33(25.78)	11(8.59)	44(34.38)
Total	N (%)	78(60.94)	50(39.06)	128(100.00)

Missing category denotes instances when ethnicity was not documented.

% indicated the % of the total cohort

b) Age and anthropometric characteristics at presentation by gender

The mean age at presentation for the cohort was 44.6 ± 11.4 years (Table 3b). There was no significant statistical difference in age at presentation between males and females, 45.2(10.64) v 43.7(12.61) years (p=0.474).

Table 3b: Age and anthropometric characteristics at presentation by gender of patients

Variable		Males	Females	All	P value
Age(years)	Mean(SD)	45.2(10.64)	43.7(12.61)	44.6(11.4)	0.474
Weight(kg)	Mean(SD)	86.28(17.28)	78.5(21.86)	83.24(19.49)	**0.030**
Height(m)	Mean(SD)	1.70(0.09)	1.58(0.10)	1.65(0.11)	**0.000**
BMI(kg/m2)	Mean(SD)	29.93(5.12)	31.14(7.63)	30.42(6.25)	0.305
Waist circumference(cm)	Mean(SD)	99.05(12.16)	100.07(18.36)	99.45(14.81)	0.730
Hip circumference(cm)	Mean(SD)	100.21(7.99)	106.02(14.49)	102.48(11.30)	**0.009**
WHR	Mean(SD)	0.99(0.07)	0.94(0.11)	0.97(0.09)	**0.017**

BMI   : Body mass index

WHR   : Waist hip ratio

The mean BMI in the females was 31.14(7.63) kg/ m^2, and in the obese range while the mean BMI in males was 29.93(5.12) kg/m^2 and nominally in the overweight range. There was however no significant statistical difference in BMI between males and females (p=0.305).

There was no significant statistical difference between the waist circumference of males, mean 99.05±12.16cm compared to that of females, mean 100.07±18.36cm (p=0.730). The waist hip ratio (WHR) was increased in both males and females above the normal standard according to WHO definition ǂ and males had a significantly higher ratio compared to females (p=0.017) as their hip circumference was lower.

c) Anthropometric characteristic comparing Caucasians to Mixed Ancestry

Table 3c below shows a statistically non-significant difference in BMI between Caucasians and Mixed Ancestry patients (p=0.453) however, Caucasians were overweight [¥] while patients with a Mixed Ancestry were obese[ǂ]. The mean WHR was above normal irrespective of ancestry[ǂ]. There was no statistically significant difference in WHR between the two ethnicities (p=0.367)

Key

[¥] Overweight = BMI $\geq$ 25kg/m^2 but less than 30kg/m^2

[ǂ] Obesity   = BMI $\geq$ 30kg/m^2

[ǂ] Abnormal WHR is $\geq$ 0.9 in males, $\geq$ 0.85 in females

Table 3c: Anthropometric characteristics comparing Caucasians to Mixed Ancestry

	Mean Caucasian	Mean Mixed Ancestry	P-value	Valid N Caucasian	Valid N Mixed Ancestry	Std.Dev. Caucasian	Std.Dev. Mixed Ancestry
Height (m)	1.65	1.64	0.960	54	25	0.10	0.15
Weight (kg)	79.62	81.15	0.711	53	27	15.41	20.73
BMI (kg/m^2)	29.30	30.26	0.453	53	25	4.99	5.79
Waist circumference (cm)	96.78	101.46	0.183	45	24	11.48	17.32
Hip circumference (cm)	100.79	102.92	0.398	45	24	10.18	9.35
Waist Hip Ratio	0.96	0.98	0.367	45	24	0.08	0.13

BMI   : Body mass index

Std.Dev   : Standard deviation

RESULTS OF LIPID PROFILE AT PRESENTATION

The mean (SD) total cholesterol for both males and females was 8.47(5.13) mmol/L (Table 4). This was accompanied by an elevated LDL-C with a mean (SD) of 4.86(2.10) mmol/L, which again is considered high. The triglyceride levels had a median (IQR) of 6.05(3.10 -11.15) mmol/L in both male and female cases, which is considered to be elevated. The mean (SD) HDL-C for both males and females was 0.53(0.10) mmol/L. There was no statistically significant difference between males and females with regards to total cholesterol, LDL-C, HDL-C, and triglyceride levels.

Table 4: Lipid profiles at presentation

Variable		Males N=78	Females N=50	All N=128	P value
Triglycerides (mmol/L)	Median (IQR)	6.05(3.6-10.6)	6.00(2.2-12.2)	6.05(3.1-11.15)	0.940
Total cholesterol (mmol/L)	Mean (SD)	8.55(4.74)	8.35(5.73)	8.47(5.13)	0.828
HDL-C (mmol/L)	Mean (SD)	0.53(0.11)	0.52(0.10)	0.53(0.10)	0.795
LDL-C (mmol/L)	Mean (SD)	5.13(2.18)	4.50(1.98)	4.86(2.10)	0.286
Apo-B (mg/dL)	Mean (SD)	120.50(43.98)	110.58(49.83)	116.72(46.33)	0.271
Apo-A1 (mg/dL)	Mean (SD)	90.11(25.83)	95.51(26.81)	92.19(26.22)	0.291

Baseline medications were medications that the patients were taking at the time of registration and enrolment at the lipid clinic on their first day of presentation.

IQR   : Interquartile range

SD   : Standard deviation

HDL-C : High density lipoprotein cholesterol

LDL-C   : low density lipoprotein cholesterol

DESCRIPTION OF COMORBID CONDITIONS AND ATHEROSCLEROTIC DISEASE

Table 5 below shows selected comorbidities which were documented or diagnosed on initial presentation. The proportion of males with hypertension was 39.74% and that of females was 42.00%. Males with diabetes was 41.02%, while females with diabetes was 30.00%. The proportion of males with connective tissue disease was 6.41% and that of females was 6.00%.

Table 5: Prevalence of very low HDL-C by comorbid status and gender

Comorbidities	Statistic	Males	Females	All	P value
	N (%)	78(60.94)	50(39.06)	128(100.00)	
Hypertension	N (%)	31(39.74)	21(42.00)	52(40.63)	0.7998
Diabetes	N (%)	32(41.02)	15(30.00)	47(36.72)	0.1802
Connective tissue disease - Yes	N (%)	5(6.41)	3(6.00)	8(6.25)	0.9033
Myocardial infarction	N (%)	15(19.23)	5(10.00)	20(15.63)	0.1605
CVA	N (%)	4(5.13)	1(2.00)	5(3.91)	0.3728
PVD	N (%)	9(11.54)	5(10.00)	14(10.94)	0.7856
HIV - positive	N (%)	1(1.28)	0(0.00)	1(0.78)	0.4215

% indicate the % within the individual sex category

CVA   : Cerebrovascular accident.

PVD   : Peripheral vascular disease

HIV   : Human immunodeficiency virus

Table 5 shows that at presentation, 19.23% of males reported a prior myocardial infarction compared to 10.00% of females. With regards to cerebrovascular accident (CVA), males prevalence was 5.13% compared with 2.00% in females. The reported cases of peripheral vascular disease were 11.54% in males compared to 10.00% in females. Only one male in the study was documented HIV positive. The majority of the patients in the cohort were not tested for HIV infection.

DESCRIPTION OF RESULTS OF SOCIAL HABITS

a) Smoking characteristics according to gender

Table 6a shows that 37.18% of males were current smokers at presentation compared to 34.00% females. Males started smoking at the mean age of 17.57(3.40) years while females started at 18.56(3.74) years. The males smoked an average of 16.83(11.12) cigarettes per day compared to the females who smoked 12.59(6.50) cigarettes per day. 21.09% of males reported they had quit smoking as compared to 8.59% of females. Average years quit smoking by males was 9.71(10.75) compared to 7.1 (9.07) years by females. 44.00% of females never smoked, p value = 0.008 (statistically significant) while males who never smoked was 21.79%.

Table 6a: Smoking characteristics according to gender

Characteristic		Males	Females	All	P value
	N (%)	78(60.94)	50(39.06)	128(100.00)	
Smoking current	N (%)	29(37.18)	17(34.00)	46(35.94)	0.715
Smoking current - Age started (years)	Mean(SD)	17.57(3.40)	18.56(3.74)	17.93(3.52)	0.375
Smoking current -Cigarettes/day	Mean(SD)	16.83(11.12)	12.59(6.50)	15.26(9.81)	0.159
Previous smoker	N (%)	27(34.62)	11(22.00)	38(29.69)	0.128
Previous smoker - Years quit	Mean(SD)	9.71(10.75)	7.1(9.07)	8.97(10.24)	0.491
Smoking never	N (%)	17(21.79)	22(44.00)	39(30.47)	**0.008**

SD   : Standard deviation.

b) Alcohol consumption characteristics by gender

More males reported consuming alcohol than females (32.05% vs 20.00%, p=0.135). Amongst those consuming alcohol, males reported consuming a significantly higher amount of alcohol per week as compared to females (**p=0.014**)

Table 6b: Alcohol consumption characteristics by gender

	Male N=78	Female N=50	p	N (%) Male	N (%) Female
Alcohol consumption (grams/week)	89.76	18.00	**0.0146**	25(32.05)	10(20.00)

DESCRIPTION OF RESULTS OF MEDICATIONS

Table 7 shows baseline medications at first presentation. Lipid modifying medications (statins and/or fibrates) were prescribed to 32.81% of the overall cohort (Tables 7a and 7b). The most common statin was Simvastatin which was used by 21.09% of the patients as shown in Table 7a.

Very few patients were on fibrate therapy, 7.03% (Table 7b). Atenolol was the most commonly used beta blocker in the cohort (Table 7c). The most commonly used diuretic was hydrochlorothiazide (Table 7d). In the cohort, diabetes was mainly managed with metformin (17.19%), while gliclazide (11.72%) was the most used drug under the sulphonylurea group (Table 7e) and premix insulin (9.38%) under the insulin subtypes was the most used (Table 7f).

Table 7: Baseline medications of the patients as documented on the referral notes or self-reported.

	Statin therapy	Fibrate therapy	Beta blocker	Diuretic	Metformin	Sulphonylurea	Insulin
Yes	33	9	34	23	18	23	15
No	95	119	92	105	22	19	33

Note: Hypoglycaemic medication only recorded for those with diabetes

Table 7a: Statins at baseline

Atorvastatin	N (%)	2(1.56)
Simvastatin	N (%)	27(21.09)
Pravastatin	N (%)	4(3.13)

Table 7b: Fibrates at baseline

Bezafibrate	N (%)	6(4.69)
Gemfibrozil	N (%)	3(2.34)

Table 7c: Beta blockers at baseline

Atenolol	N (%)	31(24.22)
Carvedilol	N (%)	1(0.78)
Bisoprolol	N (%)	1(0.78)
Other	N (%)	1(0.78)

Table 7d: Diuretics at baseline

HCTZ	N (%)	15(11.72)
Furosemide	N (%)	8(6.25)

HCTZ: Hydrochlorothiazide

Table 7e: Sulphonylurea and metformin at baseline

Glibenclamide	N (%)	6(4.69)
Gliclazide	N (%)	15(11.72)
Glimepiride	N (%)	2(1.56)
Metformin	N (%)	22(17.19)

Table 7f: Insulin subtypes at baseline

Basal	N (%)	1(0.78)
Premix	N (%)	12(9.38)
Basal bolus	N (%)	1(0.78)
Other	N (%)	1(0.78)

Premix insulin is a combination of intermediate and regular insulin. The only premix used was Actraphane insulin due to its availability in the public sector of South Africa.

