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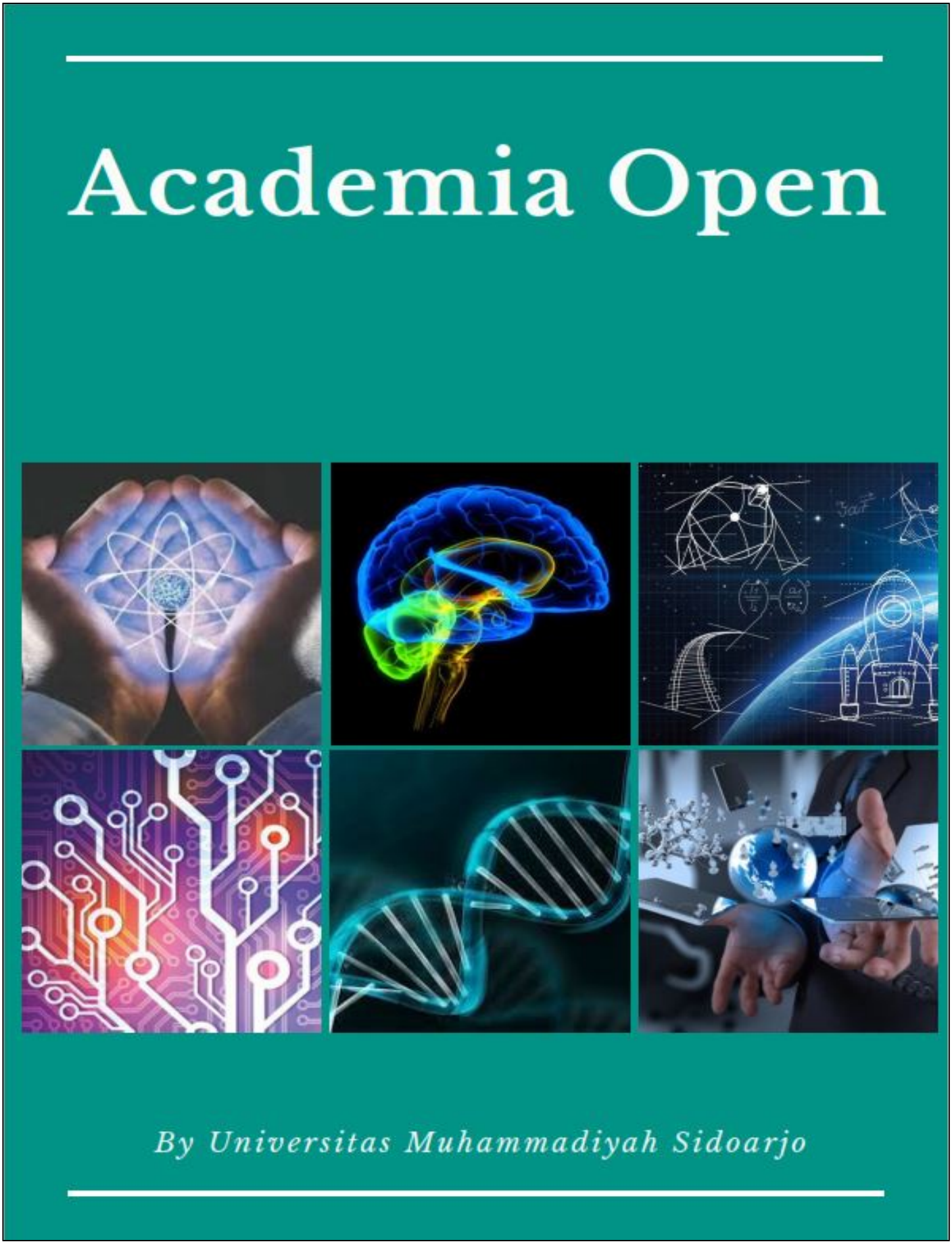

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Toxoplasma Infection Alters Lipid Profiles Challenging Cardiovascular Norms

Infeksi Toksoplasma Mengubah Profil Lipid yang Menantang Norma Kardiovaskular

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Abstract

This study conducted at Baquba Teaching Hospital from January to April 2024, investigated the lipid profiles of 75 participants (50 patients with Toxoplasma and 25 controls) aged between 50 and 60 years. Existing research suggests a potential link between Toxoplasma infection and alterations in lipid metabolism; however, specific effects on different lipid fractions are not well-understood. This study aimed to explore the association between Toxoplasma infection and changes in lipid profiles, particularly focusing on whether infection status correlates with variations in high-density lipoprotein (HDL) levels. Venous blood samples were collected pre-breakfast and processed to obtain serum, which was analyzed for lipid profiles. The findings indicated that lipid levels generally increased in Toxoplasma patients compared to controls, except for a decrease in HDL levels. These results suggest that Toxoplasma infection could influence lipid metabolism, potentially impacting cardiovascular risk. Further research is needed to understand the mechanisms underlying these changes and their clinical implications.

Highlights:

- Toxoplasma infection is linked to significant changes in lipid profiles.
- The study identifies a unique decrease in HDL levels among infected individuals.
- Implications suggest potential cardiovascular risks associated with Toxoplasma.

Keywords: Toxoplasma, Lipid Metabolism, Cardiovascular Risk, HDL Decrease

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Introduction

The protozoan parasite infection One of the most prevalent parasite infections in humans and other warm-blooded animals is Toxoplasma gondii. It can be found all throughout the world, from Australia to Alaska. Approximately one-third of the human race has encountered this parasite [1]. Most adults are not very ill from it, but in congenitally infected children, blindness and mental retardation can occur; in individuals infected after birth, blindness can occur; and in immunocompromised individuals, the disease can be fatal. The two main risk factors for T. gondii infection are eating or drinking anything contaminated with oocysts and consuming raw or undercooked animal products.

