EFFECTS OF AIR POLLUTION ON THE NEURONAL ACTIVITIES OF RICKSHAW-PULLERS AND DRIVERS OF DHAKA CITY

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DECLARATION

This dissertation has been submitted to the University of Dhaka in partial fulfilment of the requirements for the degree of Master of Philosophy (M Phil) in Biochemistry. This study has been carried out in the Department of Biochemistry, University of Dhaka. No part of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other University or other institutes of learning.

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MR. ATWAR RAHMAN, Assistant Judge, Judge Court, Rajbari, My Desireable Personality Who Introduced me to the Reality of this World with Gratitude and Love.

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ABSTRACT

Dopamine β-hydroxylase (DBH) is a neurotransmitter (catecholamine) synthesizing enzyme which catalyzes the formation of norepinephrine from dopamine. It was first isolated and characterized in 1960. The catecholamine neurons of the brain regulate high level of neurological functions. The catecholamines act not only as neurotransmitters but also as hormones. They are important bio-chemically, clinically, pharmacologically and they regulate the brain functions-emotion, behaviour, sex, sleep, mood and thinking of higher animals including human.

Recently it has been found that, the air pollution of Dhaka city is one of the height in the world. The lead concentration is 465 ngm/m^3 which is the most dangerous for human health. Many people are affected by air pollution. But upto now no research has been done on the air pollution affected people at various physiological conditions.

Neurotransmitter mediating enzyme Dopamine-β-hydroxylase, its cofactor (ascorbic acid and copper) and other bio-chemical parameters such as Zinc, vit A, glucose, TP, ALB, urea, creatinine, sGOT, sGPT, ALP, bili, lipid profile (CH, Tg, HDL & LDL) CKMB, phosphate, Ca^{++}, Na^{+}, K^{+} and etc were measured at three different age groups of taxi-drivers and rickshaw-pullers in the Dhaka city in comparison with rural individuals were used as control.

It was found that the DBH activities were decreased in group-I, group-II and group-III in the serum of taxi-drivers and rickshaw-pullers in comparison with rural individuals. For group-I the mean DBH activity was 20.9 nmol/min/ml in the serum of taxi-drivers, 22.5 nmol/min/ml in the serum of rickshaw-pullers where the activity of DBH in the serum of rural individuals was 29.5 nmol/min/ml.

For group II – the mean DBH activity was 24.5 nmol/min/ml in the serum of taxi-drivers, 25.0 nmol/min/ml in the serum of rickshaw-pullers where the activity of DBH in the serum of rural individuals was39.4 nmol/min/ml.

For group III- the mean DBH activity was 21.3 nmol/min/ml in the serum of taxi-drivers, 22.9 nmol/mim/ml in the serum of rickshaw-pullers where the activity of DBH in the serum of rural individuals was 34.2 nmol/min/ml.

Cofactors of DBH ascorbic acid (Vit-c) levels were decreased in three different age groups of taxi-drivers and rickshaw-pullers in comparison with rural individuals used as control.

Another cofactors copper contents were increased in three different age groups of taxi-drivers and rickshaw-pullers than rural individuals. Zinc levels were also increased in the serum of taxi-drivers and rickshaw-pullers.

The glucose levels and liver enzymes of sGOT sGPT were increased in the taxi-drivers and rickshaw-pullers than rural individuals. Lipid profiles cholesterol and Tg were decreased in the serum of taxi-drivers and rickshaw-pullers than rural human.

The ion of Na^+ and K^+ slightly increased in the taxi-drivers and rickshaw-pullers than rural individuals within normal range. And other bio-chemical parameters were not significantly increased or decreased in three different age groups of taxi-drivers and rickshaw-pullers.

CHAPTER-1
INTRODUCTION

Introduction

Now air pollution is the most serious problem over-all the whole world. The definition of air pollution is given by engineers joint council of USA "Air pollution means the presence in the outdoor atmosphere of one or more contaminants such as dust, fumes, gases, mists, odour, smoke, or vapour in quantities with characteristics and duration's such as to be injurious to human, plant or animal life or property or which unreasonably interfere with the comfortable enjoyment of life and property[1].

1.1 Normal composition of the atmosphere[2]:

The atmosphere has broadly speaking three categories of constituents (a) major (b) Minor, and (c) Trace. For Pollution free dry air at ground level, the components may be expressed as percent by volume as follows (with parentheses)

Table : 1 (a) Major components :

Name	Formula	Percent of amount (%)
Nitrogen	N_2	78.09
oxygen	O_2	20.94
water-vapour	$H_2O\uparrow$	0.1-1.5

Table: 1 (b) Minor components :

Name	Formula	Percent of amount (%)
Argon	Ar	9.34×10^{-1}
Carbondioxide	Co_2	3.25×10^{-2}

Table : 1 (c) Trace component :

Name	Formula	Percent of amount (%)
Neon	Ne	1.82×10^{-3}
Helium	He	5.24×10^{-4}
Methane	CH_4	2×10^{-4}
Krypton	Kr	1.14×10^{-4}
Nitrous oxide	NO_x	2.25×10^{-5}
Hydrogen	H_2	5×10^{-5}
Xenon	Xe	8.7×10^{-6}
Sulphurdioxide	SO_2	2×10^{-8}
Ozone	O_3	trace
Nitrogen oxide	NO_x	1×10^{-5}
Ammonia	NH_3	1×10^{-6}
Carbonmonoxide	CO	1.2×10^{-5}
Iodine	I_2	Trace

1.2 Classification of air pollutants[3] :

Air pollutants can be classified as follows :

(I) Natural contaminants, eg natural fog, pollen grains, bacteria and products of volcanic eruption.

(II) Aerosols (particulate)

eg, dust, smoke, mists, fog and fumes,

(III) Gases and vapours.

The gases and vapours, which are important air contaminants are shown in table. 2 (a)

Table : 2 Air contaminants :

No	Group	Examples
1.	Sulphur compounds	SO_2, SO_3, H_2S, mereaptans
2.	Nitrogen compounds	NO, NO_2, NH_3
3.	Oxygen compounds	O_3, CO, CO_2
4.	Halogen compounds	HF, HCl
5.	Organic compounds	Aldehydes, Hydrocarbon (CH_4)
6.	Radioactive compounds	Radio active gases.

1.3 Sources of air pollutants[4] :

An inventory of air contaminants is a necessary first step towards control of air pollution. Air pollutants can be either natural or may be the result of various activities of man like industrial operations. The industrial contaminants can be either by products of external combustion like smoke, dust and sulphur oxides or by products of internal combustion like the reactions in petrol and diesel engines.

Further, the emissions can be either Primary pollutants or secondary Pollutants. The various sources of pollutants can also be broadly grouped under either stationary sources or mobile sources.

1.4 Stationary and mobile sources[4] :

Emissions (air pollutants) may be classified by source, as stationary or mobile. Another method of classifying emission sources is by:

(I) Point sources (Large stationary sources)
(II) Area sources (Small stationary sources and mobile sources with indefinite routes)
(III) Line sources (mobile sources with definite routes)

Table : 3 A picture of emission inventory source classification.

Total sources

Stationary sources		Mobile sources	
Point sources	Area sources	Line sources	Area sources
I) Industrial processing	I) Residential heating coal, gas, oil.	I) High way vehicles	I) Motor vehicles light-duty, Medium-duty, Heavy-duty
II) Power plants	II) Industrial & commercial heating coal, oil, gas.	II) Railroad locomotives	II) Railyard locomotives.
III) Fuel combustion.	III) On site incineration	III) Channel vessels	III) Port vessels.
IV) Solid waste disposal municipal incinerators open burning	IV) Open burning		IV) Aircraft. (Airport)
V) Miscellaneous.	V) Evaporative loses		V) Miscellaneous.
	VI) Miscellaneous.		

1.5 Source of primary and secondary air pollutants[6] :

Air pollutants are mainly classified into two categories : Primary it emitted directly into the atmosphere by a stationary or mobile sources, and secondary, it formed in the atmosphere as a results of physical and chemical processes such as hydrolysis, oxidation and photochemistry. **Among the primary pollutants are-**

I Carbonmonoxide (CO)

II Hydrocarbons (CH)

III Volcanic organic compounds (VOC_s)

IV Oxides of sulphur (SO_x)

V Oxides of Nitrogen (NO_x) &

VI Particulate matter including dust and soot and compounds of lead.

Among the secondary pollutants including

(I) Nitrogen dioxide (NO_2)

(II) The entire class of photochemical oxidants (including O_3) &

(III) Acidic dispositions.

CO_2 has no direct adverse effect on human health but its buildup contributes to the green house effect. Other green house gases, such as nitrous oxides, methane, chlorofloro carbon (CFC) and ozone also trap heat and thus contribute to global warming and potential climatic changes. It is estimated that different green house gases presently contribute to overall global warming roughly in the following proportions

Carbondioxide (CO_2) → (49-55)%
Chlorofloro carbons (CFCs) →(14-25)%
Methane (CH_4) →(12-18)%
Nitrous gases and other gases (13-19)%]

1.6 Smoke and haze in Dhaka's Concrete Jungle[6] :

The long queer of the victims of the pall of yellow-orange haze that blankets Dhaka city the capital of Bangladesh. Dhaka, which is both the nation's administrative

capital and business hub has a total estimated population of more than nine million, which is an unbearable burden for the city's limited facilities. The population is expected to swell to 16 million by the year 2015, making Dhaka the seventh most populous city in the world.

Now a days on any working days, a filthy grey haze, composed of vehicular emissions and dangerous chemicals form the smoke of factories, blankets, the city. The smog causes the eyes to water, and coats the lungs with layers of microscopic, noxious soot, which is composed of dangerous particulate matter.

Emissions from all types of motor vehicles like cars, jeeps, buses, trucks, mini-buses, two stroke engine-powered vehicles (auto rickshaws, tempos, mini-trucks) and motor cycles have been unabatedly polluting the city's air, aircraft, railway engines, industrial plants, power plants, brick fields, open incineration, solid waste disposal sites and dust particles are also contributing to the air pollution.

The principal pollutants from gasoline-powered internal combustion engines are carbon monoxide (CO), hydrocarbons (CH), nitrogen-oxides (NO_x), sulphur dioxide (SO_2), particles of lead (Pb) compound and unburned carbon particles (soot). Emissions form diesel engines are smoke, carbon monoxide, unburned carbon, nitrogen oxides and sulphur dioxide.

Transportation accounts for more than 46% of the total pollutants produced per year and hence remains the principal source of air pollution.

Table : 4 Amount of Primary pollutants (million tones/ year) from different sources[7].

Pollutant sources	Weight of pollutant produced						Total weight of pollutant produced by each sources
	CO	NOx	CH	SOx	part		
					≥20μ≥3μ		
Transportation	69.7	10.1	10.8	0.8	1.2	1.0	93.6
Fuel combustion (stationary sources)	1.2	11.8	1.4	21.9	4.6	1.3	42.2
Industrial processes	7.8	0.7	9.4	4.1	6.3	2.7	31.0
Solid waste disposal	7.8	0.6	1.6	0.1	1.1	-	11.2
Miscellaneous	8.5	0.4	6.3	0.1	1.3	-	16.2
Total of each pollutant produced	95.0	23.6	29.5	27.0	19.5	-	194.6

The Department of Environment (DOE) has procured sophisticated equipment to detect air polluters in the city, particularly the auto mobiles that belch out black smoke. DOE has also set up modern laboratories to determine the nature and quantum of air pollution. But the fact is that the pollution level of air in Dhaka city has reached such a peak that no equipment is necessary to detect it. Any body whose sense organs are working normally will, after a few breaths, realize how polluted the air is!

According to an assessment made by the DOE, 80% of the 2,00,000 automobiles that ply on Dhaka streets are faulty and emit black smoke in excess of the prescribed limit.