Chapter 4

ANALYSIS OF HDL IN RELATIONSHIP TO VARIOUS VARIABLES

In this chapter, HDL levels were analysed in relationship to various variables. The following variables were analysed;

- Age
- Gender
- BMI
- Waist circumference
- Diabetes
- Smoking
- Alcohol
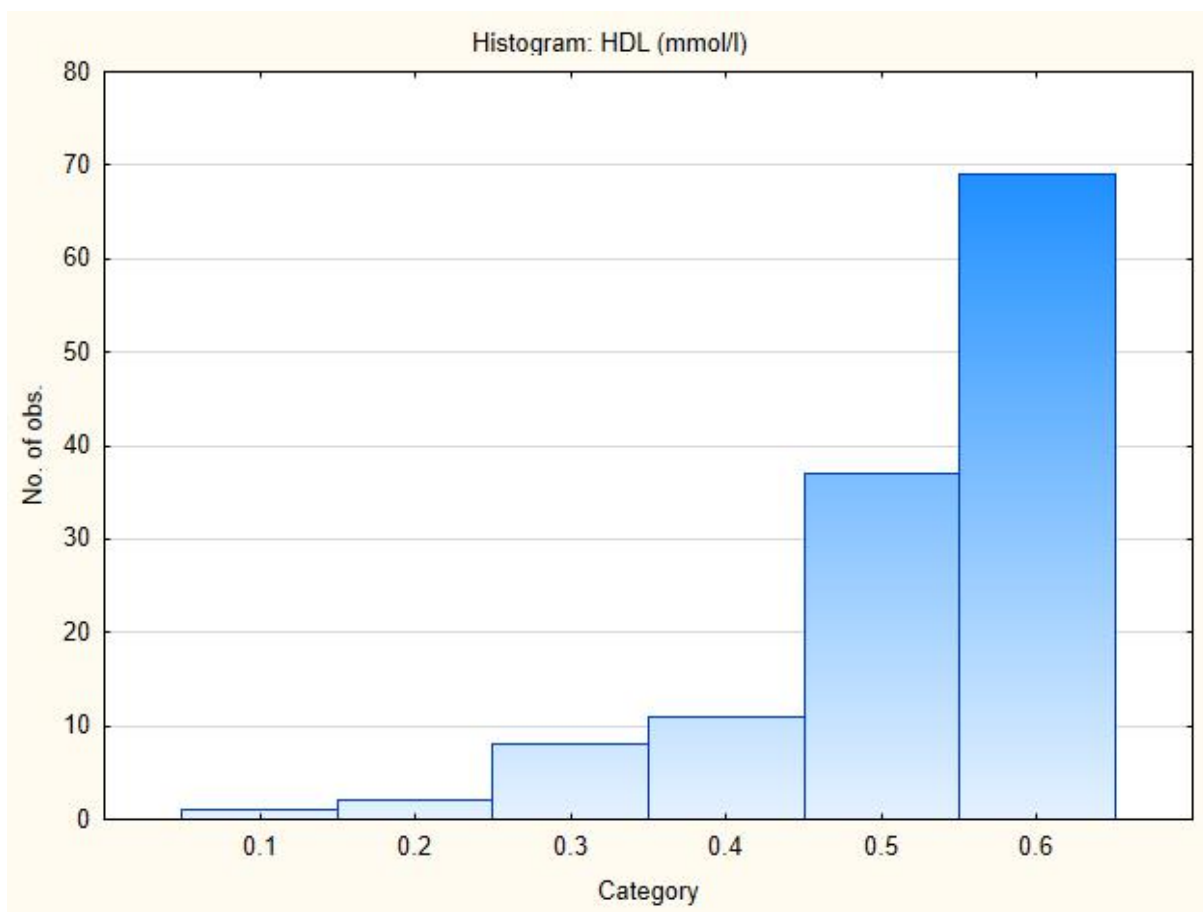
FIGURE 2:   HISTOGRAM SHOWING DISTRIBUTION OF HDL-C

Histogram: HDL (mmol/l)

Figure 2 shows that most patients in this study had an HDL-C in the range of 0.4-0.6 mmol/L.

FIGURE 3: HDL-C LEVELS AND AGE

Scatterplot of Age at presentation against HDL (mmol/l)
VeryLowHDLCholestero_DATA_LABELS_2020-11-20_1422 112v*128c
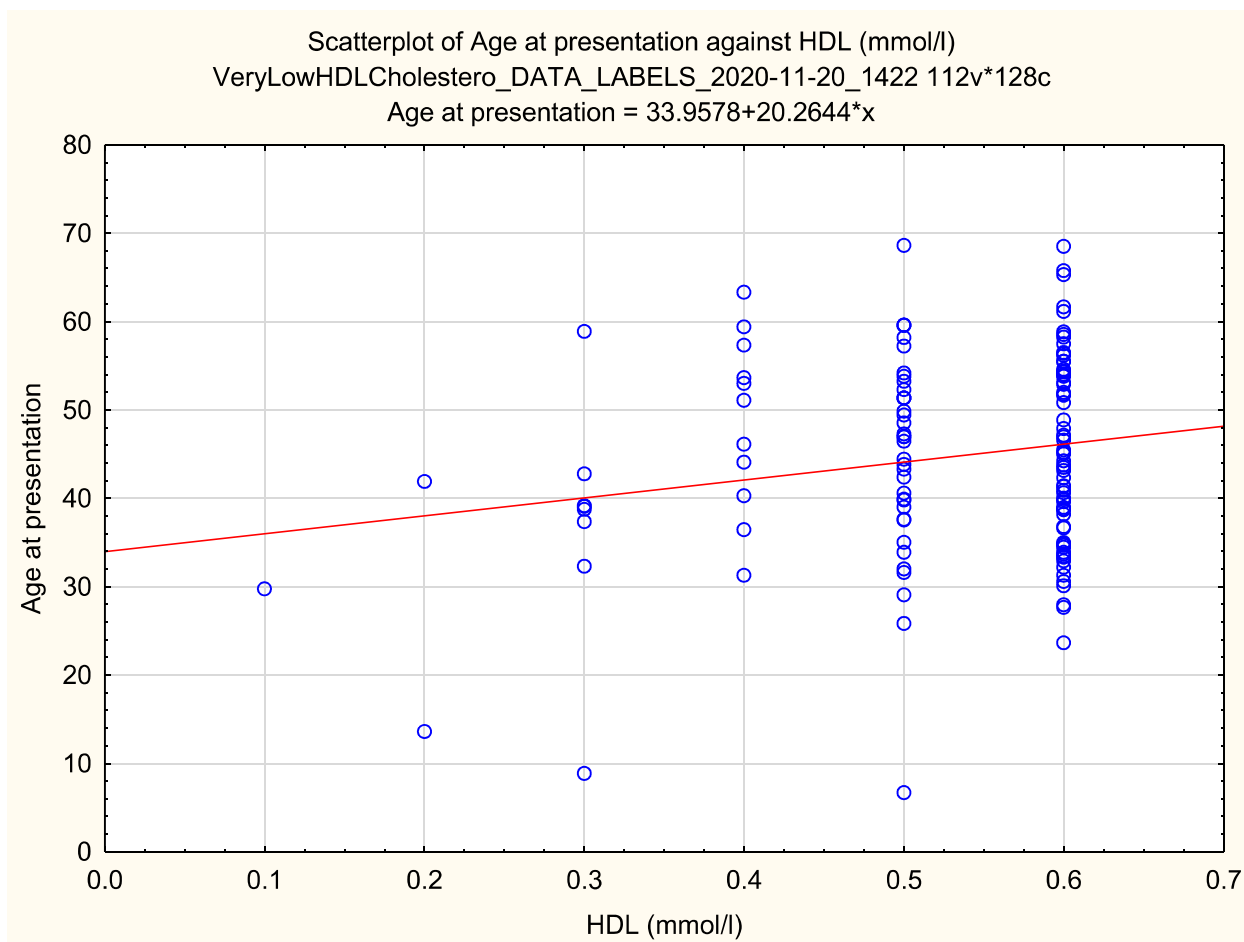Age at presentation = 33.9578+20.2644*x

Figure 3 above is showing age in years at presentation against HDL-C (mmol/L). There was no relationship between age at presentation and HDL-C.

FIGURE 4:   HDL-C AND GENDER

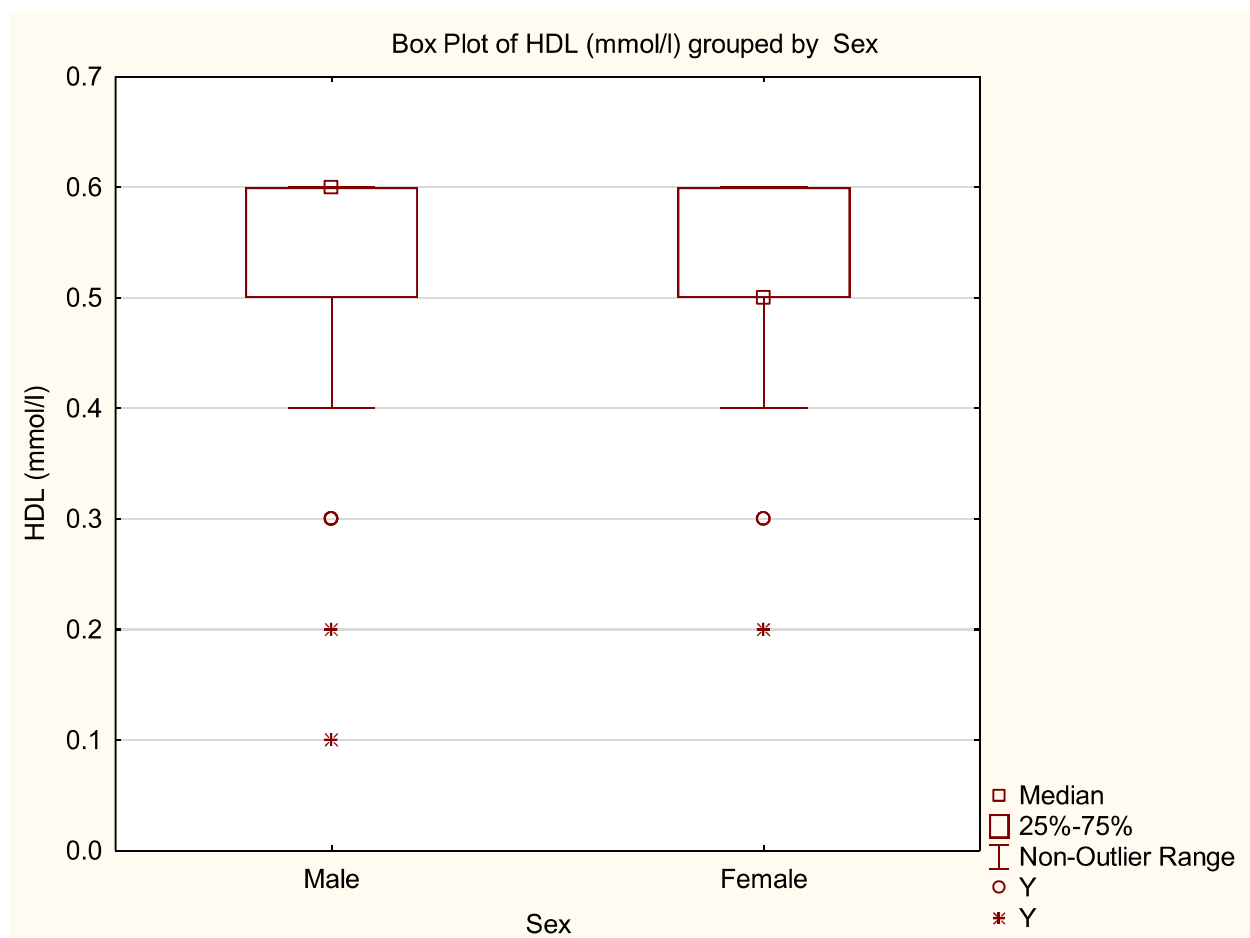Box Plot of HDL (mmol/l) grouped by Sex

Figure 4 shows HDL-C at presentation by gender. There is similar interquartile range between males and females. Males have more extreme outliers than females. The maximum values for both males and females are the same at 0.6 mmol/L, this was due to the entry criteria of an HDL-C of 0.6mmol/L or less. Males had a higher median of 0.6mmol/L compared to females who had a median of 0.5mmol/L.