1. Toxoplasma Gondii and Lipid Profile

Toxoplasma gondii, a member of the Apicomplexa eukaryotes, is the source of toxoplasmosis, an infection. Toxoplasmosis is a common disease that affects warm-blooded animalsand humans alike. The economy and public health are both seriously threatened by this protozoan parasites[2]. Because T. gondii infections can result ina variety ofclinical outcomes, include abortions, mental impairmente, hydrocephalus, retinochoroiditis, andeven death and With potentially fatal encephalitis in AIDS patients receiving organ transplants and immunosuppressive medication, they nevertheless represent a major risk topublic health [3], [4]. According to estimates, T,gondii infections impact between 30% and 65% of the world's population [5]. Human seroprevalence of T,gondiiin infections rises withage, but itis lower in colder climates and does not differ much between genders [6]. In Iraq, attention to Toxplasmosishas disease recently sharply increased as a result of multiple investigations [7], [8]. Many researchers have been interested in the role that serum biochemical parameters play in human infections with certain parasites. These measures comprise total protein, total globulin, total albumin, (LDL)high-density lipoprotein , (VLDL)very-low density lipoprotein , (LDL)low-density lipoprotein, triglycerides, and cholesterol levels [9]. Studies conducted in vitro showed that these parasites may proliferate in lipid, rich, serum-free medium [10]. Assessing the levels of specific biochemical markers in individuals who test positive or negative for Toxoplasma is the goal of this study.

Methods

A. Standard Kits

Kit Name	Company	Origin
T0TAL CH0LESTER0L	CR0MATEST(LINEAR)	Spain
HDL-CH0LESTER0L	CR0MATEST(LINEAR)	Spain
LDL_ CH0LESTER0L	CR0MATEST(LINEAR)	Spain
VLDL_CH0LESTER0L	CR0MATEST(LINEAR)	Spain
TRI GLECERIED	CR0MATEST(LINEAR)	Spain
BL00D GLUG0SE	SlGMA	USA

Table 1. *The used kits*

B. Equipment and Tools

Equipment	Company	Origin
CENTERlFUGE	HERAEUS-CHRlSTGMBH	GERMANY
EPPENDORF TUBE	AFlCO-DlSP	JORDEN
MlCROPlPETTE	GlLSON	FRANCE
MULTl-CHANNEL MlCROPIPETTE	GlLSON	FRANCE
DEEP-FREEZE	GORENJE	SERBlA
SEMl-OUTO ANALYSER	MINDRAY	MALlZlA
lNCUBATlON	WTEBlNDER	GERMANY
GELTUBE	XlNLE	CHlNA
TlPS	AFlCO-DlSP	JORDEN
VACUUM EDTATUBES	AFlCO-DlSP	JORDEN
DlSPOSABLE SYRINGES	A.D MEDlCAL DEVlCE	UAE
COBAS lNTEERA400PLUS	ROCHl	GERMANY

Table 2. ***Instruments with their company and the origin***

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C. Study Design

The study is designed on 75 person (50patients and 25 control) with Toxoplasma at range (50-60 y) between January 2024 and April 2024 in the (central care unit) of the Baquba Teaching Hospital (B.T.H), the total number inthis study include conservatives 40 men and35 women .

The patients groups include :

1. Gender (30 male and 20 female)

2. Age of patients groups (50-60 years)

D. Blood Sample Collection

Blood is taken from each participant early in the morning, before to breakfast. Venous blood samples ranging from 10 to 15 milliliters were obtained from both patients and controls, and the samples were stored in a gel tube at room temperature for fifteen minutes (anticoagulant not included). After that, the serum was split into four aliquots by centrifugation at 3000 xg for fifteen minutes, and it was refrigerated at -20 °C until it was needed for a biochemical analysis.

1. Determinations of Human Choleisterols

a. Principle

The following is the reaction strategy for the enzymatic technique as described by Allain et al. :

Cholesterols esters → Cholesterols+free fattyacids

cholesterols + O2 → Cholesten 4 one3+H2O2

2H2O2 + Phenols + PAP → Quienoneimine (pink) + 4 H 20

b. Reagent Preparations

Remove the aluminum cap with a non-sharp instrument. Quickly fill vial R1 with the contents of vial R2. Gently stir until fully dissolved. Vial R3: Ready used .

c . Assay Procedoure

1) Manaus Methods

Keep the reagent and the samples on room temperatures.

Reagents	1000 uL
BIank, Standard, ControI orspecimen	10 uL
Blend. Allow to stand for five minutes at 37°CELSLUS or ten minutes at room temperatureS.At 500 nm (480–520), note absorbances in relation to the reagent blanks.For one hour, the color remains consistent.	

Table 3. ***The reagent and the samples on room temperatures***

a) User validation is required for performances involving manual procedures.

b) Applications for Kenza and other applications are available upon request.

2. Determinations of Human LDL-Cholesterols

a. Principe

Low-density lipoproteins (LDL) are specifically precipitated by polyvinyl sulfate in whole serum, which is the basis for the separation procedure used in this technique, Centrifugation is used to settle the precipitant, and the clear supernata is then tested for residual cholesterol from the remaining lipoproteins (VLDL+ HDL). Calculating LDL-cholesterol involves deducting the sample's total cholesterol from the supernatant cholesterol fractions.

b. Reagents Preparations

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Until the date of expiration indicated on the label, all of the kit chemicals remain stable. During use, keep the vials tightly closed, shielded from light, and free from contaminations.

c. Procedures

1) Precipitating

a. Samples and reagents should be brought on room temperatures.

b. Pipette in centrifuge tubes that have been labeled

Samples or Standards	0.2 mL
Precipeitations Reagean	0.1 m
Ratio = Samples / Reageant = 1/0.5 Dil.factors = 1.5	

Table 4.

c. Vortex, then let rest at room temperature for ten minutes.

d. You can centrifuge for two minutes at 12,000 rpm or for ten minutes at 6000 rpm.

e. For the purpose of measuring cholesterol, removed of aliquot from the supernatant.