With growing urbanization and influence. the number of vehicles is also rising rapidly, causing more and air pollution. Narrow roads, traffic congestion, poor quality of fuel and improper traffic congestion, poor quality of fuel and improper traffic management are further aggravating the air pollution problem.

1.7 Number of vehicles in the Dhaka city[8] :

The Department of environment (DoE) and other concerned agencies and organizations have identified the two stroke engines used in auto rickshaws, tempos, mini-trucks and motor cycles and leaded petrol as the main culprits responsible for polluting Dhaka's air. Besides many dilapidated vehicles including 40-years old truck, are still plying in the city stoats emitting toxic gases at a dangerous rate.

Among the polluting vehicles, the two stroke engine-powered auto-rickshaws have been identified as the worst polluters. At present there are about 35000 baby-taxies in Dhaka city, according to a Bangladesh Road Transport Authority (BATA) Source. The official revealed that the two stroke engine of a baby taxi emits 13 times more smoke than a 4 stoke engine of the same size. The two stroke petrol engines are less fuel-efficient and release about 30-100 times more unburned hydrocarbons and more carbon monoxides than 4 stroke or diesel engines.

According to available government statistics, the numerical growth of auto rickshaws and tempos in Dhaka city in 1999 was 29% cars, jeeps 20%, minibus 6%, and buses only 0.6%. It is estimated that the current demand for buses in Dhaka city is more

than 5,000 against an existing fleet of 1,500. Then again only1300 of the existing number actually ply in the streets of which 1,100 are old and dilapidated. There are fewer than 200 buses of improved quality.

1.8 Slow-Poisoning of Dhaka city dwellers[8] :

The citizens of metropolitan Dhaka are being slow-poisoned by air pollution. But the city dwellers are not aware that they are being slowly poisoned by lead particles dispersed in petrol which is used as fuel. The fuel when burnt, release invisible lead particles in to the air.

According to estimate made by BAEC (Bangladesh atomic energy commission) 50 tones of lead are emitted into Dhaka city's air annually and emission reaches its height level in the dry season from Nov-January. Earlier reports from scientific say the density of lead in the air of Dhaka city in the dry season reaches 463 nanograms /m^3 (one nanogram is one billionth of a gram) the height in the world.

The lead concentration in the polluted air at mexico city is 383 nanograms and in Mumbi, India it is 360 nanograms per cubic meter.

According to another survey, the concentration of lead in the blood of most people in Dhaka city is higher than the tolerable limit of eight parts per million (PPM). lead concentration in the blood of automobile drivers and office-goers was found to be as high as 120 PPM!

1.9 Areas Worst Affected[8]:

The worst affected areas in Dhaka city include Hatkhola, Manik Mian Avenue, Tejgaon, Farmgate, Motijheel, Lalmatia, Shahabagh, and the interdistrict bus terminals survey conducted between December 1999 and June 2000 showed that the concentration of suspended particles at Farmgate goes up to as high as 2,465 micrograms/m3 as against the allowable limit to 400 micrograms/m3. The concentration of So_2 and No_2 at the spot however was still bellow the permissible limit of 100 micrograms per cubic meter.

Table-5 (a): Amount of air pollutants in the Farm gate area from December to January (1999-2000)[9]

Month	Amount of pollutants of air μg/m^3		
	SPM	SO_2	No_x
December	1797.80	71.53	24.90
January	1849.36	73.24	25.30
March	1773.70	73.10	30.00
April	887.60	65.13	27.00
May	508.40	60.09	32.31
June	665.00	51.22	25.55

Table - 5 (b) : Amount of Air pollutant lead (pb) particle in the air of Tejgaon & Farmgate area in (1999-2000)[9]

Area	Month	Amount of pb particle ngm/m^3
Tejgaon	November	30.02
	February	75.73
	March	61.18
Farmagate	November	175.43
	February	251.84
	March	122.78

Table -6 (a): Sulphur and lead content of different fuels available in Bangladesh are shown[9].

No	Different fuels	Amount of sulphur (%)
1.	Motor-gasoline premium	max 0.1 mass %
2.	Motor-gasoline regular	max 0.1 mass %
3.	High speed diesel (HSD)	max 0.1 mass %
4.	Low sulphur HSD	max 0.5 mass %
5.	Light diesel oil	max 1.8 mass %
6.	Flight sulphur furnace oil	max 3.5 mass %
7.	Kerosene	max 1.0 mass %

Table - 6 (b): Amount of lead (pb) in different fuels in Bangladesh[9].

No	Different fuels	Amount of pb gm/L
I.	Motor-gasoline premium	max 0.84 gm/L
2.	Motor-gasoline regular	max 0.5 gm/L

1.10 Effects of air pollution on human health[11] :

Air pollution adversely affects the respiratory tract and causes –

(I) Eye Irritation

(II) Nose and throat irritation

(III) Irritation of the respiratory tract.

(IV) Gases like H_2S, NH_3 and mercaptans cause ordure nuisance even at low concentrations.

(V) Increase in mortality rate and morbidity rate.

(VI) A variety of particulate particularly pollens, initiate asthmatic attacks.

(VII) Chronic pulmonary diseases like bronchitis and asthma, are aggravated by a high concentration of So_2, No_2 particulate matter and photochemical smog.

(VIII) CO Combines with the Hemoglobin in the blood and causes cardiovascular and pulmonary diseases.

(IX) HF causes diseases of the bone (fluorosis) and mottling of teeth.

(X) Carcinogenic agents cause cancer.

(XI) dust particles respiratory diseases like silicosis, asbestosis, etc.

(XII) Certain metals such as pb may enter the blood and cause poisoning.

1.11 Mechanism of action of air pollutants :

The effects of air pollution of human health generally occur as a result of contact between the pollutants and the body, Normally, bodily contact occurs at the surfaces of the skin and exposed membranes. Contact with exposed membranous surfaces is of utmost importance because of their high absorptive capacity compared to that of the

skin. Air borne gases, vapours, fumes, mist and dust may cause irritation of the membranes of the eyes, nose, throat.,larynx, tracheo-bronchial free and lungs, some irritants even reach the mucosa of the digestive tract.

1.12 Effects of radioactive fallout[11]:

The biological effects of radiation may be somatic or genetic damage. In somatic damage, the exposed individual is affected, while in genetic damage the future generations become the victims.

Radioactive fallout from testing of nuclear weapons causes:

(I) Cancer.

(II) Shortening of life span.

(III) Genetic effects of mutation.

One significant point we have to note about the effect of radio active fallout is, it causes long range effects affecting the future of man and hence the future of out civilization.

1.13 Specific pollutants emitted vehicles that affect on human health[12]:

1.13.1 Sulphur dioxide (SO_2):

SO_2 is an irritant gas which affects the mucous membranes when inhaled. Under certain conditions, some of the air-borne sulphur dioxide gas is oxidized to sulphur trioxide.

$$2SO_2+O_2 \rightarrow 2SO_3$$

Each of these two gases, in the presence of water vapour or water form sulphurous or sulfuric acid respectively. SO_3 is very strong irritant, much stronger than SO_2 causing severe bronchospasm at relatively low levels of concentration.

$$SO_2+H_2O \rightarrow H_2SO_3$$

$$SO_3+H_2O \rightarrow H_2SO_4$$

1.13.2 Carbon monoxide (CO):

Carbon monoxide has a strong affinity for combining with the hemoglobin of the blood to form carboxy-hemoglobin (COHb).

$$CO + Hb \rightarrow COHb.$$

This reduces the ability of the hemoglobin to carry O_2 to the body tissues. CO has about two hundred times the affinity of O_2 for attaching itself to the hemoglobin. So that low levels co can still result in high levels of COHb. Carbonmonoxide also affects the central nervous system. It is also responsible for heart attacks and a high mortality rate.

1.13.3 Oxides of nitrogen (Nox):

Both oxides of nitrogen, No and NO_2 are potential health hazards. Animal mortality studies indicate that NO_2 is about 4 times more toxic than NO. No cases of human death from NO poisoning have been reported, at the concentrations found in the atmosphere. NO is not an irritant and is not considered a heath-hazard. The greatest toxic potential of NO is its ability to undergo oxidation to the more toxic NO_2.

The proven effects of NO_2 on humans and animals are confined almost entirely to the respiratory tract and occur only with NO_2 levels higher than those now prevailing in the atmosphere. A higher NO_2 dose results in the following effect sequence, nasal irritation, breathing discomfort, acute respiratory distress, pulmonary edema, and finally death.

Even the mildest effects such as mucous membrane irritation, do not occur at the prevailing atmosphere NO_2 concentrations. The odor threshold range for NO_2 in human is reported to be (1-3) ppm. Concentration above 12 ppm resulted in eye and nasal irritation in a small number of volunteers. In these cases, the rascal irritation was more inters than that of the eyes. Concentrations of NO_2 greater than 100 ppm are lethal to most animal species and 90 percent of the resulting deaths are due to pulmonary edema.

1.13.4 Hydrogen sulphide and Mercaptans:

Hydrogen sulphide is a foul smelling gas. It is well known for its rotten egg like odour. Other sulphur compounds that are of interest in air pollution mainly because of their strong odorous are methyl mercaptan (CH_3SH) and ethyl mercaptan (C_2H_5SH).

1.13.5 Ozone (O_3):

Ozone is a gas that has an irritant action in the respiratory tract, reaching much deeper into the lungs that the oxides of sulphur.

1.14 Particulates[13] :

Particulate pollutants enter the human body almost exclusively through the respiratory system. Particulate matter that enters and remains in the lungs can exert a toxic effect in the different ways:

(I) Particulate that are themselves inert may interfere with clearance mechanisms in the respiratory tracts and prevent or slow the removal of other harmful particulate.

(II) Particulate may carry absorbed or adsorbed gas molecules and thus enable than to reach and remain in the sensitive areas of the lungs.

(III) Particulate may be intrinsically toxic and as a result, directly affect the body. such particulates are rarely found in the air in high concentration. However, many toxic materials are present in trace amounts. Much of the concern about these substances is related to the possibility of their concentrations increasing beyond current levels.

1.14.1 Fluorides :

Fluorides present in air range from those which are extremly irritant and corrosive like hydrogen fluoride to relatively non-reactive compounds, But fluorine is a cumulative poison even under condition of prolonged exposure and in sub-acute concentrations.

1.14.2 Lead

The main source of lead in urban atmospheres is the automobile. It creats urban concentration of inorganic lead of about 1-3 $\mu g/m^3$, with high values in areas of heavy traffic. Inorganic lead acts as an agent which causes a variety of human health disorders. The effects include gastro-intestinal damage, liver and kidney damage, abnormalities in fertility and pregnancy, and mental development of children gets affected.

1.15 Carcinogenic agents,

Carcinogenic agents are responsible for cancer. For example, the poly-cyclic organic compounds, 3-4 benzpyrene. The origin of these compounds is in the incomplete combustion of hydrocarbons and other carbonaceous materials. They are also reported to be present in exhaust discharges from I.C engines. In addition to poly-cyclic organic hydrocarbons are also carcinogenic.

1.16 Insecticides :

Insecticides are not only harmful for insects but also poisonous for man, eg DDT (P.P dichlorodiphenyl trichloro ethane).They can affect the central nervous system and may attack other vital organs.

1.17 Radio active Isotopes:

The important radio active isotopes that may reach ambient air are iodine 131, phosphorus-32, cobalt-60, Radium 226, carbon-14, sulpher-35, calcium-45 and uranium. The major sources of radio active air pollutants are.

(I) Number reactors
(II) Experimental accelerators
(III) Scientific and medical used of radio active isotopes.
(IV) Agricultural and industrial use of radio active isotopes as tracers, and
(V) Testing of nuclear bombs in the atmosphere.

The serious health effects are anaemia, leukaemia and carcer. Radioactive isotopes also cause genetic defects and sterility, as well as embryo detects and congenital malformations. It also shortens the life span of an individuals.