FIGURE 5: HDL-C AND BMI

Box Plot of HDL (mmol/l) grouped by BMI Category
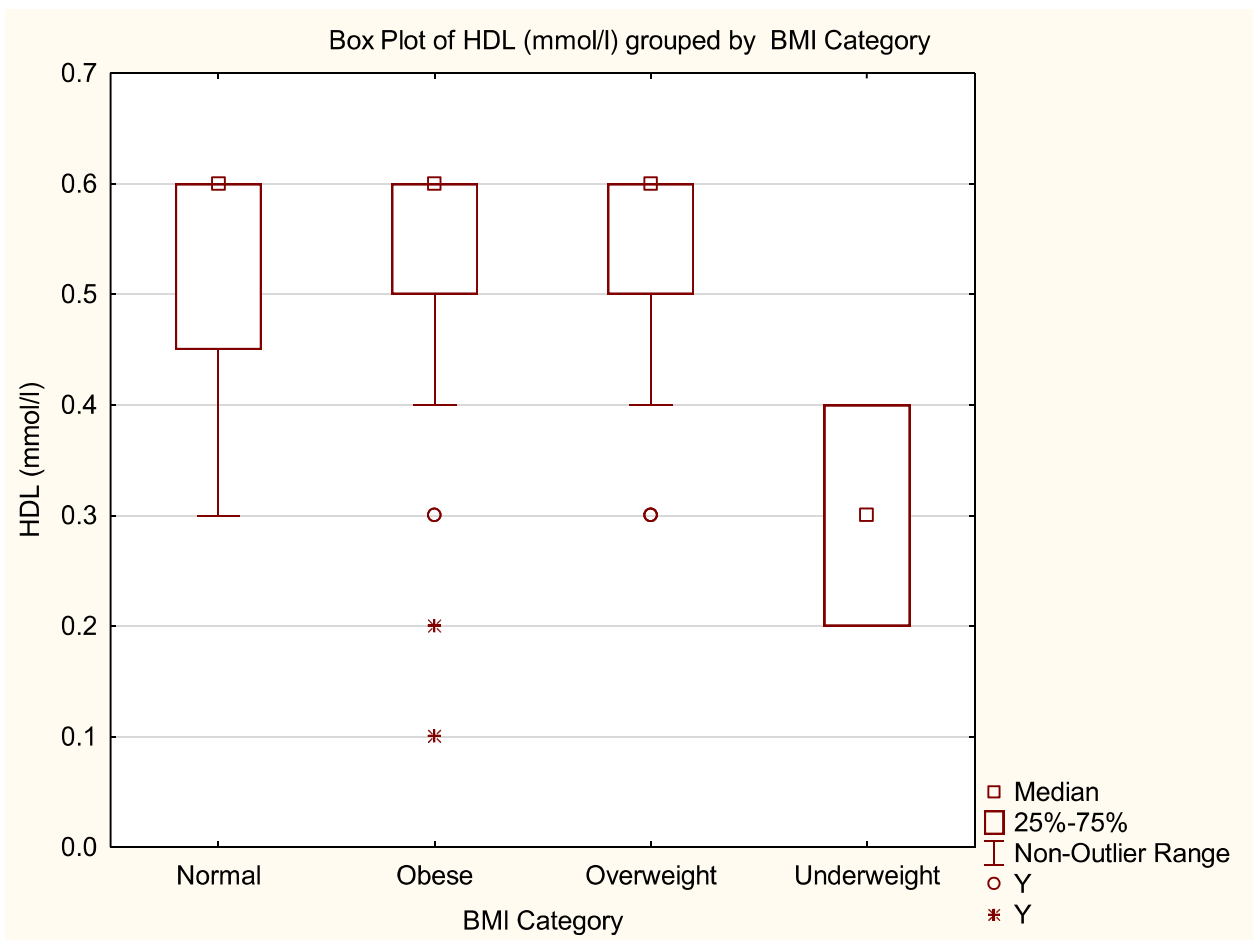
Key

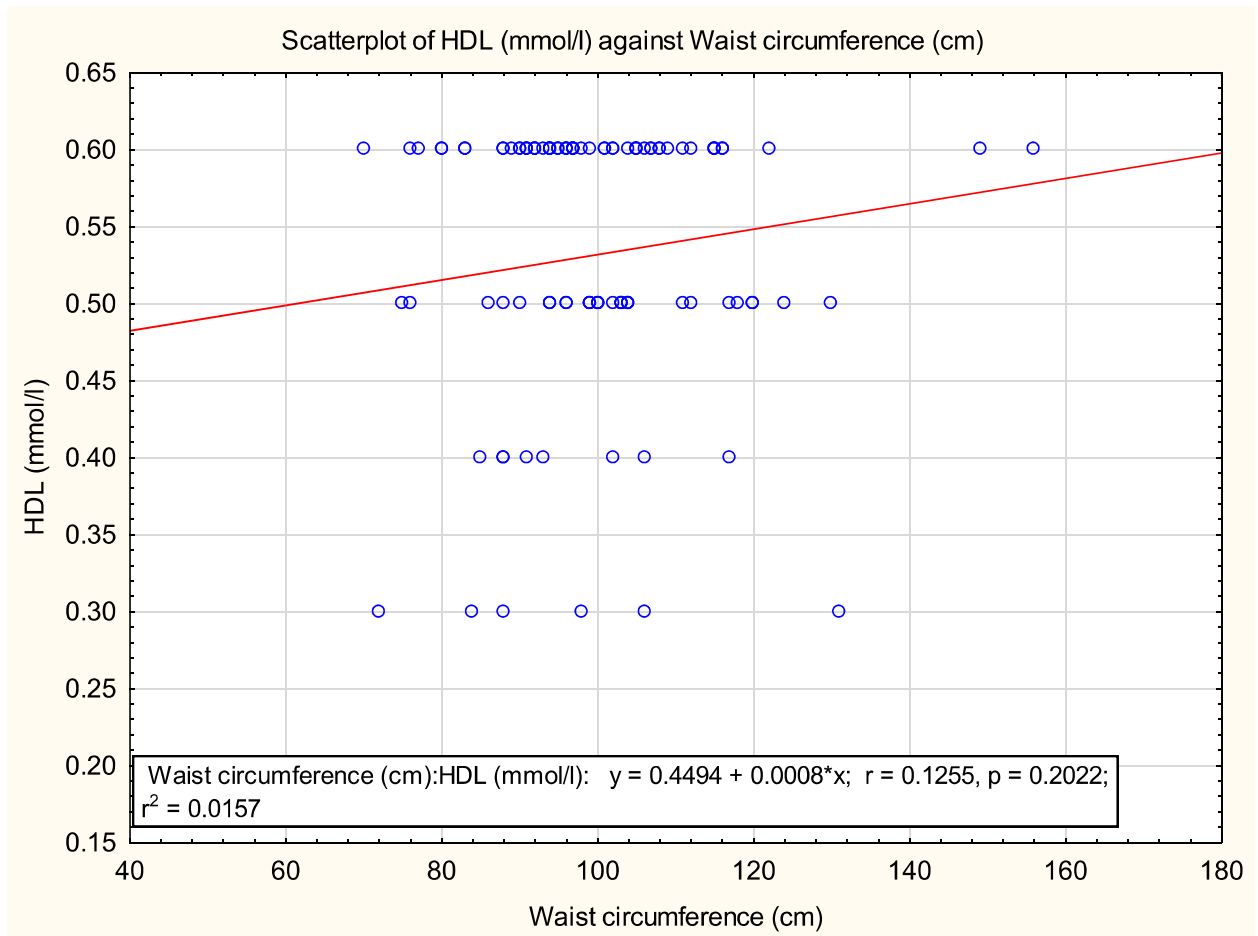Underweight : BMI < 18kg/m2

Normal : BMI 18-25 kg/m2

Overweight : 25 > BMI < 30kg/m2

Obese : BMI > 30kg/m2

Figure 5 shows that the medians are equivalent for all categories except for underweight. There was no difference in interquartile range between being overweight and being obese.

FIGURE 6:   HDL-C AND WC

Scatterplot of HDL (mmol/l) against Waist circumference (cm)

Waist circumference (cm):HDL (mmol/l): $y = 0.4494 + 0.0008 \cdot x$; $r = 0.1255$, $p = 0.2022$; $r^2 = 0.0157$

HDL-C   : High density lipoprotein cholesterol

WC   : Waist circumference in centimetres

Figure 6 above is a scatterplot of HDL against waist circumference. It shows that there was no relationship between HDL-C and waist circumference.

FIGURE 7:   HDL-C AND DIABETES MELLITUS

Box Plot of HDL (mmol/l) grouped by Comorbidities (choice=Diabetes)

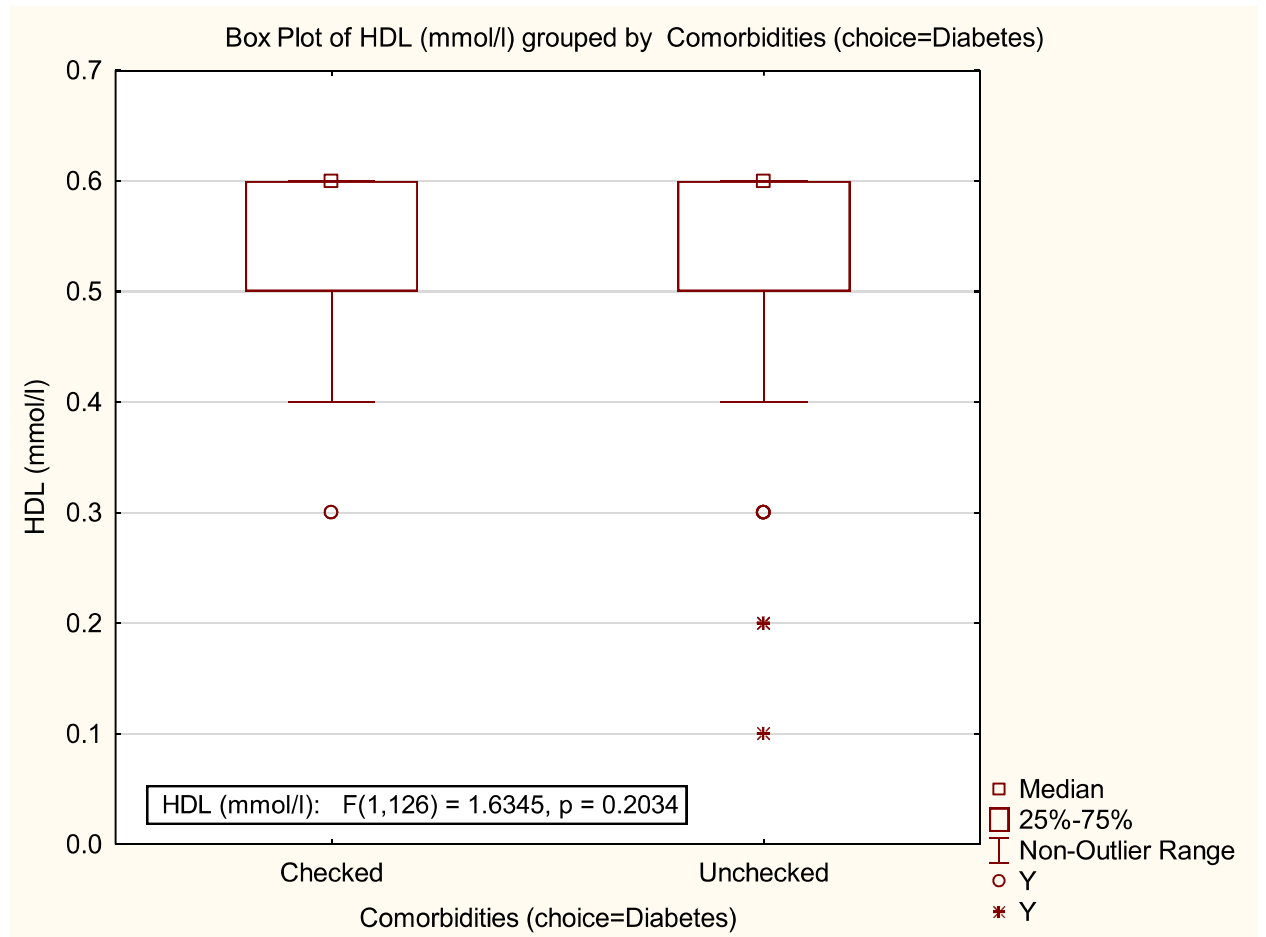HDL (mmol/l): $F_{(1,126)} = 1.6345$, $p = 0.2034$