2) Calorimetry

a. Allow the kit's and the cholesterol MR's components to come to room temperatures.

b. Set up two sets of tests so that the total cholesterols in the sample and the cholesterols that is still in the supernatant may be measured simultaneously. Observe the insert's directions for total cholesterols.

c. Pipette into tubes with labels

TUBES	Biank	Samples Supernats	Standards Supernats
Monoureagent	1.0mL	1.0mL	1.0mL
Supernates	~	50µL	~
Standard	~	~	50µL

Table 5. ***Pipette into tubes with labels***

Under light protection from light, the color remains constant for at least half an hour

3. Determinations of Human Trigyclerides MR

a. Principle

The foundation of method1,2 is the enzymatic breakdown of serum or plasma triglyceride by lipoprotein lipase (LPL), which yields glycerol and free fatty acids (FFA). Adenousine triphosphate (ATP) phosphorylates glyceruol in the presence of glycerolkinase (GK), resulting in the formation of glycerol-3-phosphat (G-3-P) and adenousine diphousphate (ADP). Glycerophosphate Oxidase (GPO) oxidizes G-3-P to produce hydrogen peroxide and dihydroxyacetone phosphate (DHAP). When4-aminoanteipyrine (4-AA)and phenoul are coupled with hydrogen peroxide (H2O2) under the action of peroxidase (POD), a red chromogen is created that is proportionate to the amount of triglycerides present in the sample.

Trieglycerides + 3H2O → Glyceroul + 3FFA

Glyceroul+ATP → Glyceroul-3-P+ADP

Glyceroul-3-P+O2 → DHAP+H2O

4-AA+4Phenoul → Quinouneimeine+H2O

b. Reagents Preparations

Both the Standards and the Monoreagents are prepared for usage.

c. Procedurs

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1) Let the samples and reagents come on room temperature.

2) Pipette into tubes that have been labeled

TUBES	Biank	Samples	CAL. Standards
R1.Monoureagent	1,0mL	1,0mL	1,0mL
Samples	~	10L	~
CAL.Standards	~	~	10L

Table 6.

3) Stir, then leave the tubes for either five minutes at 37ºciuisi or fifteen minutes on room temperature (16–25ºC).

4) After calibrating the samples and standards on 500 nm, compares their absorbance (A) with the reagenet blank.

Result and Discussion

A. Lipid Profiles and Glucose Biomarkers Among Study Groups

Patients with toxoplasmosis exhibited significantly higher mean levels across multiple parameters compared tohealthy controuls. Specifically, inpatient with toxoplasmosis, themean levels for glucose were 179.74 ± 14.33 mg/dL, compared to 111.68 ± 8.68 mg/dL in healthy controls (P = 0.001). Triglycerides (TG) levels were 204.88 ± 33.92 mg/dL in patients and 122.88 ± 29.14mg/dLin controuls (P~0.001). Tutal cholesterols (Total Ch) levels showed a mean of 267.90 ± 20.66 mg /dLin patient and 169.08 ± 17.63mg /dLin controul (P = 0.001). HighDensity Lipouprotein (HDL) level was significantly lowerin patient (13.92 ± 3.87 mg/dL) compared to controls (35.96 ± 3.01 mg/dL) (P~0.001). (LDL)LowDensity Lipoiprotein levels were higher in patients (163.60 ± 17.98 mg/dL) compared to controls (118.84 ± 5.49 mg/dL) (P = 0.001). Very Low-Density Lipoprotein (VLDL) levels was higherin patients (40.98 ± 6.78 mg/dL) compared to controls (17.12 ± 3.81 mg/dL) (P = 0.001). These findings highlight the significant metabolic and lipid profile alterations associated with toxoplasmosis, as detailed in Figure 1.

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S.t.s	Case	N.	Me an	Std.Deviation	Std.Error Mean	*P valoue*
Glucose	Patients	50	179.7400	14.32966	2.02652	0.001
	Control	25	111.6800	8.68293	1.73659	
T.G	Patients	50	204.8800	33.91504	4.79631	0.001
	Control	25	122.8800	29.13520	5.82704	
Total.Ch	Patients	50	267.9000	20.66274	2.92215	0.001
	Control	25	169.0800	17.62555	3.52511	
HDL	Patients	50	13.9200	3.86950	.54723	0.001
	Control	25	35.9600	3.00666	.60133	
LDL	Patients	50	163.6000	17.97845	2.54254	0.001
	Control	25	118.8400	5.48999	1.09800	
VLDL	Patients	50	40.9760	6.78301	.95926	0.001
	Control	25	17.1200	3.81139	.76228	
P valoue < 0.05 * *Valoues are expressedas mean ±standard error(SE)*				* *Indepenedent-sample Ttest*		

Figure 1. ***Comparative Analysis of Laboratory Test Results in Patients with Toxoplasmosis and healthy Controls***