1.18 Dopamine-β-Hydroxylase (DBH)[14]

Dopamine--β-Hydroxylase (3,4- Dihydroxyphenylethylamine, ascorbate: Oxygen oxidoreductase (β-hydroxylating), [EC 1.14.2.1] Trivial name: Dopamine-β-hydroxylase, Dopamine oxidase). Dopamine--β-hydroxylase, which catalyzes the conversion of dopamine to norepinephrine, is an ascorbate requiring copper-containing oxygenase.[15] Enzyme activity is stimulated by the addition of dicarboxylic acids, such as fumaric acid (Fig.1.)

Dopamine-β-hydroxylase was first isolated and characterized in 1960[16] and subsequently, purified to a single homogeneous protein.[17]

1.19 Tissue Distribution of DBH

DBH is an intraneuronal enzyme in the sympathetic nervous system and enzyme activity was found in the adrenal medulla,[16] in the brain[18] and various sympathetically innervated organs such as the heart.[19,20] It is currently believed that this enzyme is localized primarily in the membrane of the amine storage granules. In the adrenal medulla[21] and in the sympathetic nerves[22] DBH was found in the norepinephrine containing granules. About 50% of the activity in the catecholamine-containing granules were easily solubilized either by freezing-thawing or by sonication.[23] The rest of the enzyme activity as firmly attached to the granules, and the use of a detergent, cutscum, was necessary to solubilize the enzyme.

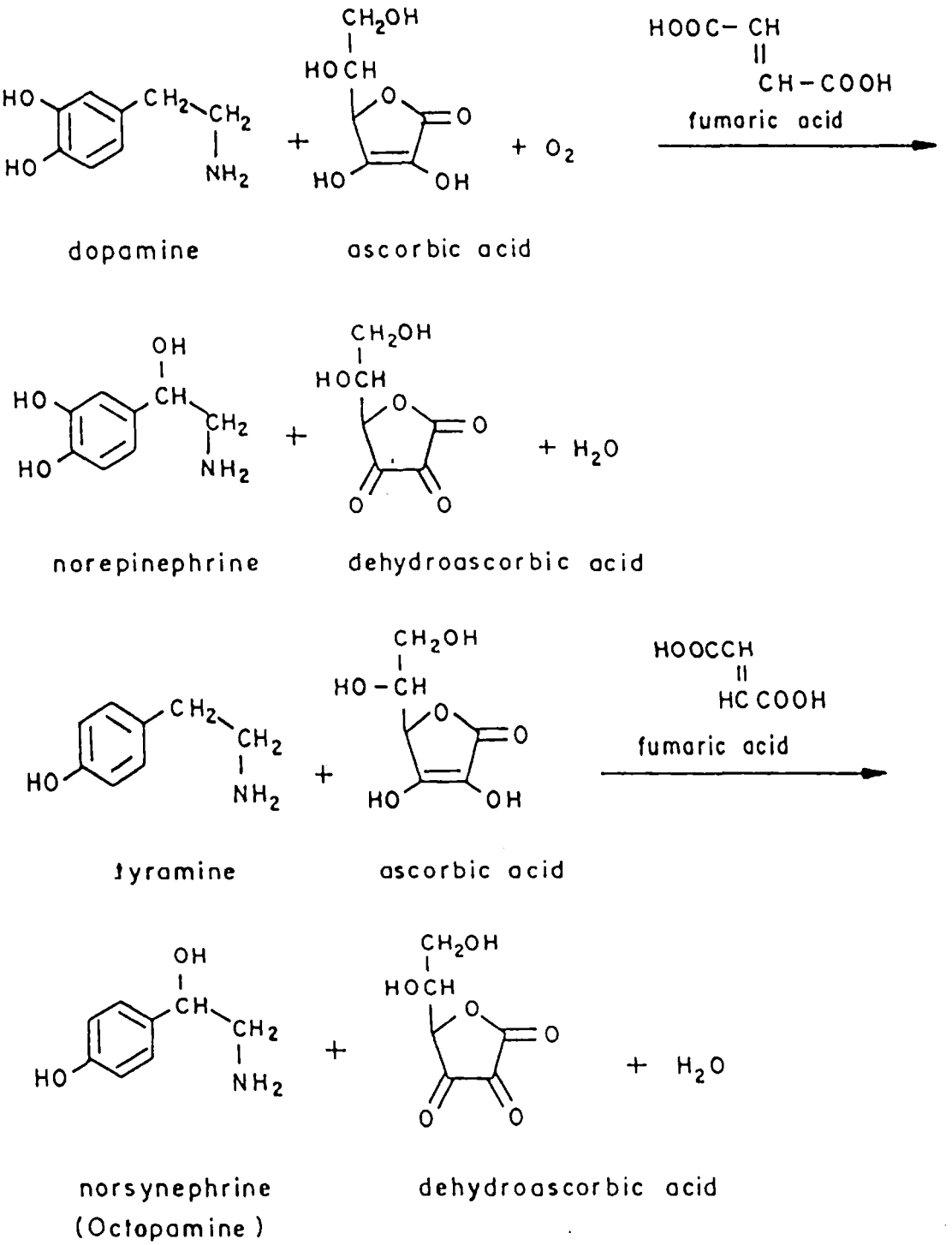


Figure : 1 Reaction of DBH [24]

1.20 Properties of DBH

1.20.1 Molecular weight

The enzyme purified from the bovine adrenal glands by the method of Friedman & Kaufman (1965) was a homogenous protein as determined by electrohoresis on starch gel (pH 6.5 and 8.9) and by disk electrophoresis (pH 9.5). The enzyme had a $S_{20,w}$ of 8.93 and the molecular weight, determined by equilibrium-ultra centrifugal analysis was 2.9×10^5. From both these values, it was calculated that the enzyme has fractional ratio of about 2, which indicates that it is a relatively asymmetrical molecule.

1.20.2 Absorbance

The maximum absorbance is at 330 nm and no absorbance existed in the visible region.

1.20.3 Optimum pH

The optimum pH for conversion of tyramine to norsynephrine was 5.5 and that for conversion of dopamine to norepinephrine was 6.2.[25]

1.20.4 Copper present in the Enzyme

Although the amount of copper in the enzyme varied with the preparation and with the length of dialysis against copper free buffer 16-18 hr), the purest enzyme contained 0.65 to 1μg of copper per mg of protein (4-7 μmoles per mole). DBH as a cu^{2+} containing protein, with about two moles of cupric ion/mole of enzyme.[26] When a concentrated solution of the enzyme was made 1mM with respect to diethyldithiocarbamate, a yellow color developed rapidly. The spectrum of this complex in the visible region was essentially identical to that of the copper diethyldithiocarbamate complex. 98% of the copper in the enzyme was removed by treatment with cyanide and ammonium sulphate. Only the addition of cupric ions to the cyanide-treated enzyme restored enzyme activity. As additional evidence in support of the idea that DBH is a copper protein, it was shown that CO inhibits the enzyme and that is inhibition is not reversible in light.

1.20.5 Reaction Mechanism and Role of Ascorbic Acid

The purified enzyme has no detectable-SH groups and it could oxidize ascorbate to dehydroascorbate either anaerobically or aerobically. About I μmole of ascorbate was oxidized per μmole of enzyme. The reduced form of the enzyme is formed during the reaction of ascorbate and the enzyme. This reduced enzyme intermediate hydroxylated, aerobically, approximately an equivalent amount of substrate in the absence of a reducing agent. The hydroxylase as isolated by the purification procedure of Friedman and Kaufman (1965b),[27] is an inactive, oxidized form that requires interaction with ascorbate before it is capable of hydroxylating the substrate. It has been found that the protein-bound copper is the site of reduction and its role was studied by means of electron paramagnetic resonance techniques.[28] Most of the protein-bound Cu^{++} is reduced by treatment of the enzyme with ascorbte a large part of the Cu^{++} in the reduced enzyme is oxidized during the hydroxylation of the substrate. These results support the concept of a reduction oxidation role for the protein-bound copper. Based on this finding Friedman and Kaufman (1966)[29] presented the reaction mechanism of DBH as shown in (Fig.2). A kinetic study supported this hypothesis and the following ping- pong mechanism was proposed this[25] (Fig-3). It was proved that molecular is incorporated into a substrate β-phenylethylamine- a-c ^{14} to from β- phenylethanolamine contaning o^{18}.

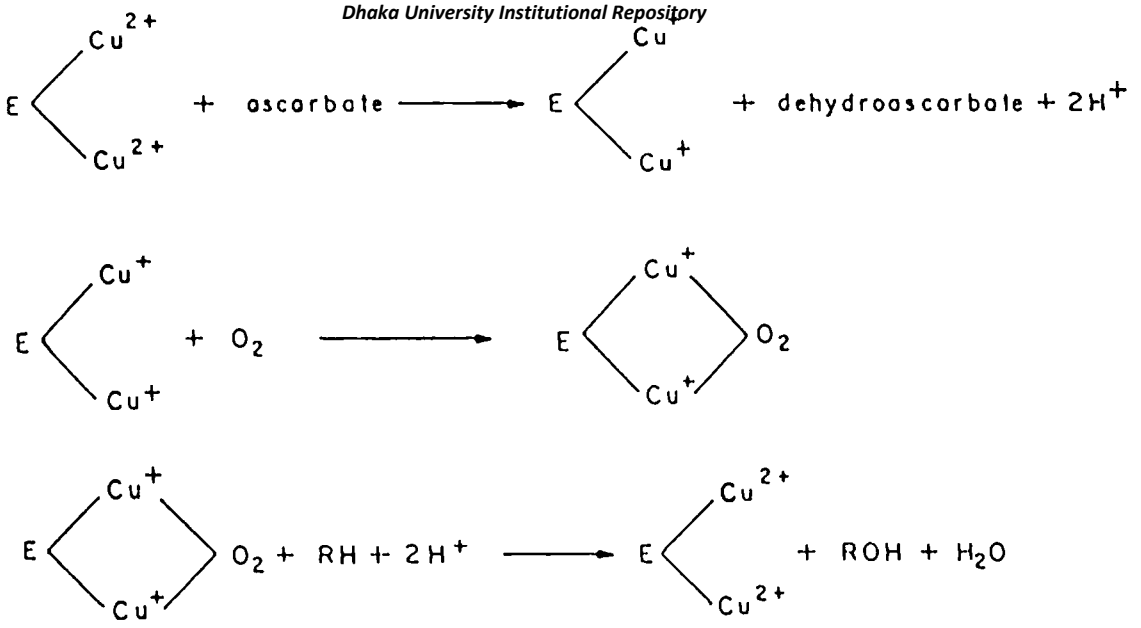
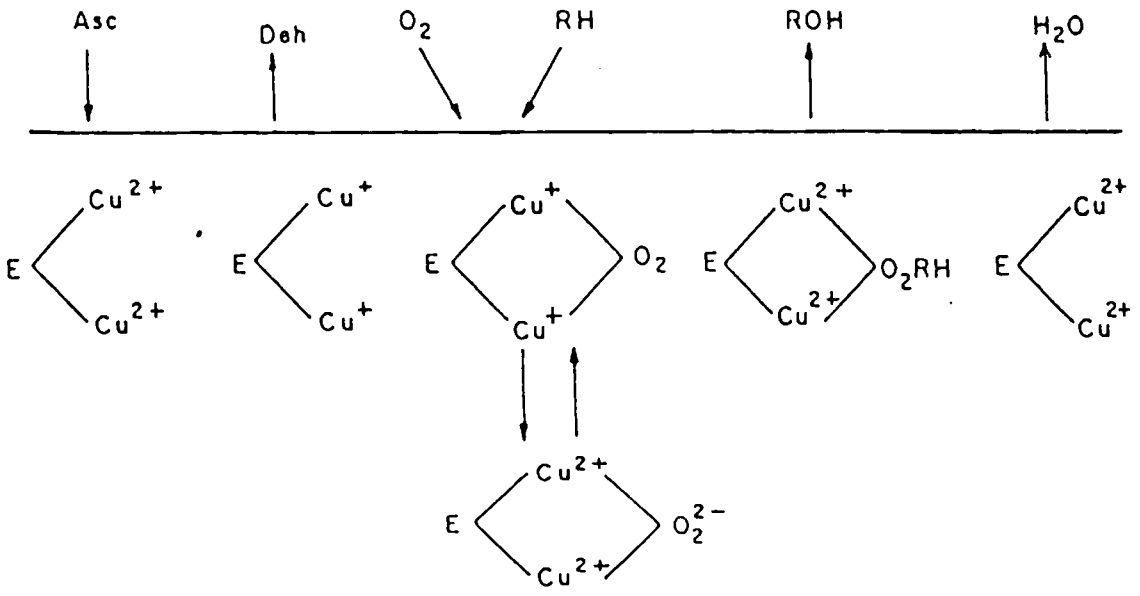


Figure 2. Reaction mechanism of DBH



RH = substrate (dopamine or tyramine)

ROH = product (norepinephrine or octopamine)

Asc = ascorbate

Deh = dehydroascorbate

Figure 3. Schematic presentation of the DBH reaction

When the reaction was carried out in a medium of H_2O with air as the gas phase, no o^{18} was found in the β-phenyleth anolamine.[6] Thus DBH was proved to be a monoxygenase.