Figure 7 above shows HDL grouped by diabetes status. It shows that there was no difference in interquartile range but non-diabetics had more extreme outliers of HDL than the diabetics.
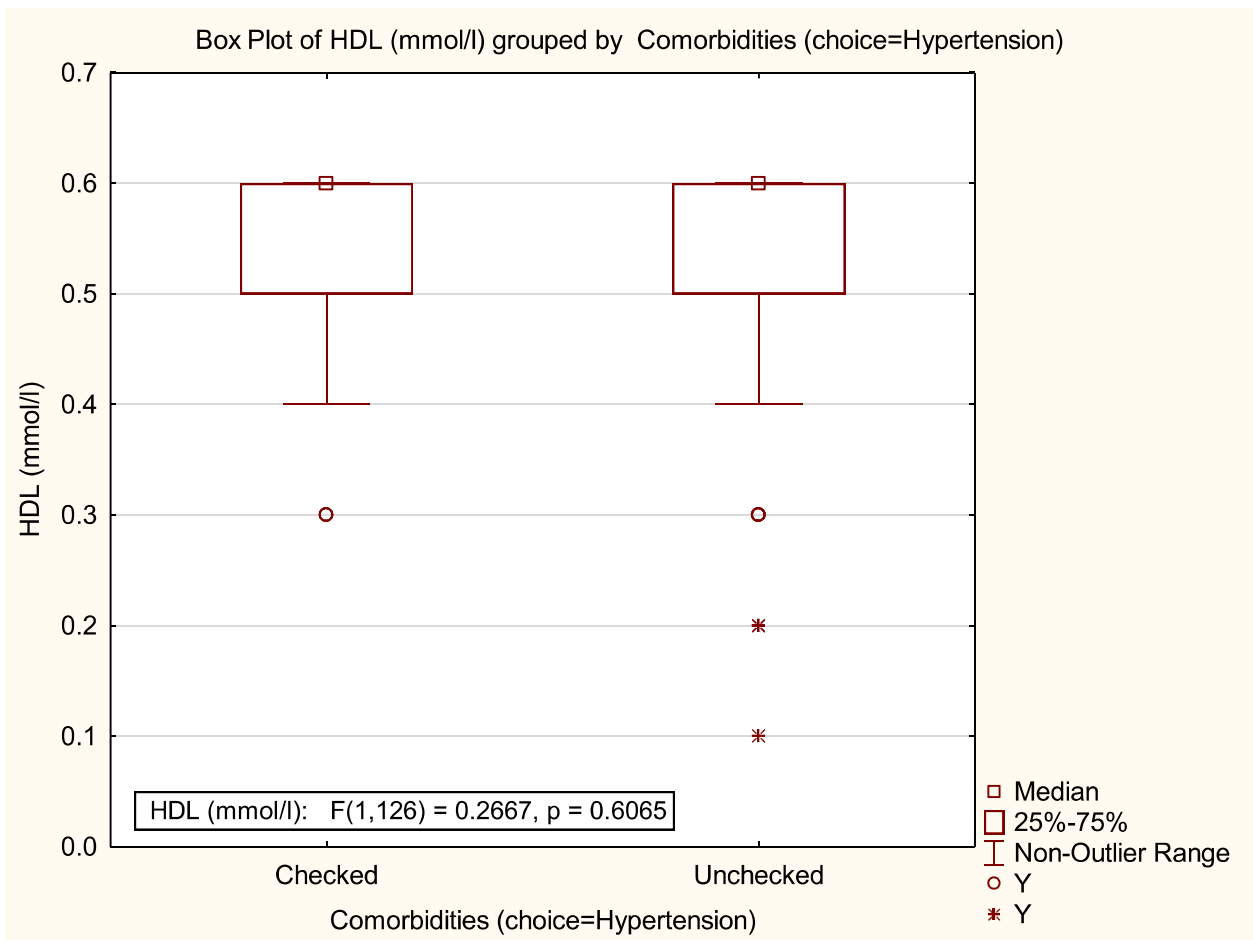
FIGURE 8:   HDL-C AND HYPERTENSION

Figure 8 above shows that there was no difference in interquartile range but non-hypertensive patients had more extreme outliers of HDL than the hypertensive patients.
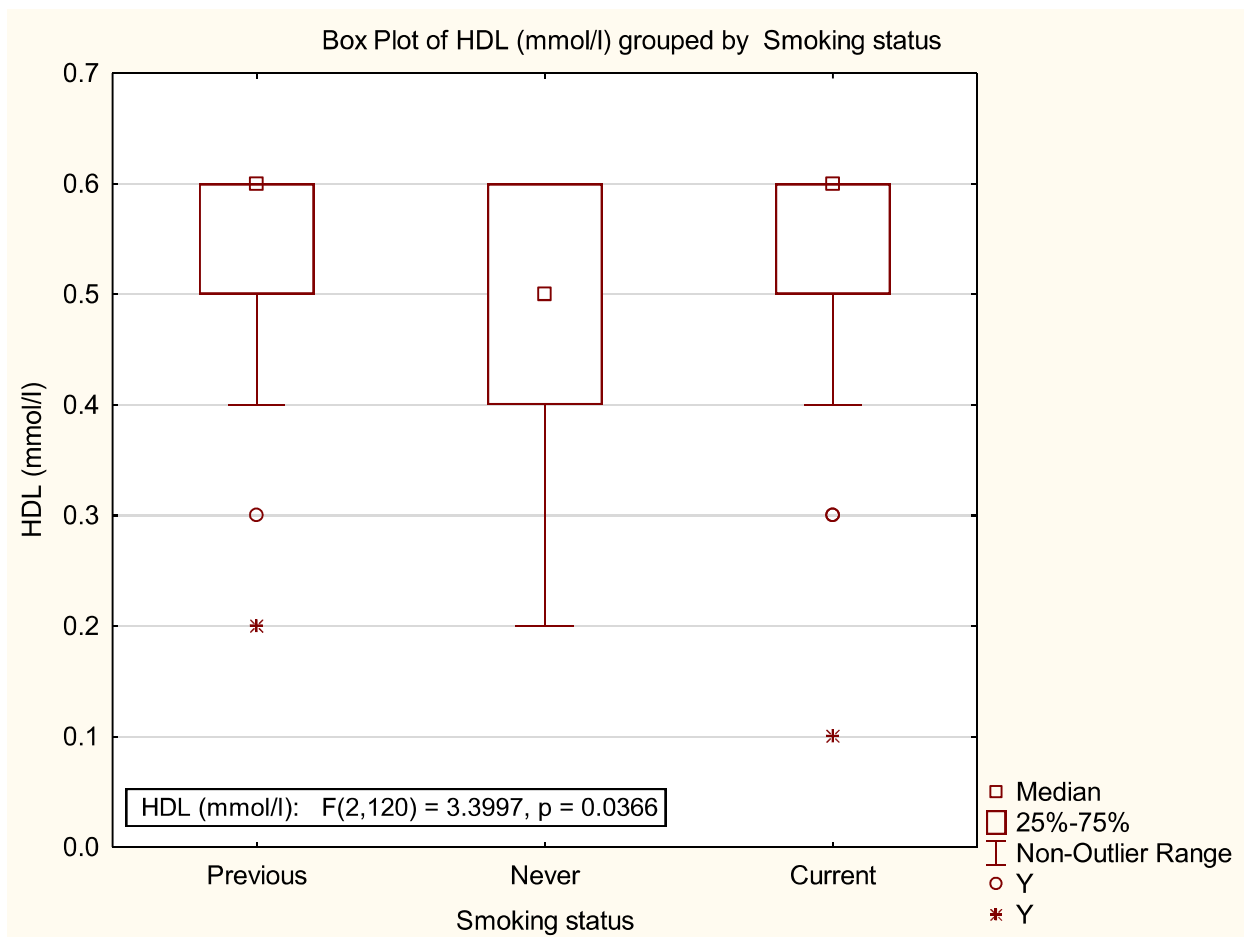
FIGURE 9:   HDL-C AND SMOKING

Box Plot of HDL (mmol/l) grouped by Smoking status

HDL (mmol/l): $F_{(2,120)} = 3.3997$, p = 0.0366

Figure 9 shows that the never smokers had the numerically lowest HDL-C levels.

FIGURE 10:   HDL-C AND ALCOHOL CONSUMPTION

Box Plot of HDL (mmol/l) grouped by Alcohol consumption

HDL (mmol/l): $F_{(1,126)} = 0.1101$, $p = 0.7406$

□ Median
□ 25%-75%
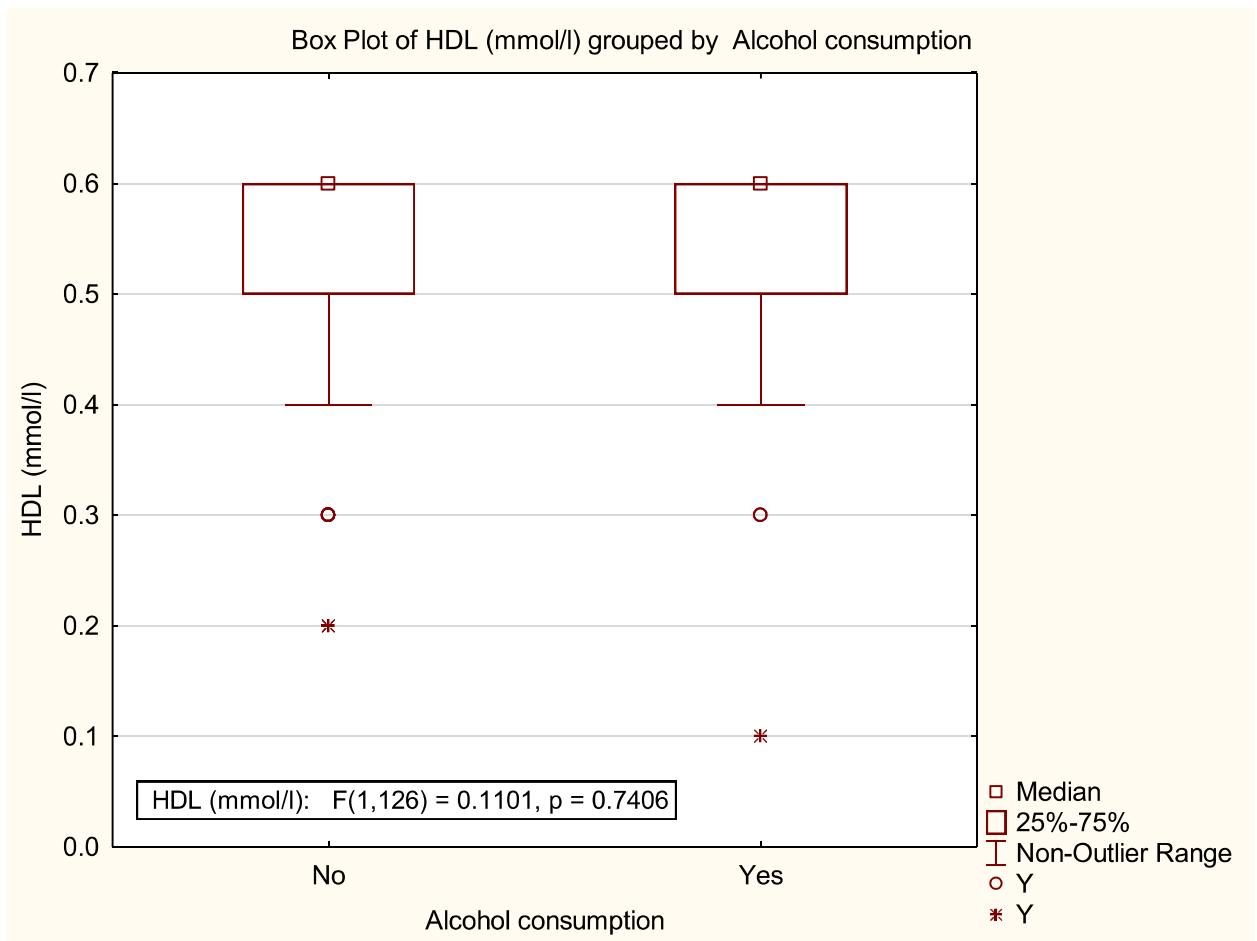⊥ Non-Outlier Range
○ Y
✳ Y