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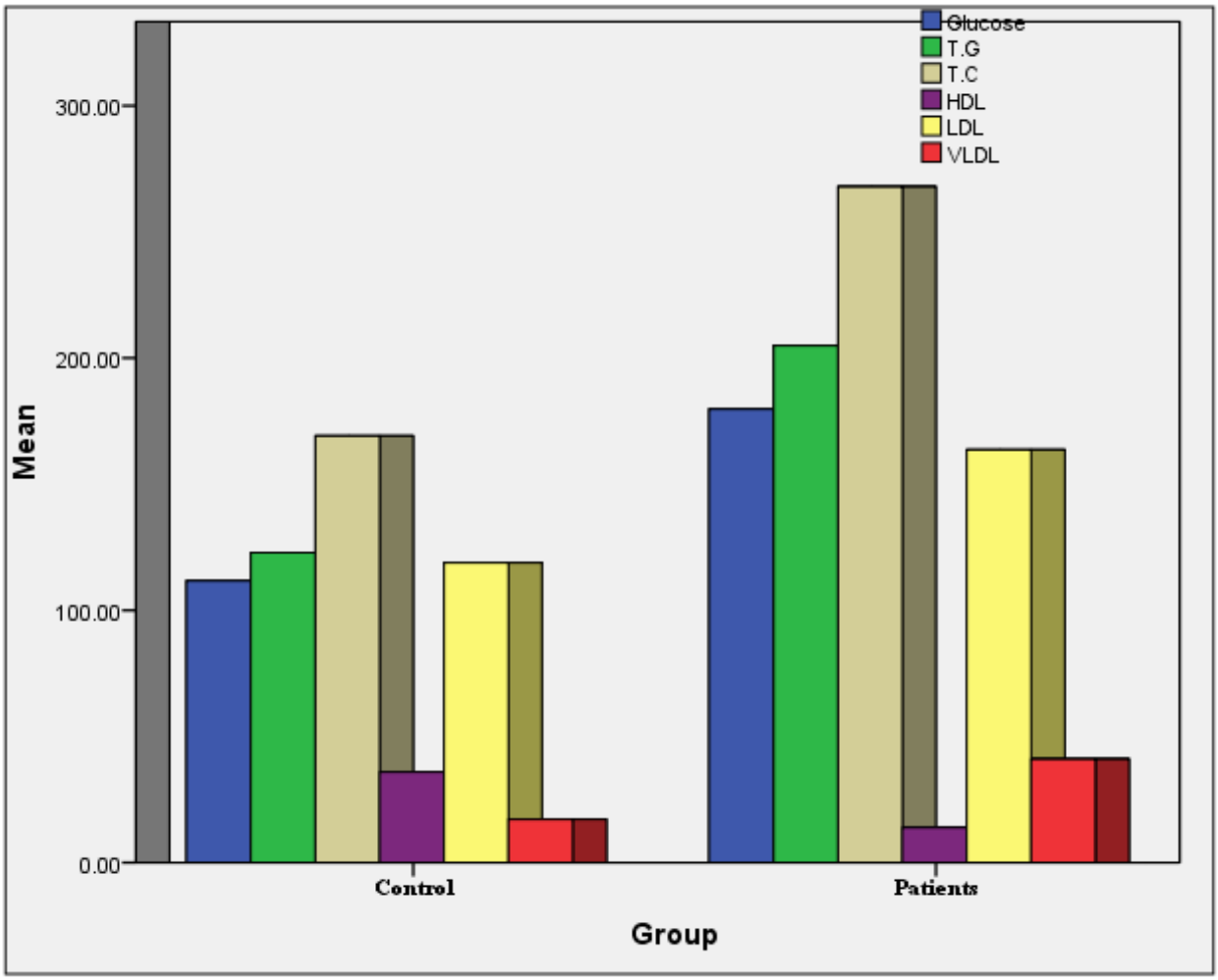

Figure 2. ***Lipid Profiles and Glucose values for control and patient group***

B. Blood Glucose

For glucose levels, patients with toxoplasmosis (N=50) had a mean of 179.74 mg/dL, compared to 111.68 mg /dLin healthy contruols (N=25) (P ~ 0.001). The findings were in agreement with Anastasia Poznyaket al. (2020),[11]

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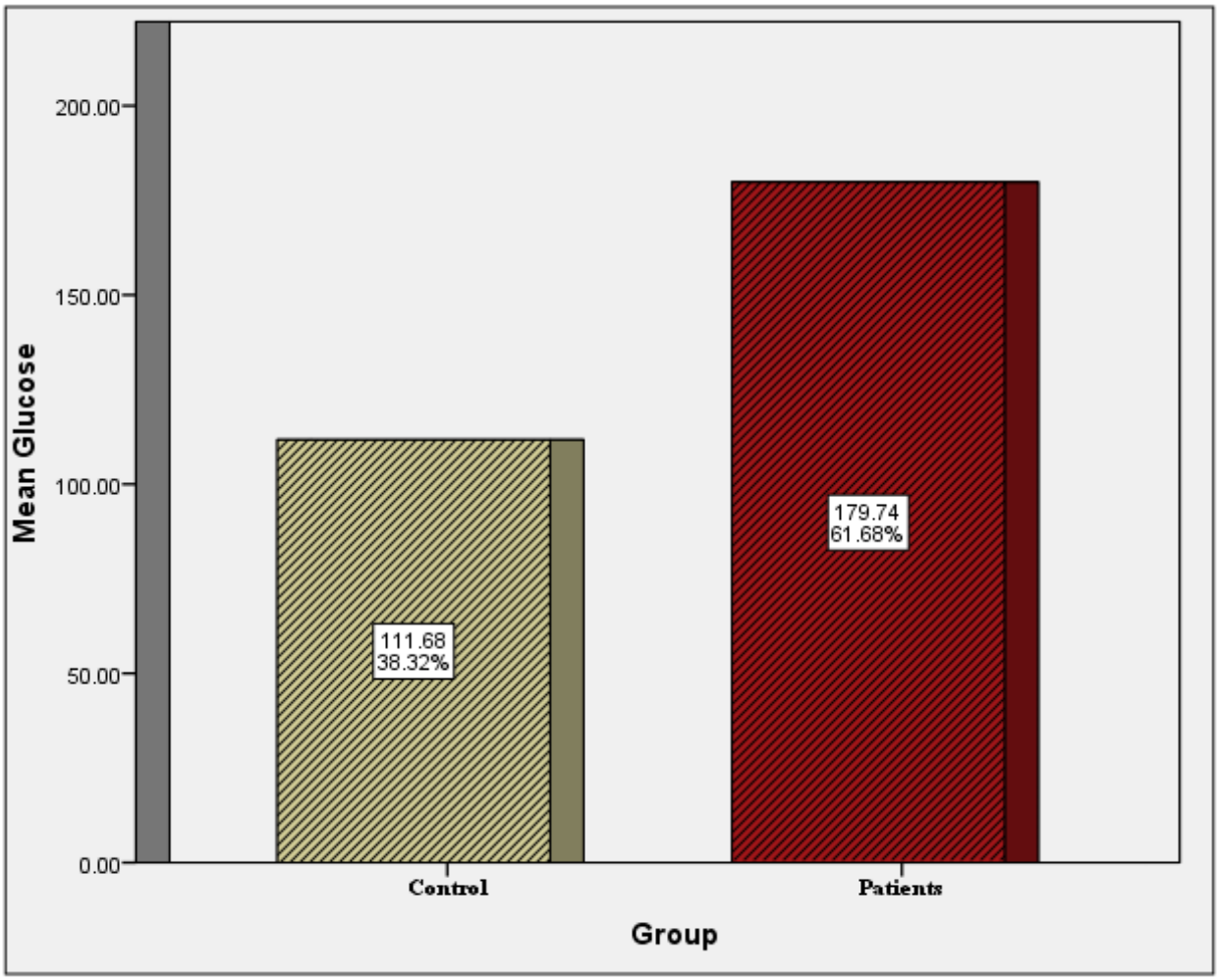