1.20.6 *Role of Fumarate*

In the presence of fumarate, the enzyme catalyze the hydroxylation of 1060 moles of dopamine per mole of enzyme per min at 25^0C[4].

1.20.7 *K_m Values*

The K_m value based on ascorbate was 6×10^{-4} M and maximal activity was obtained at 9×10^{-3}M. The Km value based on dopamine was 6×10^{-3}M, and based on tyramine, 4×10^{4}M.[30]

1.21 *Substrates of DBH*

DBH not only effects the hydroxylation of dopamine to norepinephrine, but accepts as substrates a wide variety of phenylethylamine derivatives.[31] The substrates and the products are shown in Fig. 4, phenylethylamine, tyramine, dopamine or epinie was converted to phenylethanolamine, norsynephrine, norepinephrine, or epinephrine, respectively.[31,32]

1.22 *Reaction of DBH in the Absence of Ascorbic Acid*

In the absence of ascorbic acid, the DBH catalyzed a dopamine dependent oxidation of NADH or NADPH, as measured by a decrease in optical density at 340 nm. It was postulated that in the absence of ascorbic acid the catechol grouping of one dopamine molecule is oxidized to an orthoquinone during hydroxylation of the side chain of another dopamine molecule. Thus the catechol grouping of 1 mole of the substrate serves as electron donor, forming an orthoquinone. NADH would readily react nonenzymatically with the quinone product, regenerating the substrate dopamine.[33]

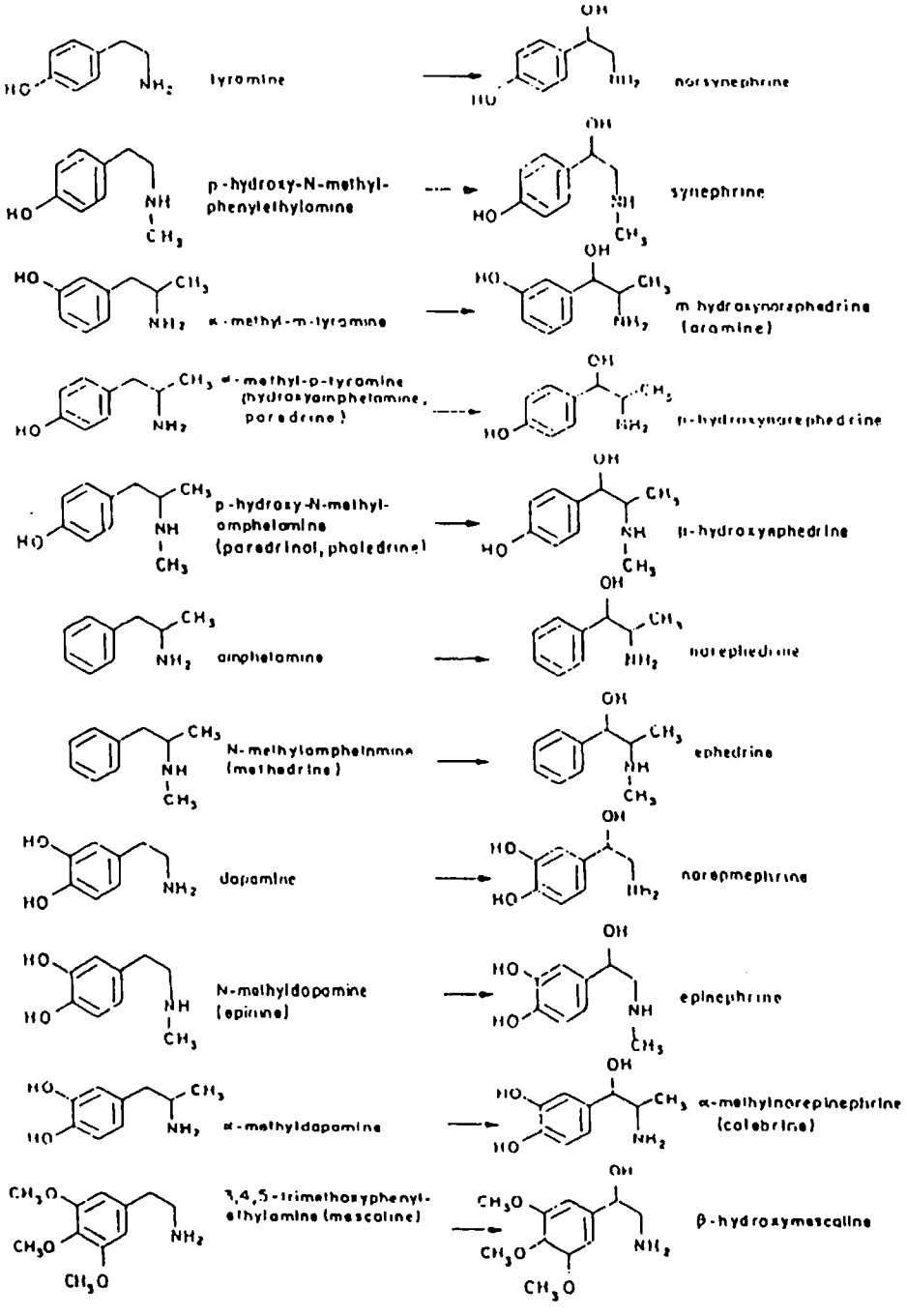


Figure 4. Substrates and products of DBH[34]

$$2\ \text{dopamine} + O_2 \xrightarrow[\text{nonezymatic}]{\text{enzymatic}} \text{norepinephrine,} + \text{"dopamine–quinone"} + H_2O$$

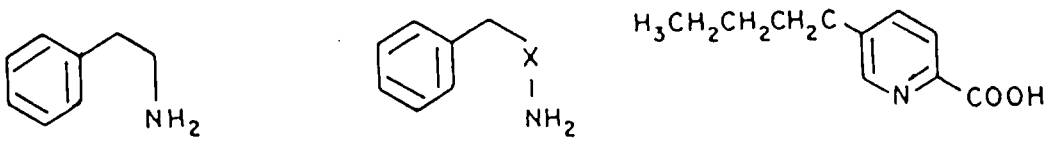
$$\text{" dopamine-quinone"} + NADH + H^+ \longrightarrow \text{dopamine} + NAD^+$$

$$\text{Sum : dopamine} + O_2 + NADH + H^+ \longrightarrow \text{norepinephrine} + NAD^+ + H_2O$$

A similar mechanism was proposed for the hydroxylation of epinine to epinephrine in the absence of ascorbic acid. However, the enzyme-catalyzed oxidatin of ascorbate was so rapid as to preclude and participation of the catechol grouping as a cofactor in side chain hydroxylation.

1.23 Inhibitors of DBH

Substrate analogues, such as benzylhydrazine and benzyloxyamine were potent inhibitors. [34,29]



Phenylethylamine

X = NH : benzylhydrazine

X = O : benzyloxyamine

Fusaric acid

Figure 5. Inhibitors of DBH

Copper-chelating agents such as diethyldithiocarbamate and alkylthio urea inhabited the enzyme in vitro and in vivo. These copper chelating agents reduced the endogenous level of tissue norepinephrine and increased that of tissue dopamine.[35-38] Tropolone and other chelating agents were also inhibitory.[39] Disulfirum via its reduced metabolic diethtydithiocarbamate was an effective inhibitor of DBH in vivo.[40,41]

Reserpine inhibited DBH by blocking the dopamine uptake mechanism in the amine storing granules where the enzyme is localized.[42-44] However, β-hydroxylation of α-methyl-m-tyramine and α-methyldopamine took place despite the blockade of the storage mechanism by reserpine.[45] Dopamine itself was shown to exhibit two properties when administered in vivo; the release of norepinephrine form sympathetic tissue and the stimulation of its synthesis.[46]

Various-SH compounds such as cysteine, glutathione and coenzyme-A, inhibited the enzyme. The inhibition was found to be due to chelating action on the protein bound copper. Cu^{2+} partly reversed the inhibition. The inhibition of the enzyme by the endogenous inhibitors in the adrenal medulla and in the brain was completely reversed by the addition of N-ethylmaleimide, which is a-SH reacting agent. N-ethylmaleimide itself did not affect the activity of the purified DBH. By the addition of N-ethylamaleimide to a crude enzyme preparation of adrenal medulla marked DBH activity was observed not only in the catecholamine-granules but also in the soluble fraction.[47] The addition of Cu^{++} or P-chloromercuribenzoate also activated the enzyme in the crude adrenal preparation.[47]

Fusaric acid (5-butylpicolinic acid) inhibited the enzyme by 50% at 10^{-7} M in vitro and showed a potent hypotensive action.[48] Picolinic acid and all 5-alkylpicolinic acids inhibited DBH reaction. The activities were dependent on the number of carbon atoms in the 5-alkyl group, and 5-pentyl compounds showed stronger inhibition then the others. By increasing the number of carbons in 5-alkyl group to four or five, the hypotensive effect became stronger, and the 5-butyl and 5-pentylpicolinic acids showed stronger hypotensive effect than the 5-propionyl, 5-ethyl, and 5-methylpicolinic acids. The inhibition of DBH by 5-alkylpicolinic acid was uncompetitive with the substrate tyramine but was competitive with the cofactor ascorbic acid. The chelation of 5-alkylpicolinic acid with copper in DBH seems to be its inhibitory mechanism.[49] Fusaric and inhibited DBH in vivo and lowered endogenous levels of norepinephrine and epinephrine in the adrenal glands, the heart, the brain and the spleen. Maximum depletion of norepinephrine and epinephrine was observed between 3 and 6 hour after the administration of fusaric acid.

1.24 Physiological aspects

Although various phenylethylamine substracts can be converted to β-hydroxyderivatives, the main DBH reaction in vivo may be the conversion of dopamine to norepinephrine. It was once suggested that the hydroxylation of dopamine is the rate limiting step in the formation of both norepinephrine and epinephrine.[50] However, the activity per gram of tissue was sufficient to lead to the formation of all the epinephrine and norepinephrine known to be present in beef adrenal medulla within 0.25 min, even assuming no loss of enzyme during the purification procedure.[16] Tyrosine hydroxylase was established as the rate-limiting step of catecholamine biosynthesis.[51] However, a potent inhibitor of DBH in vivo would be able to reduce endogenous levels of norepinephrine, though the enzyme itself could not produce the rate-limiting step.