Figure 10 shows no difference in HDL in the patients who were consuming alcohol compared to those who were not consuming alcohol.

FIGURE 11:   **SCATTERPLOT OF APO A1 AGAINST HDLC**

Scatterplot of Apo A1 (mg/dL) against HDL (mmol/l)
Apo A1 (mg/dL) = 92.2838-0.1813*x

HDL (mmol/l):Apo A1 (mg/dL):   y = 92.2838 - 0.1813*x;
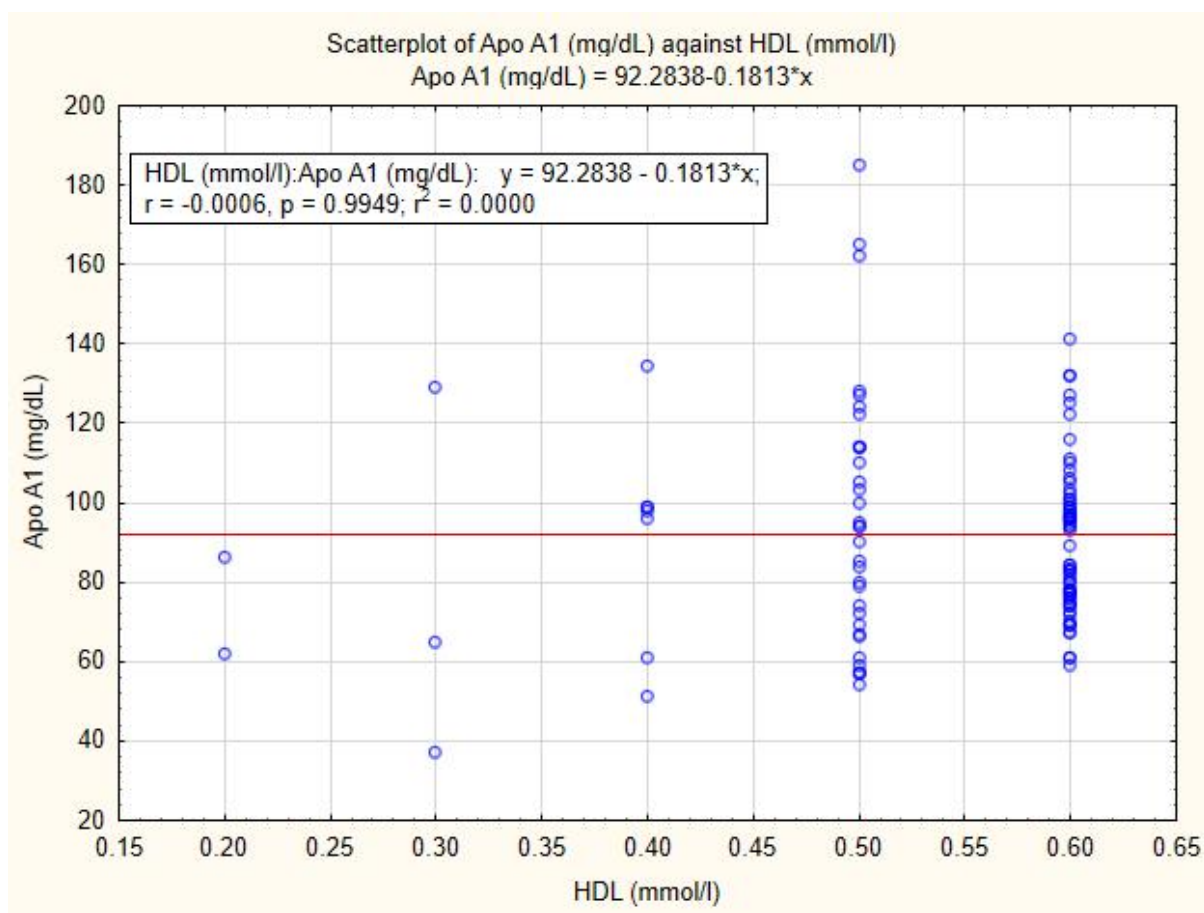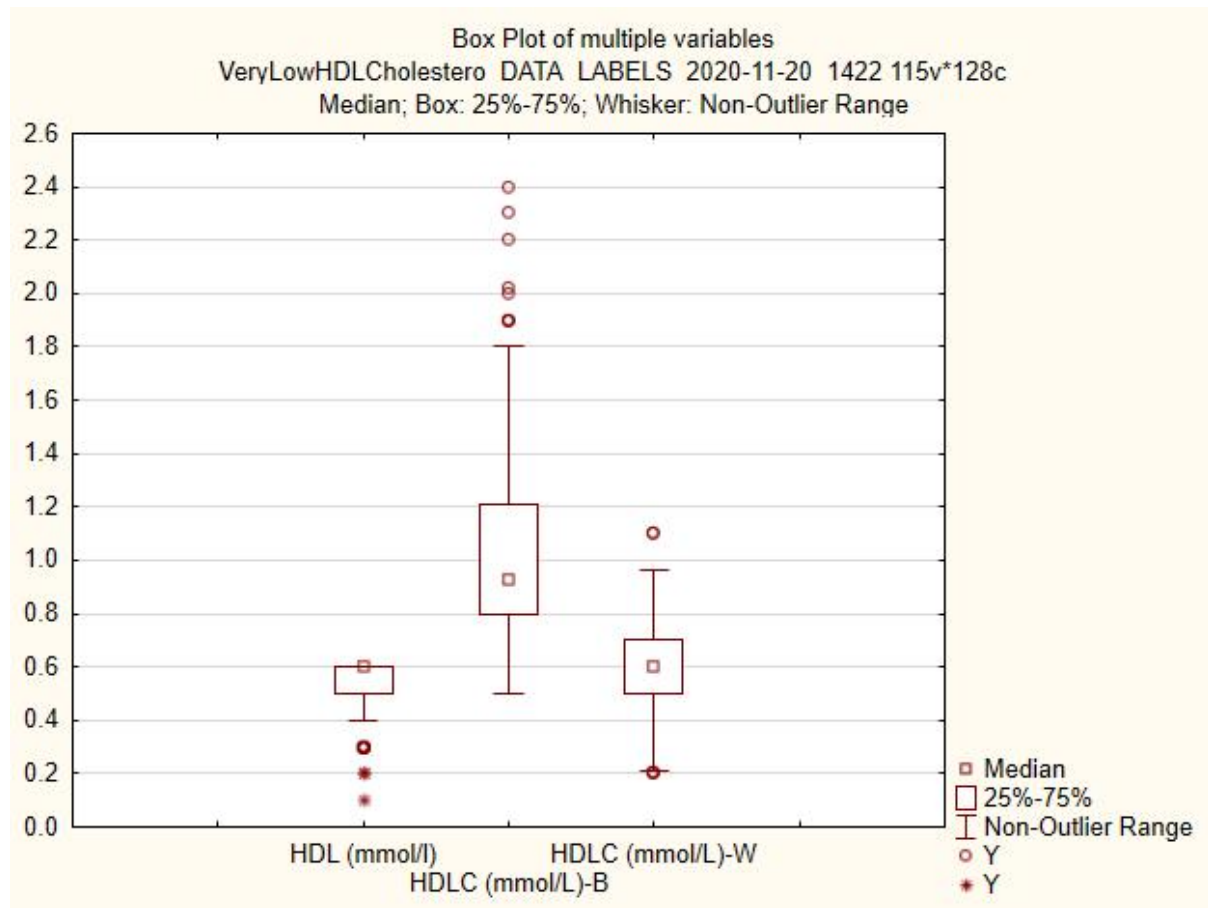r = -0.0006, p = 0.9949; r² = 0.0000

Figure 11 above shows that that in this cohort with very low HDL-C there was no linear correlation between Apo A1 and measured HDL-C.

Chapter 5

HDL-C on FOLLOW-UP

In this chapter, HDL trends from the initial presentation and the subsequent changes which occurred during follow up were analysed. The magnitude of changes between the best and worst trends were also analysed.

FIGURE 12:   BOX PLOT SHOWING HDLC ON FOLLOW-UP VISITS

Box Plot of multiple variables
VeryLowHDLCholestero DATA LABELS 2020-11-20 1422 115v*128c
Median; Box: 25%-75%; Whisker: Non-Outlier Range

KEY

HDL   : Initial HDLC on presentation

HDLC-B   : HDLC best (highest) readings on follow-up

HDLC-W   : HDLC worst (lowest) readings on follow-up

Figure 12 above shows HDLC on follow-up visits. The graph with HDLC-B shows a marked improvement from the initial HDLC, while the graph with HDLC-W shows same median values with initial HDLC levels.