Figure 3. ***Glucose values for control and patient group***

C. Triglycerides (T.G.)

Triglycerides (T.G.) levels were significantly higher in patients (mean = 204.88 mg/dL) compared to controls (mean = 122.88 mg/dL) (P = 0.001). This outcome agreed with that of Lambertini C.et al. [12] were seen to be rising in T.G.'s serum.

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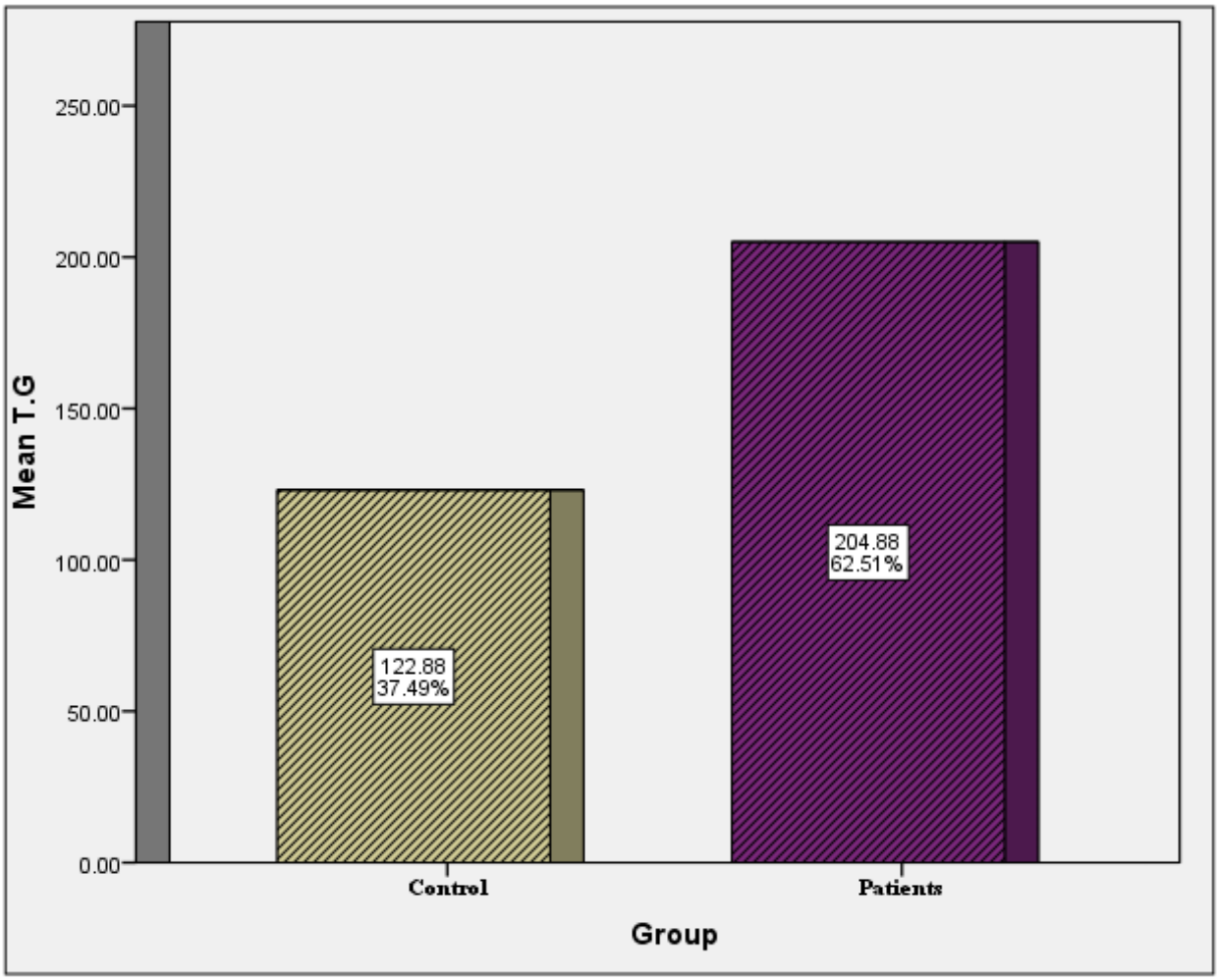

Figure 4. ***Triglycerides values for control and patient group***

D. Total C holesterol s (T C)

Total cholesterols (TC) levels were also higher in patients (mean = 267.90 mg/dL) compared to controls (mean = 169.08 mg/dL) (P = 0.001) This outcome was in agreement with Maha Radhi Abeass, et al. were seen rising in the total cholesterols serum sample.