In guinea pig adrenal DBH activity was very low at birth but reached adult values within five days after birth. The enzyme activity was unaffected by even severe ascorbic acid deficiency.[20] This eliminates the possibility of a specific role of asocorbic acid as a cofactor of DBH in vivo. Alternatively it may indicate that even a marked degree of ascorbic acid deficiency is not enough to make DBH rate-limiting in the overall conversion of tyrosine to norepineophrine. Rats that were feed a diet deficient in Cu^{2+} for 7-13 weeks showed a greatly decreased rate of conversion of parentally administered C^{14}-dopamine to cardiac C^{14}-norepinephrine as compared with control animals.[52] During the acetylcholine stimulated secretion of catecholamines in perfused isolated bovine adrenal glands, DBH was released into the perfusion fluid together with the catecholamines and a protein specifically found in the catecholamine storage vesicles (chromogranin).[53] These results support the hypothesis that during secretion from the adrenal gland, the entire soluble content of the storage vesicle discharged directly to the exterior of the cell.[54]

A specific and potent antibody to the DBH prepared from bovine adrenal medulla was produced in rabbits. the antibody markedly inhibited DBH from bovine, dog, guinea pig adrenals and human pheochromocytoma. The specificity of the antibody was demonstrated by the lack of inhibition of tyrosine hydroxylase and DOPA

decarboxylase, the other two enzyme in the pathway of catecholamine biosynthesis. The DBH in the adrenal medulla was immunologically cross-reactive with that in sympathetic nerves. Various sympathyetic nerves in the sheep, including fibers from the superior cervical and coeliac ganglia, were ligated and fluorescence antibody techniques were used to locate the enzyme. Immunosera of antigens of DBH from sheep adrenal medulla and of catecholamine-binding protein reacted strongly with the cytoplasm of cell bodies in the superior cervical and stellate ganglia. In constricated sympathetic nerves, there was a large increase in dopamine-β-hydroxylase and the catecholamine binding protein fluorescence which was localized proximal to the constriction in the region where specific catecholamine flouorescence was also observed. These results support the hypotheses that the site of synthesis of DBH is in the cell body and that it is transported to the axonal terminals by axoplasmic flow. To prove this hypothesis. The norepinephrine content and DBH activity were determined on both sides of a ligature in dog splenic nerves, and in the proximal segment of the nerve (above the ligation), there was an increase in the norepinephrine content and DBH activity.

1.25Genetics of DBH

DBH is a copper-containing glycoprotein and a 290 KDa homotetramer consisting of a 75KDa subunit with 2 of copper per subunit.

DBH is known to exist in both membrane bound and soluble forms. The residues of DBH which plays a role in anchoring of the enzyme to the membrane have not been identified; membrane attachment is speculated to be due to some post-translational modification or uncleaved N-treminal signal peptide.

T.Nagatsu in 1991[55] isolated two different cDNAs (type A&B) for human DBH and the genomic DNA, and showed that the two mRNAs were generated through alternative polyadenylation form a single gene. Type A mRNA (2.7kb) and type B mRNA (2.4 Kb) encoded the same amino acid sequence and were different only on the 3' untranslated region. Type A mRNA contained a 3'-extension of 300 bp at the end of the type B mRNA sequence. The 5'-flanking region of the gene of human

DBH contain possible transcription regulatory elements such as cyclic AMP response element (CRE), glucocorticoid response element (GRE) etc.

1.26 Neurotransmission[56]

To successfully relay a massage, a nerve impulse initiated in a neuronal cell body and conducted through its axon (up to the nerve ending) has to be transmitted to other cells in succession and finally to the target cells. This passage of impulse from cell to cell is termed neurotransmission. It form the basis of all information transfer in the body. The junction at which the cells are interconnected and through which the impulses are propagated are called synapses first named by Sherigton (1906) from the Greek word "Synapto" meaning tight clasping. Two types of synapses are found.

1.27 The electrical synapses

In which transmission takes by direct condition electricity form one cell to the next.[16]

1.28 The chemical synapses

In the case of chemical synapses nerve transmission is mediated through the action of chemical substances known as neurotransmitter. Synapses having both chemical and electrical characteristics have also been identified.

1.29 Neurotransmitters[56]

With the exception of a few cases in which neurotransmission takes place purely by electrical means the electrical synapses all other types of transmission are mediated by chemical substances the neurotransmitters (chemical synapese). Many years of research on the mechanism neurotransmission through chemical synapses has determined that chemical compounds must fulfilled the following rigid criteria to be termed as neurotransmitters.

- The substances must be stored in the nerve endings.
- Upon pre synaptic stimulation, the compounds must be released into the synaptic cleft.

- The chemicals, when applied post synoptically, must exert their effect as though the pre synaptic membrane were stimulated.
- Mechanisms must exist in the synaptic region for rapid removal of the compounds, by hydrolysis or by other means, so that the duration of their action becomes limited. substances known to prevent this removal should potentiate neurotransmitter action.
- Neurotransmissions should be blocked partially or fully by antagonists that specifically prevent, competitively or non competitively, the neurotransmitter receptor interaction.

1.30 Catecholamine

The term "Catecholamine" refers generically, to all organic compounds that contain a catechol nucleus (a benzene ring with two adjacent hydroxyl substituents) and an amine group. That is catecholamines possessing a catechol ring and a side chain of either ethylamine or ethanolamine. In practice the term catecholamine usually implies dihydroxyphenylethylamine (dopanine DA) and its metabolic products norepinephrine (NE) and epinephrine (E), are present in animals and plants.[57]

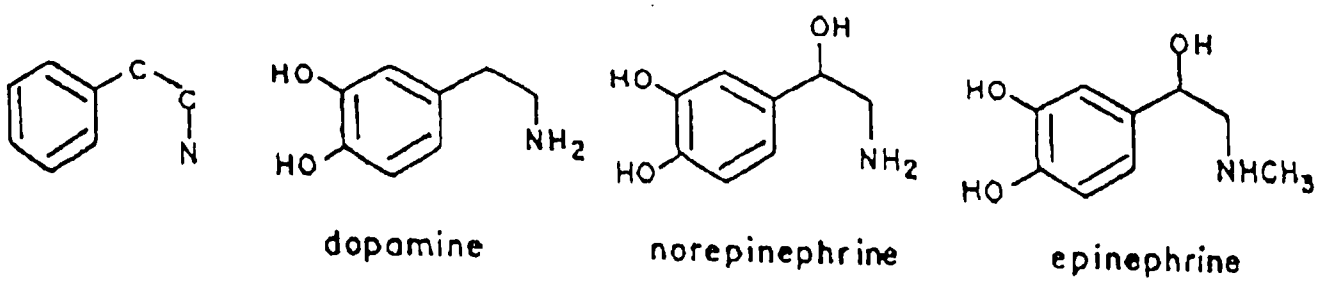


Figure 6. Structures of catecholamines

Catecholamine to be discovered as epinephrine (adrenalin) which is predominant hormone in the adrenal medulla.[58-60] Epinephrine was synthesized in 1904.[61] Simultaneously, the second catecholamine to be discovered, norepinephrine

(noradrenaline) which lacks the N-methyl group in epinephrine was also synthesized.[61] The discovery of norepinephrine in the animals, however, was not made until 40 years latter.[62] It was discovered in sympathetically innervated organs and sympathetic nerves[62] as well as Urine and the adrenal medulla.[63] The third catecholamine to be discovered, dopamine, which lacks the hydroxyl group at the β-carbon site of the norepinephrine side chain was found in animals at about the same period.[64-66] On the other hand, studies on the biosynthesis and metabolism of catecholamines and on the related enzymes began around 1930. Dopa decarboxylase which decarboxylate, DOPA to Dopamine was first discovered among the biosysthetic enzymes.[67] As a result, the following main metabolic pathway of catecholamines was proposed:Tyrosine-Dopa→ Dopamine-Norepinephrine-Epinephrine.[68] This hypothetical pathway was later proved by isotope experiments.[69] By 1964, all the enzymes of the biosynthesis of catecholamines had been discovered and the synthetic pathway and the mechanism of biosynthetic regulation were elucidated.[70,71]

1.31 Chemical Properties of Norepinephrine

CH_8NO_3 (Noradrenaline) M. Wt 169.18 Pka (25^0c) 8.82 (OH), 9.98 (NH_2). Natural (-) form had D-configuration relative to mandelic acid. Aqueous solution slowly recemize and oxidize under the influence of light and oxygen. Stable at acidic pHs, stability maximum at pH 4.0.

1.32 Tissue Distribution of Norepinephrine, the Product of DBH

In 1946 von Euler in Sweden and shortly later Holtz in Germany independently identified the presence of norepinephrine in adrenergic nerves.[72] A systemic study of extracts of various mammalian nerves by Euler and his colleagues demonstrated that there was a very close correlation between the content of norepinephrine present and the proportion of nonmyelinated to myelinated nerve fibers. Sympathetic ganglia were also found to myelinated nerve fibers. Sympathetic ganglia were also found to contain norepinephrine in a conc. Similar to that found in the postganglionic fibers. Of all the nerves studied, splenic nerve was found to have the highest content of norepinephrine.

Norepinephrine was identified by Holtz as a normal constituent of mammalian brain. In 1954 vogt demonstrated that noirepinephrine was not unformly distributed in the central nervous system. More norepinephrine is generally found in gray matter than in white matter.