FIGURE 13:   BOX PLOT SHOWING CHANGES IN HDL-C
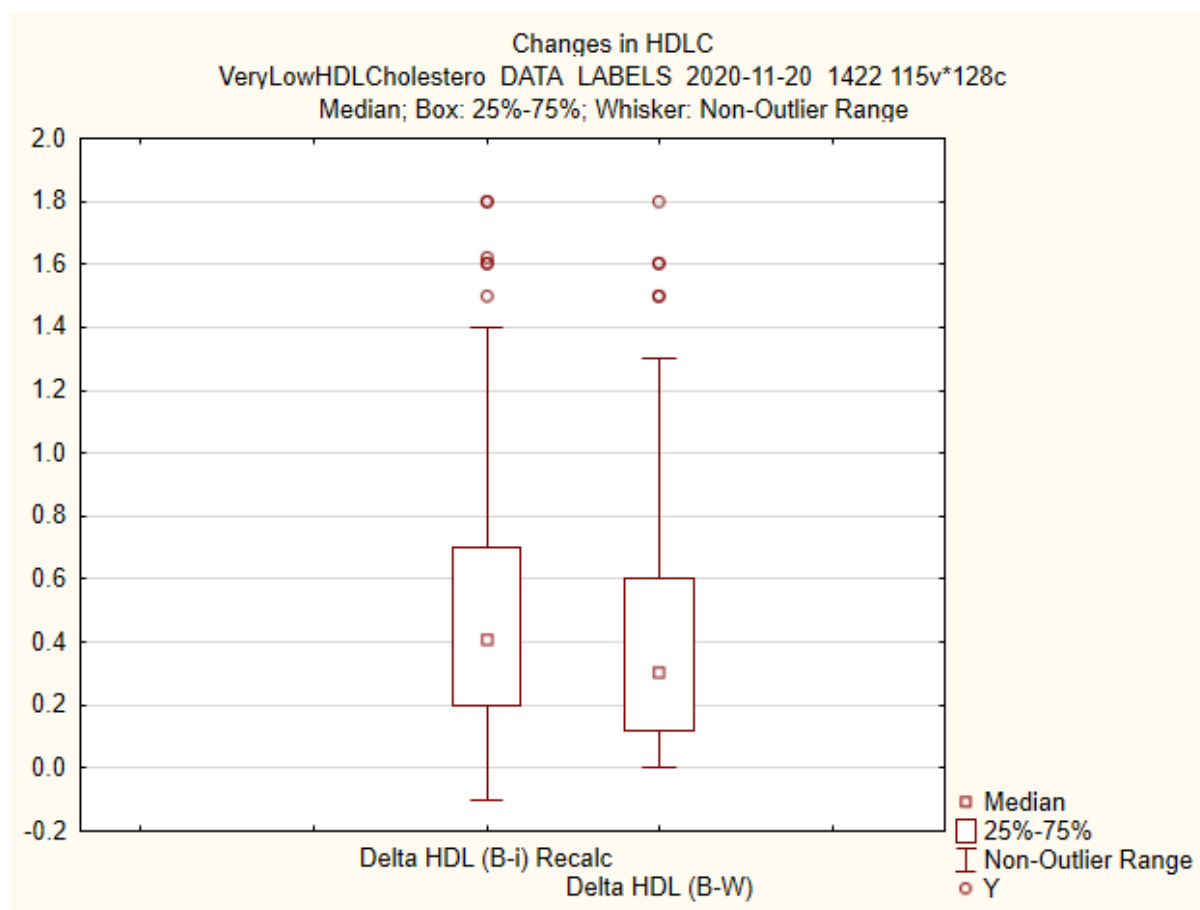
KEY

B-i   : change from initial to best

B-W   : difference between best and worst

Figure 13 shows changes in HDLC on follow-up. The median for B-i is higher than for B-W. The B-i lowest is in the negative indicating few instances where the HDL-C on follow-up dropped from the initial HDLC values.

TABLE 7: DESCRIPTIVE STATISTICS SHOWING CHANGES IN HDLC ON FOLLOW-UP VISITS

	Descriptive Statistics (VeryLowHDLCholestero_DATA_LABELS_2020-11-20_1422)								
	Valid N	Mean	Median	Mode	Frequency of	Minimum	Maximum	Lower Quartile	Upper Quartile	Std.Dev.

				Mode					
Delta HDL (B-i) Recalc	123	0.521545	0.410000	Multiple	14	-0.100000	1.800000	0.200000	0.700000	0.413617
Delta HDL (B-W)	122	0.437131	0.305000	0.000000	20	0.000000	1.800000	0.120000	0.600000	0.404711

Chapter 6

DESCRIPTION OF TRIGLYCERIDES

In this chapter, triglyceride values and their link to HDL-C were analysed. A histogram showing the distribution of triglycerides was included as shown below (Figure 14). The patients were divided into low, medium and high triglycerides (divided into tertiles) to assess the effect of triglycerides on HDL-C. This enabled a better analysis of patients with 'pure' low HDL-C versus those in whom the HDL-C may be low due to a secondary factors such as hypertriglyceridaemia.

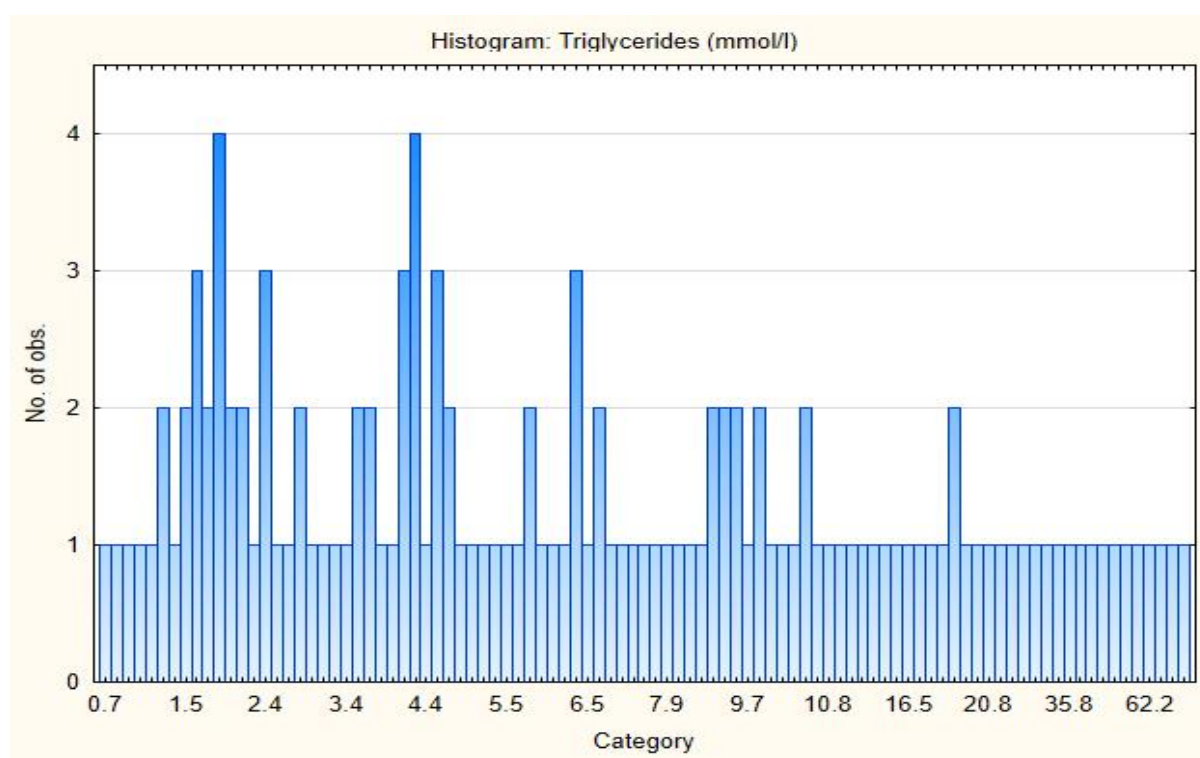**FIGURE 14:   HISTOGRAM SHOWING FREQUENCY DISTRIBUTION OF TRIGLYCERIDES LEVELS**

Histogram: Triglycerides (mmol/l)

TABLE 8: TRIGLYCERIDES RANGE OF DISTRIBUTION

	Descriptive Statistics (VeryLowHDLCholestero_DATA_LABELS_2020-11-20_1422)							
Variable	Valid N	Mean	Median	Minimum	Maximum	Lower Quartile	Upper Quartile	Std.Dev.

Triglycerides (mmol/l)	128	12.18672	6.050000	0.700000	165.0000	3.100000	11.15000	19.78482

Figure 14 shows that triglycerides were highly variable, ranging from normal values to severe hypertriglyceridaemia.

FIGURE 15:   BOX PLOT OF TRIGLYCERIDES GROUPED BY QUARTILES
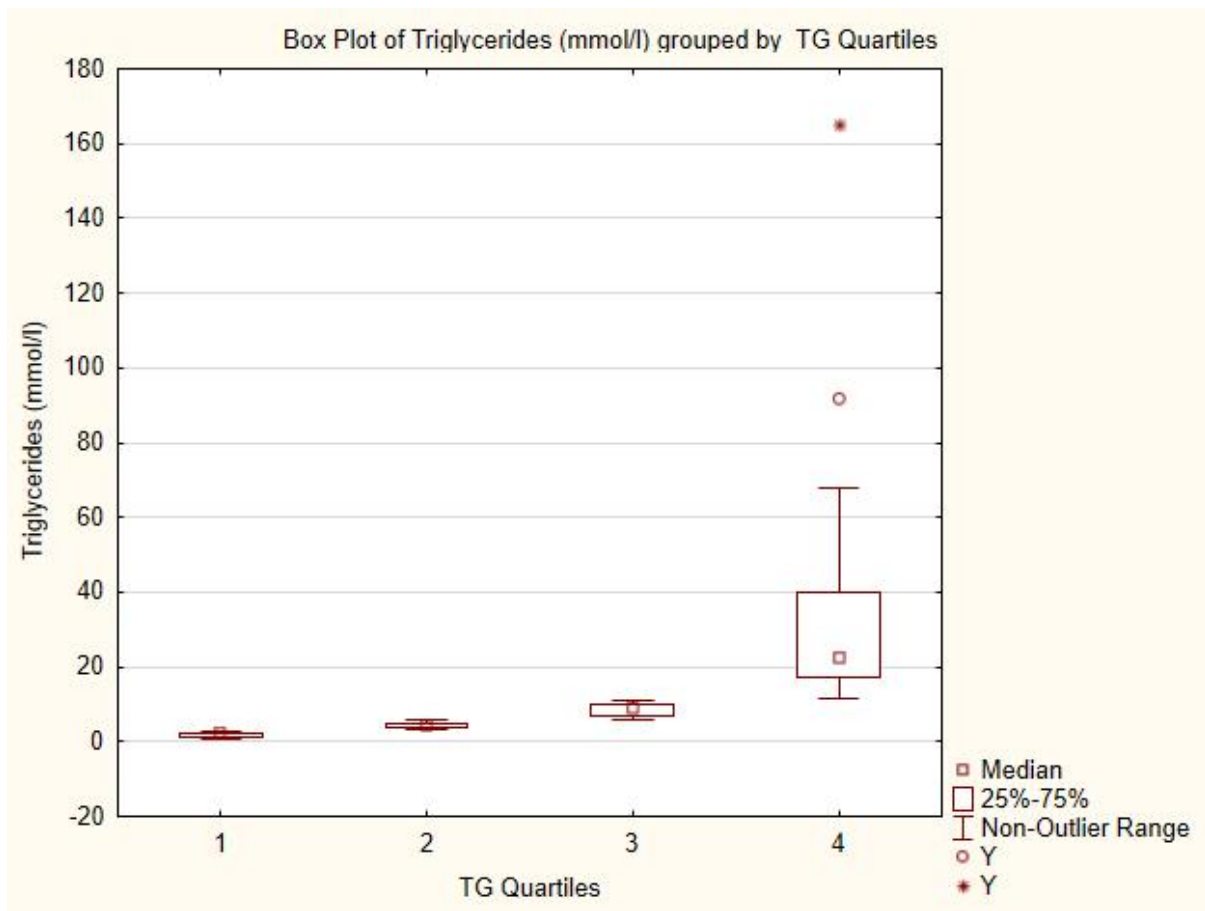

Figure 15 shows triglycerides grouped by quartiles. It shows that the values are widely spread in the fourth quartile and have an upper extreme of about 70mmol/L and outlier of 165mmol/L.