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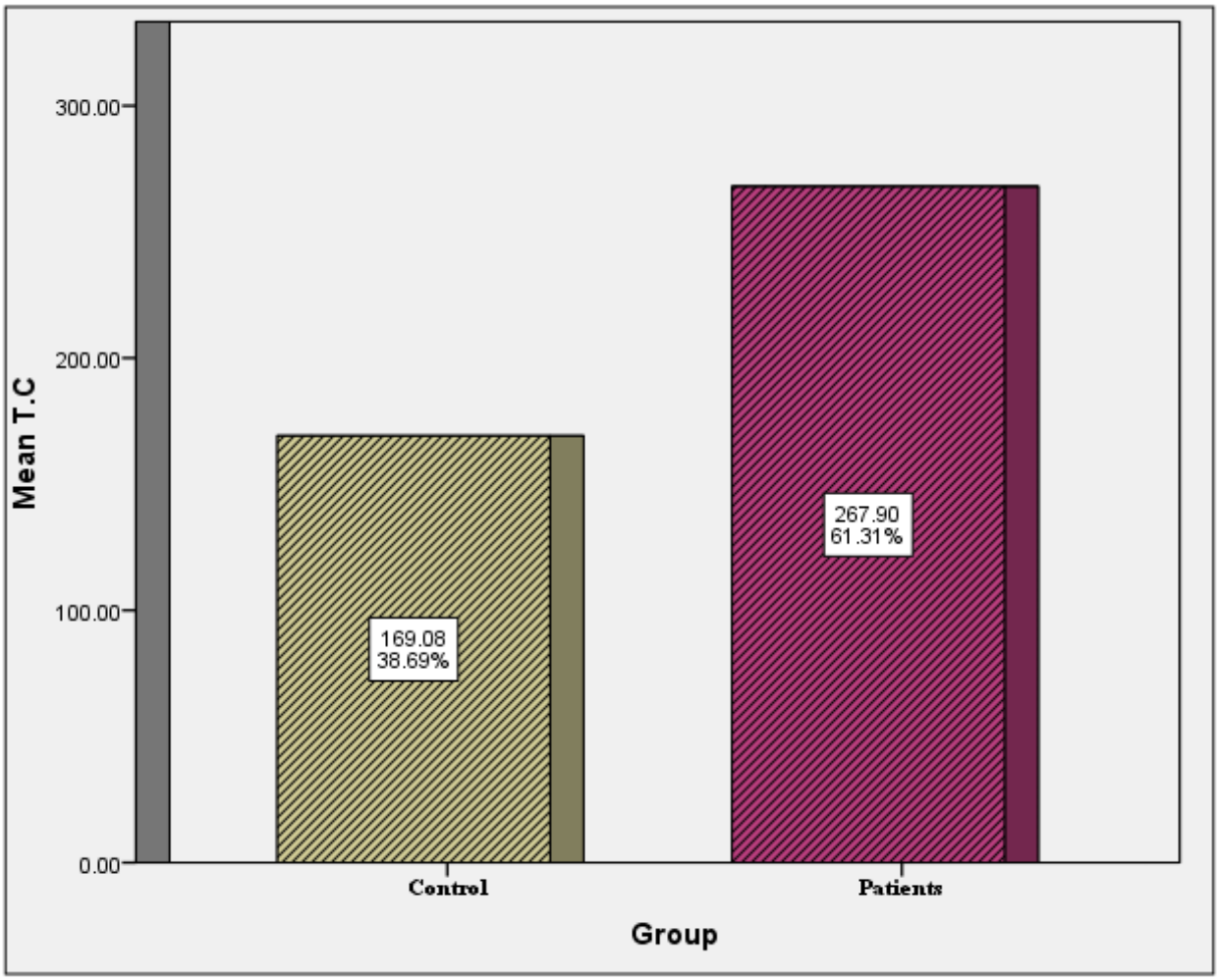

Figure 5. ***Total cholesteruol values for control and patient group***

E. High Denesity Lipouprotein (HDL)

Patients had significantly lower levels of High-Denesity Lipouprotein (HDL) (mean~13.92 mg/dL) than controls (mean~35,96 mg/dL) (P~0.001). This outcome was in agreement with Maha Radhi Abeass,,et al. [12] were found to be declining in the HDL cholesterols serum level.