1.16 Biosynthesis of Norepinephrine[58,60]

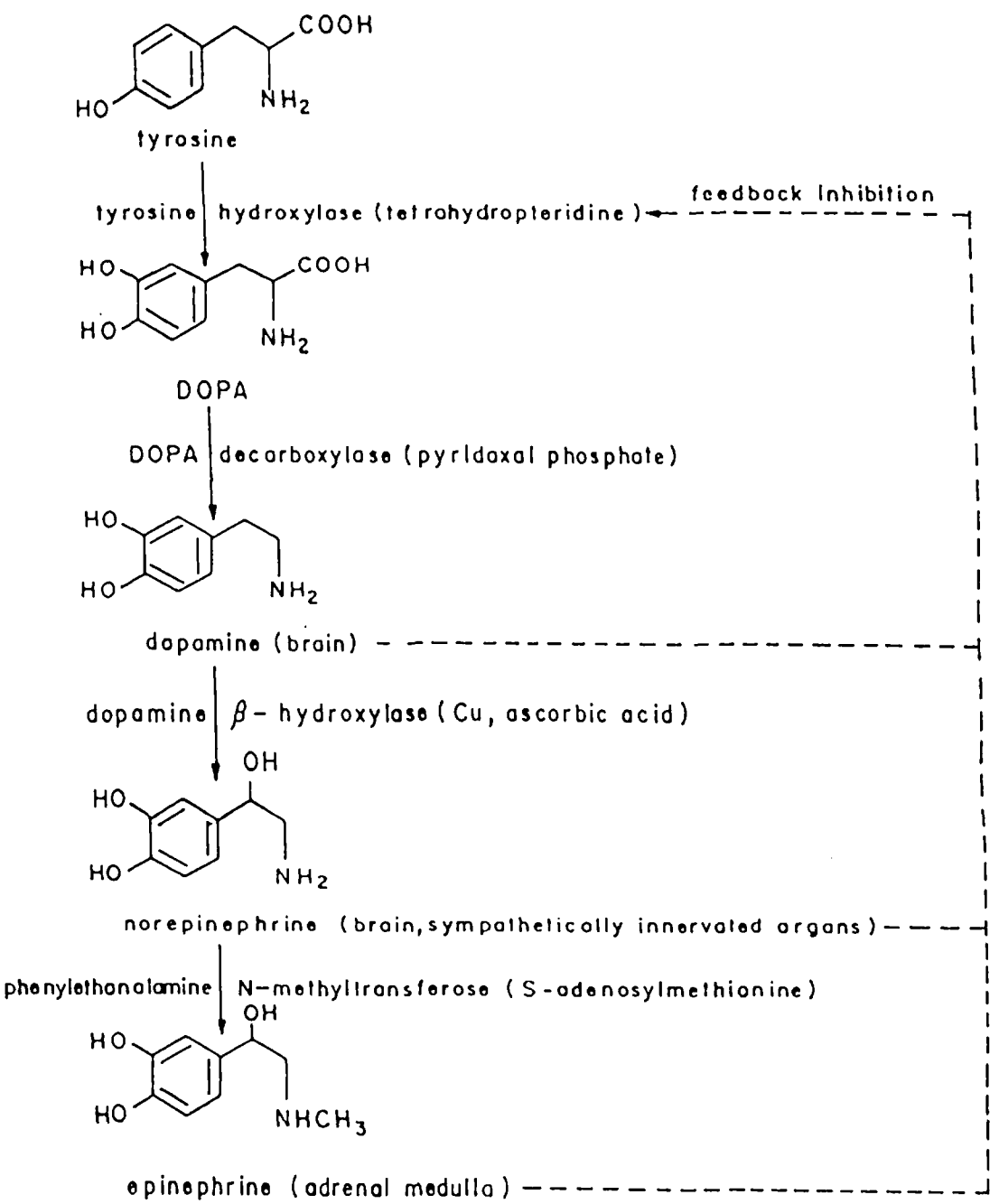


Figure 7. The main biosynthetic pathway of catecholamines and its regulation

1.33 Physiological Importance of Norepinephrine

Norepinephrine is secreted from adrenal medulla, continually in small amount. It is generally contained in sympathetic nerves discharged from the nerve endings as a chemical transmitter. Norepinephrine acts in the area in which it discharged probably by binding with a "receptor" in the target organs. Norepinephrine is therefore called a "local hormone" or a "tissue hormone". Norepinephrine and dopamine are also contained in the brain and are supposed to be chemical transmitters. Recent finding showing a high concentration of dopamine in the extrapyramidal systems of the brain strongly suggests its physiological importance in the brain. Catecholamine produce many important physiological effects in the brain, the cardiovascular system and other organs.[57] Recent studies have stressed their practical importance in nervous diseases, including parkinsonism and psychoses, such as mental depression, in heart diseases and in hypertension.

1.34 Neurotransmitter Roles of Norepinephrine

It has been accepted by most investigators that norepinephrine is in fact the sympathetic neurotransmitter in the mammalian peripheral sympathetic nervous system. At the present time it is also well accepted that norepinephrine acts as a neurotransmitter in the central nervous system although the most compelling evidence applies only the certain regions innervated by noradrenergic fibers.

In most instances norepinephrine cause inhibition, but it cause excitation in some areas.[74,75]

Many of the proposed criteria for neurotransmitter have been satisfactorily fulfilled for norepinephrine and dopamine at some specific synaptic sites in the mammalian central nervous system. Nrepinephrine released from brain slices by gross nonspecific electrical stimulation. It has also been shown that stimulation of isolated spinal cord produces a release of some norepinephrine and 5-hydroxytryptamine into the medium.

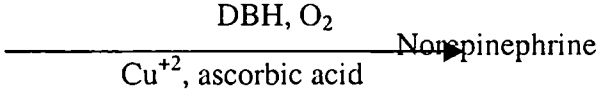
In the olfactory bulb norepinephrine may be mediator of a recurrent inhibitory synaptic pathway since (a) iontophoretically applied norepinephrine depressess spontaneous activity; (b) this effect as well as depression due to synaptic inhibition can be blocked by dibenamine, on α-adrenergic blocking agent; (c) synaptic inhibition is reduced when catecholamine are depleted by pretreating animals with response and α-methyl tyrosine; (d) norepinephrine containing nerve ending appear to be restricted to the layer in the olfactory bulb whose synaptic inhibition is most easily demonstrated.

CHAPTER-2

AIMS AND OBJECTIVES OF THIS THESIS

Aims and objectives of this thesis

Dopamine β-hydroxylase (DBH) is one of the most important neurotransmitter (Catecholamine) synthesizing enzyme. This enzyme catalyzes the reaction to produce norepinephrine (one of the most important catecholamines) in mammalian blood as well as in central and peripheral tissues. Norepinephrine, not only acts as a neurotransmitter but also as a hormone. This neurotransmitter is involved in the regulation of emotion, behaviour, sex, mood, sleep, excitation etc, of higher animals including the human beings. Catecholamine produce many physiological effects in the brain, cardiovascular system and other organs. Recent studies have stressed their practical importance in nervous diseases including parkinsonism and psychoses such as mental depression, heart diseases and in hypertension.[44]



The product of above mentioned reaction increases or decreases at different physiological condition.

There have been a lot of studies on many non neurotransmitter mediating enzyme in Bangladeshi high diabetic patients, heart disease patients, arsenic affected patients and effect of these diseases have been reported on the status of respective enzymes. But till now no study have been done on DBH activity in the serum of taxi- drivers and rickshaw-pullers were collected from the various places in the Dhaka city. Now Bangladesh is the most air pollution affected country in the world. The levels of lead (Pb) in the air of Dhaka city is 463 ngm/m^3. Carbonmonoxide (co), Hydrocarbons (CH), nitrogen dioxides (No_2), sulphurdioxides (SO_2), methane gas and highly black smoke emitted from motor vehicles like cars, jeeps, taxi, baby-taxi, buses, trucks, mini-buses etc. They have serious adverse effects on the passers-by and the drivers.

This tempted us to determine the DBH activity and its cofactors (**ascorbic acid, cu^{++}**) in the serum of three different age groups of Taxi-drivers and rickshaw-pullers in the Dhaka city. We also plan to measure the other biochemical parameters such as zinc,

total protein, albumin. liver enzymes as GOT, GPT, ALP, urea, creatinine lipid profile, serum electrolytes, vit-A level in the serum of taxi-drivers and rickshaw-pullers of Dhaka city and rural individuals.

The aim of this thesis is also to make a systematic comparative study of DBH activity and other biochemical parameters in the serum of healthy rural individuals between taxi-drivers and rickshaw-pullers in the Dhaka city.

Our present systematic studies on the levels of DBH activity, it's cofactors and other biochemical parameters might contribute to realize the future environment pollutions, treatment and control of how to prevent this pollution.

CHAPTER-3

MATERIALS AND METHODS

Materials and methods

3.1 Subjects :

Catecholamine neurotrasmitter mediating enzyme, Dopamine β- hydroxylase, its cofactors and other biochemical parameters were studied in the serum of 100 healthy rural individuals, 100 Taxi- drivers and 100 rickshaw-pullers at different age groups of 15-65 years. Among the 100 taxi-drivers, the age of 30 drivers was in the range of 15-26 years, that of 40 driver's was 27-40 years and the remaining 30 was 41-65 years. The total samples were divided into 3 Groups, namely group- 1 (15-26 years), group-2 (27-40 years) and group-3 (41-65 years). The serum samples of the rural individuals (control) were also divided in same manner.

The control samples were collected from the different villages of Manikgonj district. The villages are situated in the remote country side and there were no air polluting agents like industries, brick-fields, engine-vehicles etc.

On the other hand taxi-driver's blood samples were collected from the drivers of taxi stands in the Dhaka city. Rickshaw–puller's blood samples were also collected from different rickshaw-stands in the Dhaka city.

The taxi-drivers and rickshaw-pullers were suffering from eye irritation, irritation of the respiratory tract, nose and throat irritation. Carbonmonoxide from defective and old vehicles combines with the hemoglobin and causes cardiovascular and pulmonary disease, cancer, depression, metal poisoning etc.

3.2 Working Instruments :

i Photometer 4010 (Boehreinger Mannheim's, Mannheim, Germany).
ii Atomic adsorption Spectrophotometer (AAS, Pye-Unicam Sp9)
iii DU-70-Spectrophotometer (BACKMAN)
iv Thermostatic water bath (CHINA)
v Vacuum pump and compressor (Edwards High Vacuum Ltd.). SUSSEX

vi Hotbox oven with fan, Size-1, (GallenKamp)

vii Distilled water plant (GENRTSTO, NOTINGHAM, UK)

viii Freezer –41^{0}C (MDF-790AT, SANYOULTRALOW, JAPAN)

ix Freezer (SINGER)

x Magnetic stirrer (IKAMAG-RCT, Britain)

xi Electric Balance (Mettle, E-200, Switzerland).

xii Freeze, Dryer (WEST GERMANY)

xiii Refrigerated superspeed centrifuge ICE-B-20A.

xiv pH stick (GallenKamp)

xv Centrifuge (MSE-England).

xvi Reading Balance L-200SM (Shimadzu-JAPAN).

xvii Plastic putty (ACE Scientific supply Co. Inc.)

xviii Capillary tubes (ACE Scientific supply Co Inc.)

xix Homogenizer, Model No.4-0669011, Type NSI-12AI (Bodine Electric Company, USA).

xx Incubator Model No. W760 x-800095 (Karl Kold, West Germany)

xxi Gilford Model 240 Spectrophotometer (USA) Model 704 Hita cni dual wavelength/double beam recording spectrophotometer, USA.

xxiiMicrohematocrit centrifuge (International Model MB) (ACE Scientific Supply Co. Inc.)

xxiii Drummond Microcaps pipets (ACE Scientific Supply Co. Inc.)

3.3 *Photometric Assay of DBH Activity in Human Blood*[76]

3.3.1 Materials

a. Sigma Chemical CO. St. Louis. No. 63178.
(i) Catalase (Crystal suspension), (ii) N-ethylmaleimide, (iii) Octopamine, (iv) Ascorbic acid, (v) Sodium meta periodate, (vi) Sodium thiosulfate.

b. Calbiochem, san Diego, Calif, 92112.
(i) Tyramine hydrochloride, (ii) Disodium fumarate, (iii) Mono sodium fumarate.

c. E. Merck, West Germany

(i) Dipotassium hydrogen phosphate, (ii) Potassium dihydrogen phosphate, (iii) Glacial acetic acid, (iv) Copper sulphate, (v) Trichloro acetic acid, (vi) Sodium hydroxide.

d. J.T. Baker Chemical Co. U.S.A.
   (i) Sodium acetate, (ii) Ammonium hydroxide.

e. Abbott Laboratories Co. North Chicago, III, 60064.
   (i) Pargyline-hydrochloride.

f. British drug house, U.K.
   (i) Hydrochloric acid.

g. Dow Chemical Co.
   (i) Dowex-50 w-x4 (H+, 200-400 mesh).

3.3.2 Method

3.3.2.1 Preparation of Reagents

(a) Preparation of 1M sodium acetate buffer: Stock solution of IM sodium acetate ($CH_3COONa.\ 3H_2O$) and IM acetic acid (CH_3COOH) were prepared by using distilled water. From this stock solutions. 1M acetate buffer, pH 5.0 was prepared according to the procedure of Sorensen (1909). A buffer preparation was preserved 4^0C for use in all the experiments of this work.

(b) Preparation of 0.2M N-ethyl maleimide solution : 0.1M N-ethyl maleimide (M.W.125.1) solution was prepared by dissolving 0.5004g of N-ethylmalemide in 20 ml 0.01N HCL.

(c) Preparation of 0.2M sodium fumarate solution : 0.2M sodium fumarate (M.W. 138.06) solution was prepared by dissolving 0.13806 g of sodium fumarate in 5 ml of distilled H_2O.

(d) Preparation of 0.2M ascorbic acid solution : 0.2M ascorbic acid (M.W. 176.13) solution was prepared by dissolving 0.3522g of ascorbic acid in 10ml of H_2O.

(e) Preparation of 20mM-pargyline hydrochloride solution : 20mM pargyline hydrochloride (M.W. 195.68) solution was prepared by dissolving 0.0078272 g of pargyline hydrochloride in 2ml distilled water.