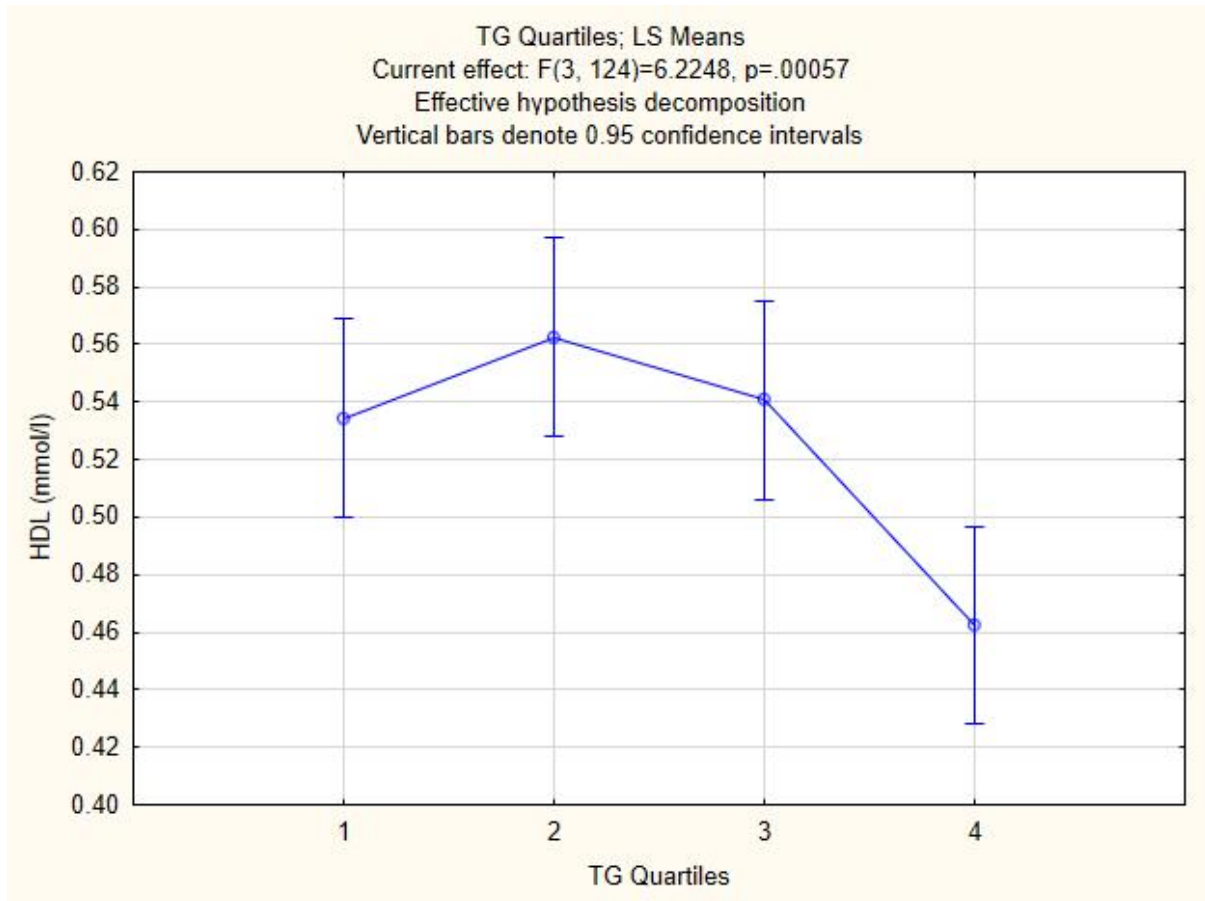**FIGURE 16:   GRAPH SHOWING HDL LEVELS PLOTTED AGAINST TG QUARTILES**

TG Quartiles; LS Means
Current effect: F(3, 124)=6.2248, p=.00057
Effective hypothesis decomposition
Vertical bars denote 0.95 confidence intervals

Table 9: Descriptive statistics between HDL and TG quartiles

Variable	Aggregate Results Descriptive Statistics (VeryLowHDLCholestero_DATA_LABELS_2020-11-20_1422)								
	TG Quartiles	Valid N	Mean	Median	Minimum	Maximum	Lower Quartile	Upper Quartile	Std.Dev.
HDL (mmol/l)	1	32	0.534375	0.600000	0.300000	0.600000	0.500000	0.600000	0.093703
HDL (mmol/l)	2	32	0.562500	0.600000	0.400000	0.600000	0.500000	0.600000	0.060907
HDL (mmol/l)	3	32	0.540625	0.600000	0.200000	0.600000	0.500000	0.600000	0.094560

HDL (mmol/l)	4	32	0.462500	0.500000	0.100000	0.600000	0.400000	0.600000	0.131370

Figure 16 shows that above the second TG quartile there is an inverse relationship between levels of HDL and TG.

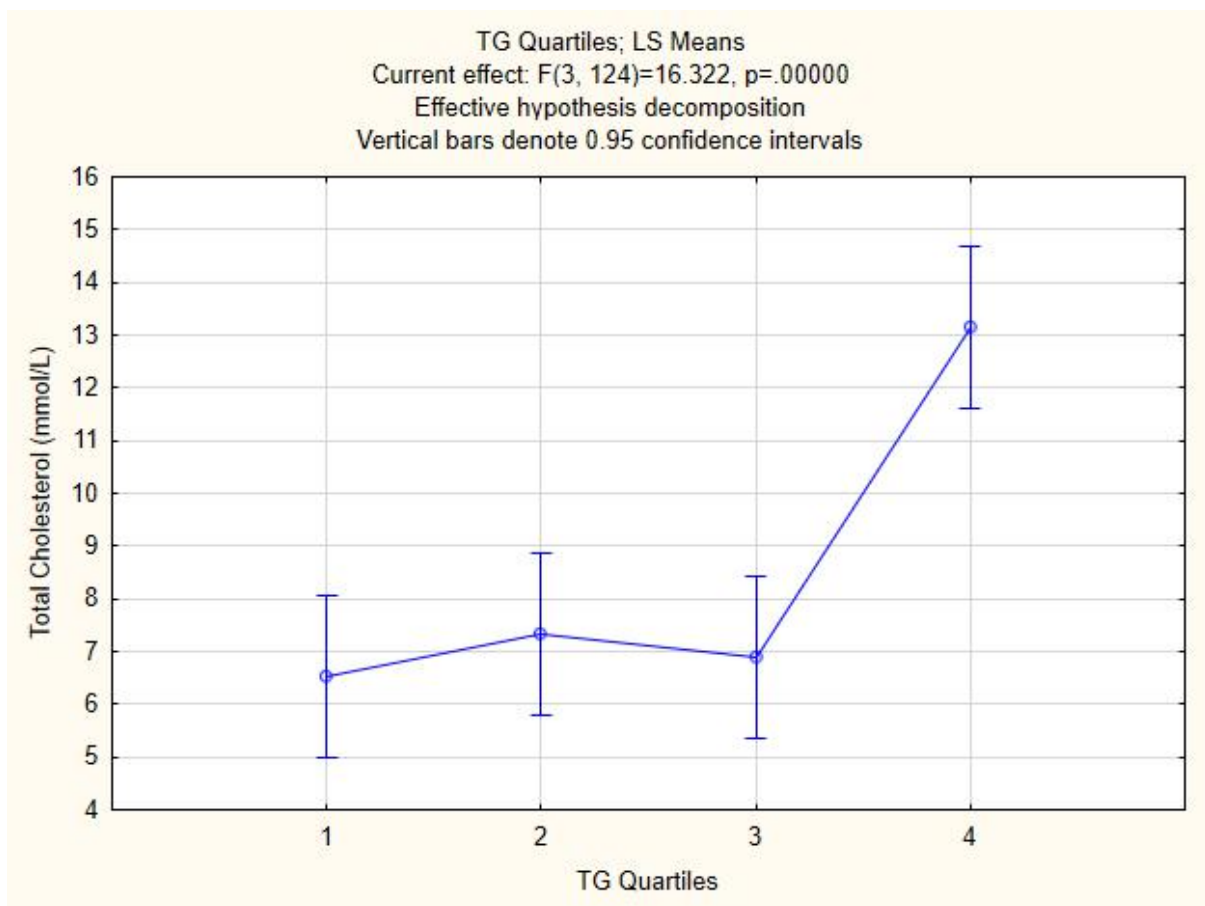**FIGURE 17:   GRAPH SHOWING TOTAL CHOLESTEROL PLOTTED AGAINST TG QUARTILES**

Figure 17 shows a direct relationship between total cholesterol and TG quartiles. This is more pronounced after the third quartile.

Chapter 7

In this chapter, age of onset of various cardiovascular complications were analysed. These included age of myocardial infarction, age of angina pectoris, age of cerebrovascular accidents as well as age of peripheral vascular disease. This is shown in the box plot below.

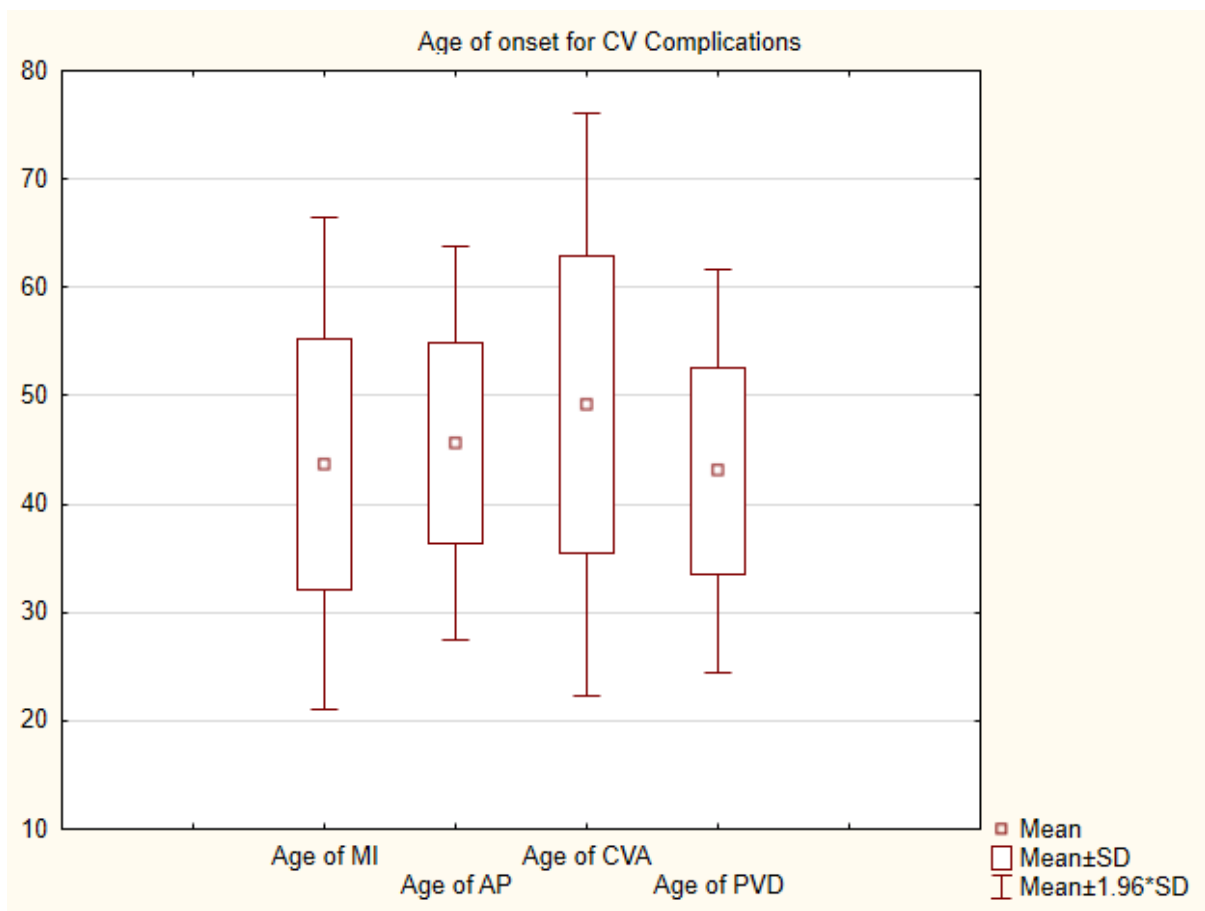**FIGURE 18:   BOX PLOT FOR AGE OF ONSET OF CV COMPLICATIONS**

Figure 18 shows that the onset of cardiovascular complications was in the twenties age group while the extreme onset of cardiovascular complications was found in the seventies as shown in the patients with CVA above.