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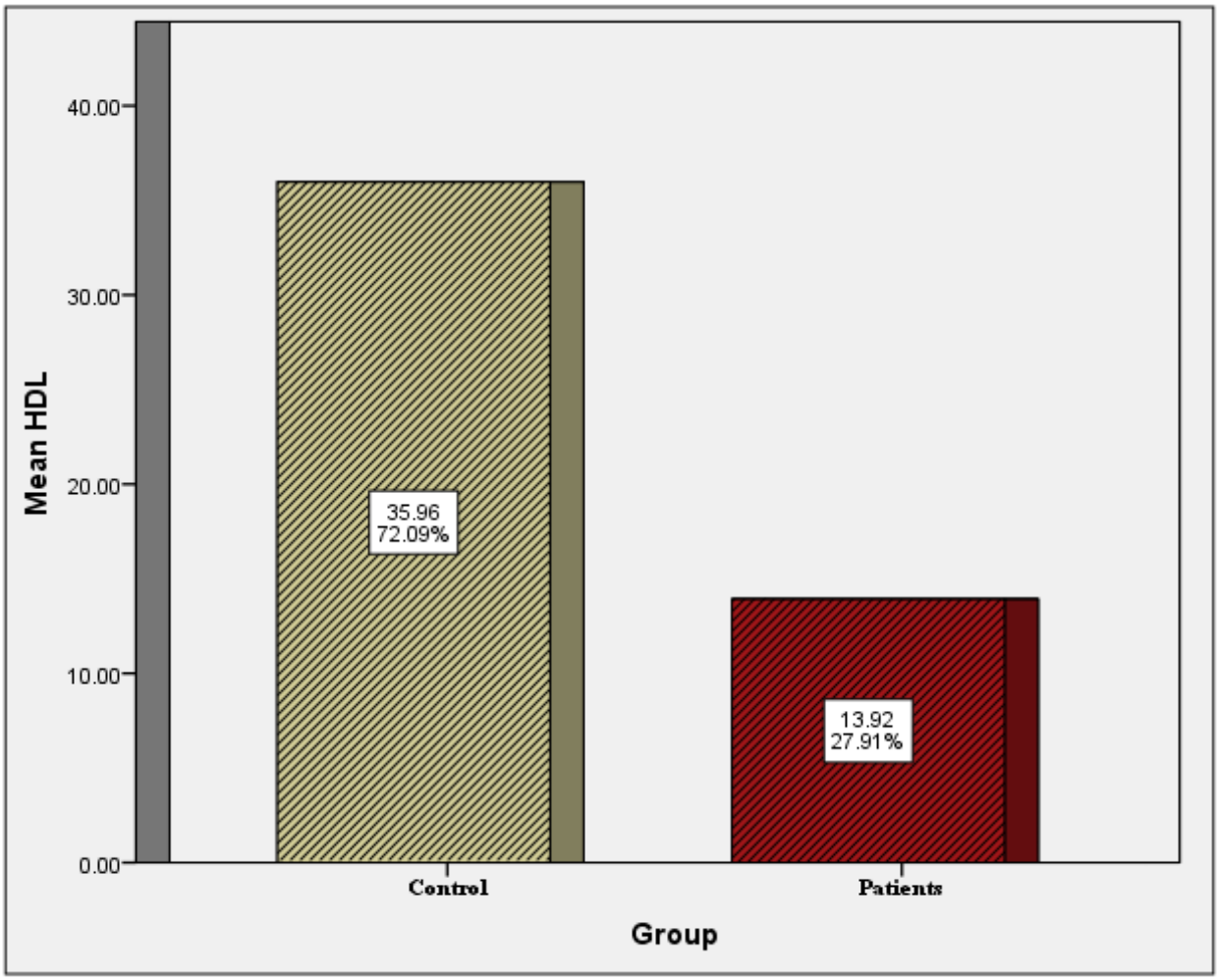

Figure 6. ***HDL cholesterols values for control and patient group***

F. Low Denesity Lipouprotein (LDL)

Patients had greater mean levels of low-denesity lipouprotein (LDL) (163.60 mg/dL) than controls (mean = 118.84 mg/dL) (P = 0.001). This outcome was consistent with Lambertini C.,et al. [13] were shown to be rising in the LDL serum.

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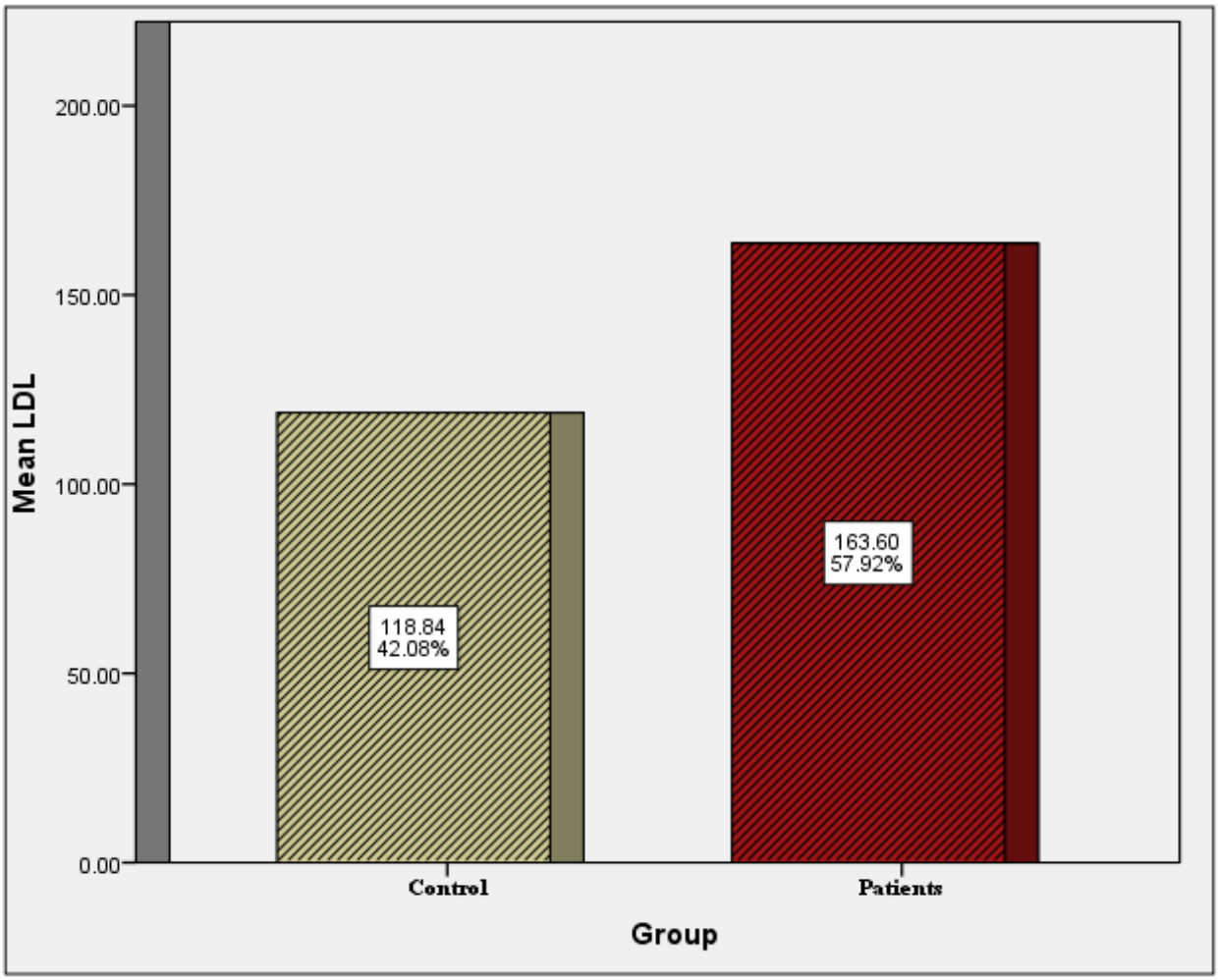