(f) Preparation of 1,500 Units of catalase in 30 µl of aqueous solution (lmg/ml): This solution was prepared by dissolving 0.2g of catalase in 10ml of distilled H_2O.

(g) Preparation of 0.4M tyramine solution : 0.4M tyramine (M.W. 173.5) solution was prepared by dissolving 0.0694 g of tyramine in l ml of H_2O.

(h) Preparation of 2nM octopamine solution : 2nM-octopamine (M.W. 189.6) solution was prepared by dissolving 0.001896 g of octopamine 10ml of distilled water.

(i) Preparation of 3M trichloroacetic acid (TCA) solution: Trichloroacetic acid (M.W. 169.39) solution was prepared by dissolving 49.017 g of TCA in 100ml distilled water.

(j) Preparation of 2% $NaIO_4$ Solution : 2% $NaIO_4$ solution was prepared by dissolving 0.4g of $NaIO_4$ in 20ml distilled water.

(k) Preparation of 10% $Na_2S_2O_5$ Solution: 10% $Na_2S_2O_5$ solution was prepared by dissolving lg of $Na_2S_2O_5$ in 10ml of distilled water.

(i) Preparation of 4 M NH_4OH Solution : The original NH_4OH solution is 8.285M. The 4M NH_4OH solution was prepared by diluting 2.07 times of original NH_4OH solution.

(m) Preparation of 20 µM $CuSO_4$ solution : 20 µM copper sulfate solution was prepared by dissolving 0.00124 g of $CuSO_4$ in 5 ml dist. H_2O.

(n) Preparation of 1N HCl : The original concentrated HCl is 11.92. IN HCl was prepared by diluting 11.92 times of original solution.

(o) Preparation of lM NaOH : 4 g of NaOH was measured and placed in a measuring cylinder. The total volume was made 100ml by adding distilled water.

3.3.2.2 Preparation of Enzyme

(i) Human serum DBH collection : Human serum DBH was obtained either by venipuncture or by capillary tubes form fingertip blood. Samples obtained by venipucture about 10:00 hours were placed on ice, then centrifuged at 10,000g for 10min and the serum removed. Collected serum was used as enzyme. The enzyme activity in human serum is fairly stable and specimens can be stored either in the refrigerator or deep freeze for considerable periods of time.

3.3.2.3 *Activation. of Dowex-50W-x4, (H^+, 200-400 mesh)*

The steps of Dowex-50 W-x4 activation are as follows:

i. Weight Dowex-50 (H+, 200-400 mesh) 20g.
ii. Wash with distilled water three times.
iii. Wash with 400ml 1N HCl for 30 min.
iv. Wash with distilled water three times.
v. Wash with 1M NaOH 400 ml.
vi. Wash with distilled water with stirring for 20 minutes each, three times
vii. Wash with in HCl 400 ml.
viii. Wash with distilled water.
ix. Wash with 1M NaOH 400ml.
x. Wash with distilled water.
xi. Wash with in HCl 400ml.
xii. Wash with distilled water to pH 3-4.
xiii. Suspended in 1M Na-acetate buffer pH 5.0 and adjusted to pH 5.0 with 1M Na-acetate solution.
xiv. Re-suspended in 1M Na-acetate buffer pH 5.0 and adjested to pH with 1M sodium acetate solution.
xv. Resuspended by 2 volume of Na-acetate buffer (p^H5.0).
xvi. Activated Dowex-50 was preserved at 4^0C for use in all the experiments.

3.3.2.4 Procedure

The standard assay system was prepared as follows: The standard incubation mixture (total volume 1.0 ml) contained -50µl of human serum or plasma, as enzyme; & H_2O

to dilute to 400µl; sodium acetate buffer (1 mol/liter, pH 5.0), 200µl; sodium fumarate (0.2 mol/liter), 50µl; copper sulphate (20 µM), 50 µl; ascorbic acid (0.2 mol/liter, freshly prepared) 50µl; catalase (img/ml), 50µl (1500U); tyramine (0.4 mol/liter), 50µl; paragyline (20 nmol/liter), 50µl; & N-ethylmaleimide (0.2 mol/liter) 100µl. The various regents used to make up the final reaction mixture can be premixed into one regent (600 µl total volume) & added to the enzyme (diluted serum). A sample of boiled enzyme preparation was used as blank. A tube of the enzyme (diluted serum) was placed in a boiling water bath so that the average temperature was 95^0C for 5 mins. 20µl of an internal standard solution containing 2.00 nanomoles of octopamine were added to another blank incubation mixture. The reaction mixture was incubated at 37^0 for 60 mins in a water bath with continual shaking, the action contents were exposed to air. The incubation was stopped by adding 0.2 ml of 3M TCA, and the mixture was centrifuged at 2000 rpm for 10 mins. The supernatant fluid was transferred to a small column of Dowex-50 (H+, 200-400 mesh) (packed voluime 0.2ml) that had been prepared in a disposable Pasteur pipette (o.5cm×10cm). The tube precipitate were washed with 1ml of water & the washing were also transferred to the column. The column was washed two more times with 2ml of water and then the adsorbed amines were eluted with 1.0 ml of 4 molar NH_4OH. Octopamine in the elute was converted to p-hydroxybenzaldehyde by adding 0.10 ml of $NaIO_4$ solution (20 g/liter). Excess periodate was then reduced by adding 0.10 ml of $Na_2S_2O_5$ solution (100 g/liter). Absorbance was measured against at 330 nm in a microcuvette with a 1-cm light path by use of spectronic-21 spectrophotometer.

3.3.2.5 Calculation

$$\text{Octopamine formed (nanomoles/min/ml)} = \frac{\text{Absorbance of experimental} - \text{Absorbance of blank.}}{\text{Absprbance of standard-Absorbance of blank.}} \times 2.00$$

3.4 Estimation of Ascorbic Acid

Di-nitrophenylydrazine method with modification by Lowry *et al.* 1945.[77]

3.4.1 Materials

a. E. Merck, West Germany:
   (i) Trichloroacetic acid
   (ii) Sulphuric acid
   (iii) Copper sulphate
   (iv) Glacial acetic acid

b. Sigma Chemical Co. St. Louis
   (I) Thiourea
   (II) Ascorbic acid
   (III) Meta phosphoric acid

c. British drug house, U.K.
   (i) 2,4-dinitrophenylhydrazine

3.4.2 Method

3.4.2.1 Preparation of Reagents

(a) Trichloroacetic acid (TCA), 5.0% solution : TCA (5.0g) was dissolved in a small amount of water and the final volume was made (100.0 ml) with distilled water. The solution was kept in a refrigerator.

(b) Sulphuric acid solution, 65%: Distilled water (30.0 ml) was taken in a beaker and placed in ice cool water bath. Concentrated sulphuric acid (70.0 ml) was added drop-wise with stirring.

(c) Thiourea solution, 5.0%: Thiourea (5.0g) was dissolved in a small amount of distilled water in a (100 ml) volumetric flask. Distilled water was added to make up the volume. The solution was stored in a refrigerator.

(d) Copper sulphate solution, 0.6% : Copper sulphate (0.6g) was taken in a (100.0 ml) volumetric flask and dissolved in distilled water and the volume was made

(e) Acetic acid solution, 10.0% : Acetic acid (20.0 ml) was taken in a glass bottle and then (180.0 ml) distilled water was added

(f) Metaphosphoric acid 5.0%, in 10.0% acetic acid solution : Anhydrous meta phosphoric acid (5.0g) was dissolved in 10.0% acetic acid solution using a magnetic stirrer and was made 100.0 ml with 10% acetic acid solution.

(g) Sulphuric acid, 9N : Distilled water (75.0 ml) was taken in a beaker, placed in an ice cool water bath and concentrated sulphuric acid (250 ml) was added drop-wise.

(h) 2.4-Dinitrophenylhydrazine solution : Dinitrophenylhydrazine (2.2g) was dissolved in (100.0 ml) 9N shlphuric acid.

(i) Dinitrophenylhydrazine-Thiourea-Copper sulphate (DTC) Reagent : The reagent was prepared by mixing one volume 5.0% thiourea, one volume 0.6% copper sulphate solution and 20 volumes 2.2% 2,4-dinitrophenylhydrazine. The reagent was stored in a refrigerator and filtered before use.

(j) Stock Standard, 1.0 mg/ml : Ascorbic acid (500.0 mg) was taken in a crucible and heated in an oven at 105^0C for 1 h and kept overnight in a dissector. The preheated ascorbic acid (100.0 mg) was dissolved in 10% acetic acid containing 5.0% metaphosphoric acid and the volume (100.0 ml) was made up.

(k) Working Standard : The stock ascorbic acid soluton was diluted 100 fold with 5.0% TCA to obtain a solution containing 1.0 mg/ml.

3.4.2.2 Procedure

An aliquot of 0.2 ml serum was taken in a 5.0 ml test tube, 0.8 ml 5.0% TCA was added and mixed immediately using a vortex mixer. The mixer was then centrifuged at 3000 r.p.m for 10 min. An aliquot of 0.6 ml supernatantwas taken and 0.25 ml of DTC solution was added. The tube was covered with parafilm and then incubated at 60^0C for 1 hour in a water bath. Immediately after the incubation, the test tube was chilled in an ice cool water bath. One millilitre 65% sulphuric acid was then added drop-wise and mixed well. Incubated at roon temperature (25^0C) for 30 min. Working standard solution, 0.2 ml was taken and treated as serum sample. The blank was

prepared by taking 0.6 ml 5.0% TCA and 0.25ml DTC solution and treated in the same procedure as serum. The absorbance was read at 520 nm against the blank on a spectronic-20 spectrophotometer.

3.4.3 Calculation

Milligram of ascorbic acid per 100 ml of serum

$$= \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 0.002 \times \frac{100}{0.12}$$

3.5 Procedure for Determination of Metals (Cu^{++} & Zn^{++}) from Human serum by Atomic Absorption Spectrophotometer

The Cu^{++} & Zn^{++} levels were determined by using an atomic absorption spectrophotometer (AAS, pye-Unicamspg sp9)[78]

3.5.1 Materials

a. British Drug House (BDH), U.K.
   (i) Nitric acid

b. E. Merck, West Germany :
   (ii) $ZnSO_4$, $7H_2O$. (ii) $CuSO_4$, $5H_2O$

3.5.2 Methods

3.5.2.1 Preparation of Reagents

(a) Nitric acid; 1%

(b) (i) Stock zinc standard solution : Contained 0.4397 g/l zinc sulphate in 1.0% nitric acid solution.

(ii) Working zinc standard solution (10µg/ml) : The stock standard solution was diluted 100 fold with freshly prepared deionized water. A standard series containing 50, 100, 150 and 200 µg/dl zinc was prepared by appropriate dilution of the working standard with freshly prepared deionized water.

(c) i. Stock copper standard solution : Contained 0.3929g/L copper sulphate in 1.0% nitric acid solution.

ii. working standard solution (10 μg ml) : The stock standard solution was diluted 100 fold within freshly prepared deionized water. A standard scries containing 60, 80, 100 & 120 μg/dl was prepared by appropriate dilution of the working standard with fresh prepared deionized water.

3.5.2.2 Preparation of standard curve

0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 ppm of standard zinc or copper solution were taken into different bottles and then absorbances were measured at 214nm for Zn^{++} or 324.8nm for Cu^{++}. A standard curve was prepared by plotting the optical density data against the Zn^{++} or Cu^{++} concentration.