Chapter 8

DISCUSSION

In this study we evaluated patients specifically selected for very low HDL-C on presentation to a specialised lipid clinic. Our study included more males than females and was not representative of the ethnic composition of South Africa as most participants for whom ethnicity was known were white with a low representation of black Africans.

As expected, low HDL-C correlated with features of the metabolic syndrome as well with the use of medications known to lower HDL-C (beta blockers). Unexpectedly HDL-C was not found to correlate with smoking (which tends to lower HDL-C levels) or alcohol use (which tends to raise HDL-C levels). Very few patients were on lipid-lowering medications at baseline.

The mean age of the cohort was 44.6(11.4) years. This finding raises a major concern as this age group constitutes an economically active age group. The findings indicate that this group are developing premature cardiovascular disease and related complications. There was no statistically significant age difference between males and females. In the Pan-European Survey of low HDL-C, the mean age was 62.2(11.4) [18]. HDL-C levels of <1.03mmol/L in men and < 1.29mmol/L in women were however used. Our study looked at very low HDL-C. Some cross sectional studies found that HDL-C increased with age [26, 27]. In contrast however, some longitudinal studies which evaluated the association of HDL-C with aging found that HDL-C declined or was stable with aging [28].

Males made up a larger percentage in this study accounting for 60% of the participants compared to females who constituted 40% of the study cohort. This is different from the Pan-European Study which showed that, low HDL-C was present in 33% of the males and 40% of the females, while those with very low HDL-C accounted for 14% (both genders combined) with a prevalence of a 1:1 ratio [10]. In this study, a much smaller sample was used as compared to the large sample size of the Pan-European Study and as such testing and referral patterns could have influenced the higher male percentage; men usually have higher cardiovascular risk profiles and are more likely to get their lipids checked [34]. Other studies have used different cut-off levels between the two sexes, which was not done in this study. This is because it had been shown that generally females have higher HDL-C levels than males [35, 36]. One reason for this is the influence of oestrogen and testosterone on the activities of hepatic lipase. Hepatic lipase plays a role in HDL metabolism and its levels are inversely related to those of HDL. Oestrogen and testosterone respectively tend to decrease and

increase hepatic lipase levels. As a result, women tend to have higher levels of HDL than men [11].

Caucasians had almost twice the prevalence of very low HDL-C as the Mixed Race while Africans had the lowest prevalence. However, the majority of patients attending the GSH lipid clinic have Mixed Race ancestry given the demographics of the Western Cape and referral patterns to the hospital. This overrepresentation of Caucasian patients cannot be explained by anthropometric characteristics of Caucasians since they actually have lower BMI and lower WHR as compared to the Mixed Race (Table 3c). It is also probable that other factors might have been at play like genetics, exercise habits and diet. To date, there are no known previous studies comparing prevalence of very low HDLC between Caucasians and Mixed Race or Africans. However, in The Minnesota Heart Survey, African men and women had higher HDL-C than Caucasians after controlling for lifestyle characteristics [29]. In addition to this, a review by Zoratti (1998) showed that African males had lower triglycerides and higher HDL-C than Caucasian males [30]. Findings in this study cannot be generalised in the South African context since patients were from a specialised clinic who would have been referred after meeting a certain referral criteria as described in chapter 2. In addition there is vast differences in racial proportions in each province and therefore the high prevalence of Caucasians might not apply to other provinces.

In this cohort, we found that the proportion of males and females with hypertension and diabetes was relatively high. 39.74% of males and 42% of the females had hypertension. Males with diabetes was 41.02%, while females with diabetes was 30.00%. In contrast, the proportion of males with connective tissue disease was low at 6.41% and that of females was also low at 6.00%. The metabolic syndrome has been associated with low levels of HDL-C which is consistent with our results [12]. In normal state HDL exerts various anti-atherogenic properties, including reverse cholesterol transport, anti-oxidative and anti-inflammatory capacities. However, these properties are compromised in patients with diabetes mellitus (DM), due to oxidative modification and glycation of the HDL protein as well as the transformation of the HDL proteome into a pro-inflammatory protein. This association therefore increases cardiovascular risks and manifests as premature events. Only one male in the study was documented to be HIV positive. The majority of the patients in the cohort were not tested for HIV infection. This is because HIV status was not routinely tested at the lipid clinic However in this HIV era it could have been interesting to evaluate the association of both HIV infection and antiretroviral therapy with low levels of HDL-C.

At presentation, males had approximately twice the risk of cardiovascular complications such as stroke or myocardial infarction as evidenced by 19.23% of the males who reported a prior myocardial infarction compared to 10.00% of the females. With regards to cerebrovascular accident (CVA), male prevalence was 5.13% compared with 2.00% in females. Other factors like social habits could have contributed to this finding. In our cohort, males smoked more cigarettes per day than females and they started smoking at an earlier age than the females. Furthermore, more males reported consuming alcohol than females.

Participants that had "Never Smoked" had the lowest HDL-C levels. This is the opposite to the known effects of smoking which lowers levels of HDL-C in previous studies [14, 20]. Various factors might however have affected the results. However we could not evaluate the other

characteristics of the never smokers. Forey *et al.* (2013), in their meta-analysis study concluded that quitting smoking increases HDL-C, and that this increase occurs rapidly after quitting, with no clear pattern of change thereafter [32]. Cigarette smoking cessation was also shown to increase serum levels of HDL-C but not of total cholesterol, LDL-C or triglycerides [31]. A study by Swank *et al.* (1991) on female subjects, showed that HDL-C increased after stopping smoking while there was no significant change in total cholesterol [33].

There was no statistically significant difference in levels of HDL-C at presentation in the patients who were consuming alcohol compared to those who were not consuming alcohol. However, previous studies have shown that consumption of moderate alcohol quantities increases the levels of HDL-C [15]. In this study, we were focused on the extremes of low HDL-C where alcohol consumption did not show any effect. As this study sample was small we cannot draw concrete conclusions on this. Further research will be important to look into this interesting finding since other confounding factors like diet and exercise habits may have been involved.

Baseline lipid profile showed that very low HDL-C was associated with elevated total cholesterol and hypertriglyceridemia in many patients. On follow-up, the best HDL-C ranged from 0.5 – 1.8mmol/L, while the worst recorded ranged from 0.2-1.0mmol/L. In some few cases, for example in those who were not put on lipid lowering agents at presentation, the HDL-C decreased to below baseline(as evidenced by the negative graph (figure 12) on box plot showing changes in HDLC). The higher margins in HDL-C rise could possibly have happened on those patients who were on fibrate therapy in addition to statins whilst those who had modest increments might have been on statins alone. Studies have shown that a second drug is often required to achieve substantial HDL-C increase, either a fibrate or niacin [23, 24, 25]. In this study, fibrates were used in some patients only.

Very few patients were on lipid-lowering medications at baseline. This was due to public sector formulary restriction whereby lipid-lowering medications could only be accessed via a lipid clinic. The most common statin used was simvastatin which was used by 21.09% of the patients. The high use of simvastatin was due to public sector formulary that made simvastatin the primary statin. In other studies, more than 70% would have been put on at least one statin. However, it is known that statins have little effect on raising HDL-C levels. Meta-analysis studies have shown 5-7% changes while nicotinic acid has shown very high changes, 15-35% in HDL-C levels [16].

Atenolol was used in a relatively higher percentage in this cohort, 24.22% which is about a quarter of the cohort. This is in contrast to the negative effects of beta blockers in HDL-C metabolism as it is known that oral nonselective beta-adrenergic blockers reduce HDL cholesterol by 19% and increase triglycerides by 20-40% [19].

There was no relationship between age of presentation and level of HDL-C. Knowing the effects of HDL-C on metabolism, we expected earlier presentation in patients having lower HDL-C levels. Various multiple factors could have contributed to this result including effects of quality and particle size of the HDL-C. It has been previously noted that, the inverse relationship of HDL-C with risk of ASCVD is neither linear nor continuous [22]. In the Framingham Offspring study, cardiovascular disease risk as a function of HDL-C phenotypes

was modulated by other components of the lipid panel [21]. In this study, there was no association between HDL-C level with waist circumference and again, there was also no relationship between Apo-A1 and measured HDL-C.

The lipid profile in this study was similar to previous studies whereby hypertriglyceridemia was associated with low HDL-C. However, there were few cases of very low HDL-C having normal levels of triglycerides. Previous studies have attributed this to multifactorial factors which include some drug effects such as steroids use, genetic defects such as in Tangier disease and malignancy [17]. In the South African setting, it will be interesting to further research and find out what the association will be. Many of the subjects had markedly increased total cholesterol and LDL-C as the mean total cholesterol of the cohort was 8.47mmol/L, and many may well have had familial hypercholesterolaemia. Subjects with familial hypercholesterolaemia, particularly homozygous familial hypercholesterolaemia, tend to have low HDL-C levels [39, 40]. Our study did, however, not include any patients with homozygous familial hypercholesterolaemia.

Chapter 9

CONCLUSION

STUDY LIMITATIONS

The sample size was small. This makes further subgroup analysis difficult. Most subjects' ethnicity was unknown with only one African documented. There was missing data on some important variables which further complicated data analysis. We did not explore other important patient characteristics which have been traditionally known to impact on HDL-C levels such as diet and exercise as we did not have this data. Recent infection or inflammation can lower HDL-C as well as other medications like anabolic steroids, a detailed history of infection, concomitant medications was not available for all the subjects. The hospital (Groote Schuur), where the data was obtained, is a tertiary institution and receives referrals from the Western Cape Province whose population demographic characteristics widely differ from other provinces and hence might not be truly representative of the whole country. The clinic where the study was undertaken offers specialized services and therefore data cannot be applied to the wider population. There was no control arm due to time constraints. This would have added extra information comparing the characteristics of the extreme phenotype subjects with subjects with less severe phenotypes or normal subjects.

CONCLUSION ON THE STUDY

This study has provided important insights into the characteristics of patients with very low HDLC in Cape Town South Africa. As expected, it confirms the relationship between low HDLC and the metabolic syndrome as well as the use of medications known to lower HDL-C (beta blockers). The inverse correlation between high triglycerides and low HDL-C was also demonstrated. Unexpected was that HDL-C was not found to correlate with smoking (which tends to lower HDL-C) or alcohol use (which tends to raise HDL-C). Early initiation of lipid-modifying therapy should be encouraged as it was shown to improve on the lipid profile of patients. Further studies are needed on the patients with very low HDL-C but having normal triglycerides.

ACKNOWLEDGEMENTS

I give my special thanks to my supervisor, Professor Dirk Blom for his continued support, leadership and commitment. He dedicated his time and provided adequate supervision to ensure this thesis is completed. I am also grateful to Mr. Wisdom Basera the statistician for his assistance in setting up the Red cap data base which enabled our data entry to be smooth. A big thank you also goes to the lipid clinic staff for maintaining the records and keeping them in an orderly manner for ease of access. Last but not least, I would thank the secretary in the records rooms for giving me access to her computer and keys so that I could do my work even during odd hours of the day and on the weekends.

DECLARATIONS

Ethics Approval

Ethics approval was obtained by Human Research Ethics Committee of University of Cape Town

Consent to Publication

Not applicable

Competing Interests

The authors declare they have no competing interests.

Chapter 10

<div align="center">REFERENCES</div>

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