Figure 7. ***LDL cholesterols values for control and patient group***

G. Very Low Denesity Lipouproteib (V.LDL)

Very,Low-Denesity Lipouprotein (V.LDL) level was higherin patients (meun~40.976mg/ dL) comepared to control (meun~17.12mg/ dL) (P~0.001). This outcome was consistent with Lambertini [14] were shown to be rising in the VLDL serum.

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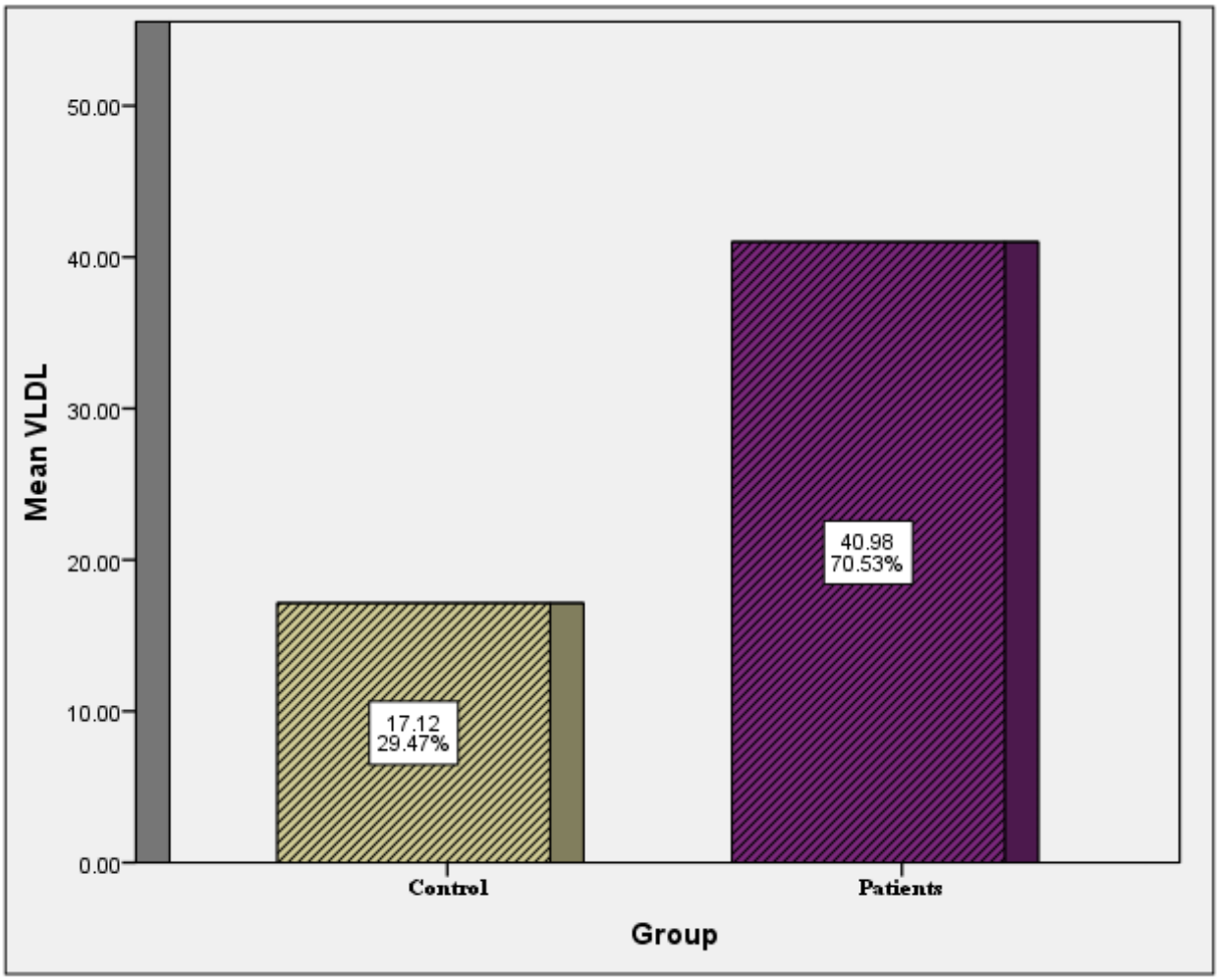

Figure 8. ***VLDL cholesterol values for control and patient group***

Conclusion

1. The results observed increasing in (Glucose) values with Toxoplasma Patients.

2. Increasing of Lipid profile (Total cholesterol , LDL cholesterol , T.G and VLDL) with Toxoplasma Patients.

3. The HDL cholesterol decreases with cases Toxoplasma Patients.

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