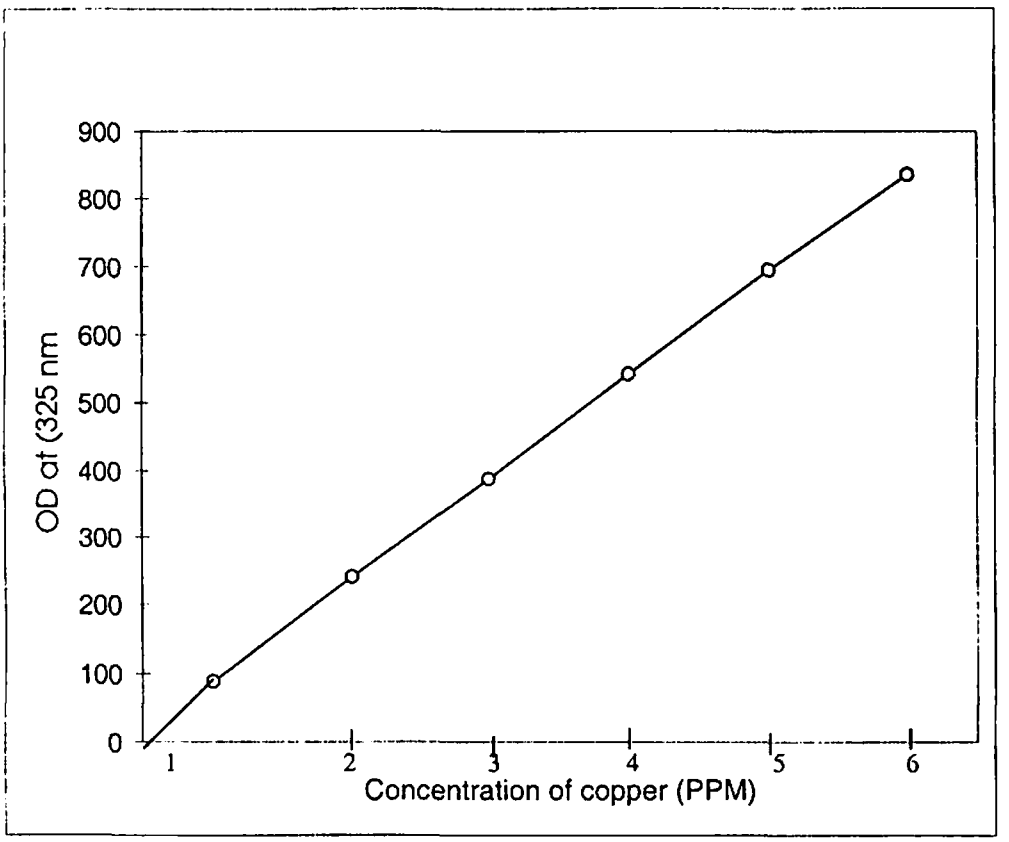


Fig: 1 Standard curve for copper

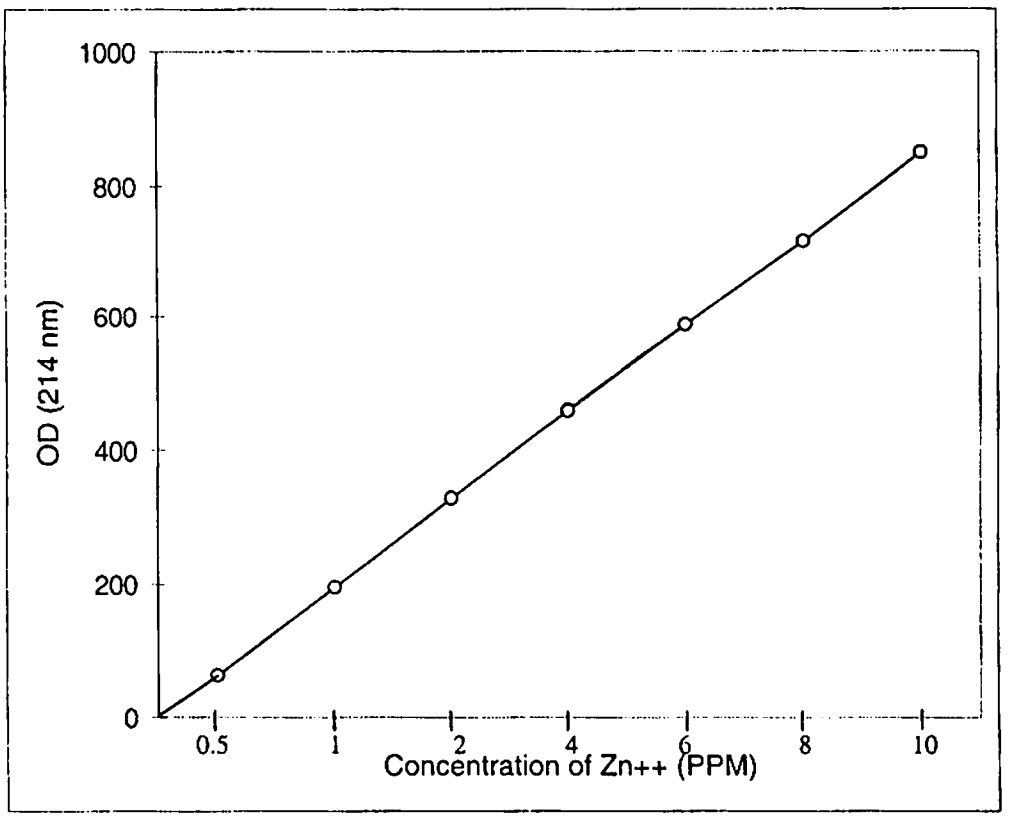


Fig: 2 Standard curve for zinc

3.5.2.3 Estimation of Zn^{++} and Cu^{++} in human serum by atomic absorption spectrophotometer

3.5.2.4 Procedure

An aliquot of 0.2 ml serum was taken in an acid washed glass tube and 0.8ml freshly prepared deionized water was added. The solution was mixed using a rota mixture. Then the absorbance were measured. The amount of Cu^{++} and Zn^{++} was calculated from the standard curve.

3.6 Estimation of vitamin-A from the serum by the method of Bieri and Tolliver with adaptation from Horustt et.al., and Huang et.at.,

3.6.1 Materials

(I) HPLC grade absolute alcohol (ethanol).

(II) Spectograde n-hexan.

(III) Nitrogen gas.

(IV) Retinyl acetate.

(V) Methanol.

3.6.2 Method

3.6.2.1 Preparation of Reagents

(i) 97% methanol

97 ml of methanol was taken in a glass bottle and then 3.0 ml distilled water was added.

(ii) Preparation of retinol

10mg of retinol was dissolved in 100 ml ethanol. The concentration of retinol in this solution was 100mg/ml. 5.0 ml of this solution was diluted to 100 ml with ethanol. Now the concentration of retinol was 5 mg/ml. 1.0 ml of this stock solution was diluted to 50 ml with ethanol so that the concentration of retinol became. 0.1 mg/ml. Where this was mixed with the 50 ml solution of retinyl acetate (internal standard) solution, the concentration of retinol became 0.05 mg/ml.

3.6.2.2 Procedure

200 ml of human serum was taken in a glass test tube and it was added to 100 ml of the internal standard (retinyl acetate 1 mg/ml in ethanol) solution and 100 ml ethanol. The contents were mixed well. For the extraction of lipid 400 ml spectrogram hexane were added and the contents mixed vigorously, intermittently for 45 seconds on a vortex mixture. The tubes were centrifuged at 1200 g for 10 minutes to separate the phases. Much of the solvent as possible was carefully drawn off from the upper phase with a 75 ml long levy pipette and transferred to a 3 ml conical conical centrifuge tube. The solvent was evaporated kunder a strain of nitrogen gas with the tube in a 60^0C water bath. For infection into the chromatograph, the lipid in the centrifuge tube was dissolved in 100 ml absolute ethanol with gentle mixing by finger tapping. About 50 ml of the solution was gently infection followed by a flush of ethanol.

3.6.2.3 Calculation

$$\text{Amount of Vit A } (\mu g/dl) = \frac{\textit{Peak area of unsknown}}{\textit{Peak area of known}} \text{ X Concentration of standard solution}$$

3.7 Estimation of Glucose content of blood by Nelson Somogyi method[79, 80]:

3.7.1 Materials :

a. E. Merck, West Germany

i. Zinc sulfate,
ii. Barium hydroxide,
iii. Sodium sulfate,
iv. Sodium bi-carbonate,
v. Rochelle salt,
vi. Sodium sulfate,
vii. Copper sulfate,
viii. Sulfuric acid,
ix. Benzoic acid

b. British Drug House, U.K.

I NH4-molybodate,

ii Di-sodium hydrogen arsenate,

iii. Glucose

3.7.2 Method

3.7.2.1 Preparation of Reagents

a. Zinc sulfate solution: 5% zinc sulfate in water.

b. Barium hydroxide solution: 28.og Ba (OH), H_2O in 500ml of hot water amdifillered after cooling.

c. Alkaline copper reagent:
Dessolve 50g anhydrous sodium carbonate 40 g sodium dicarbonate, 50g Rochelle salt and 400g anhydrous sodium sulfate in 1600 ml water and make the volume to 2000 ml (filter of required). Dissolve 75 g copper sulfate in water, add 0.25 ml sulfuric acid and make up the volume to 500 ml.

d. Aresenomolybdate reagent:
Dissolve 25.0 g NH_4 –molybdate in 450 ml water, and 21.0 ml of conc, H_2SO_4, mix, and 3.0g disodium hydrogen arsenate dissolved in 25.0 ml of water. Mix and place in the incubator at 37^0 C for 24-48 hours (if required urgently heat it to 55^0 C) keep in a glass stopped bottle.

d. Standard glucose solution :
0.1% glucose in 0.2% benzoic acid solution. For use dilute 5 ml to 100 ml with benzoic acid so that the strength is 0.05 mg/ml.

3.7.2.2 Procedure:

Place 0.1 ml of serum in 3.5 ml of water and 0.2 ml of 0.3N Ba $(OH)_2$ and 0.2ml of 5% zinc sulfate solution, shake well, filter/centrifuge. Pipette 1 ml of the filtrate (0.025 ml of serum). Take 1.0 ml of water for blank and take 1.0 ml of standard in

separate test tubes (take in triplicate). Then add 1.0 ml of water and 2.0 ml of alkaline copper reagent to each tube and heat in a boiling water bath for 10 mins. Cool quickly for 1 minute and 1.0 m of Aresenomolybdate reagent and make up to 10.0 ml with water, read at 680nm.

3.7.2.3 Calculation :

$$\text{Milligram of glucose per 100 ml of serum} = \frac{\text{Reading of sample}}{\text{Reading of standard}} \times 0.05 \times 100/0.025$$

3.8 Determination of total protein[81]:

The total protein was estimated by the method of lowery et al. In this process, protein solution was allowed to react with the Folin-coicalteau reagent to give a colored complex, which was measured spectrophotometrically.

3.8.1 Materials :

a. E. Merck, West Germany :

i. Sodium-tungstate,
ii. Sodium-molybdate,
iii. Phosphoric acid,
iv. Lithium sulfate,
v. Copper sulfate,
vi. Sodium-potassium tartarate,
vii. Sodium carbonate,
viii. Sodium hydroxide

b. British Drug House U.K.

i. Hydrochloric acid
ii. Bromine
iii. Bovine serum albumin (BSA).

3.8.2 Method:

3.8.2.1 Preparation of Reagents :

a. Folin-coicalteau reagent (FCR): It was prepared according to the method of Folin and coicalteau. To a 2000 ml flat-bottom flask covered with carbon paper were added sodium tungstate (100g) 25 g sodium molybdate (Na_2MO_4. $2H_2O$) 700 ml water, 50 ml 85% phosphoric acid and 100 ml concentrated hydrochloric acid. The contents of the flask were refluxed gently for 10 hours followed by the addition 150 g lithium sulfate (Li_2 SO_4. H_2O), 50 ml H_2O and few drop of bromine. The mixture were then boiled for 15 minutes in the fume hood without a condenser to remove excess bromine, cooled and diluted to 1 liter. This reagents was stored in freezer in amber colored reagent bottle and was diluted with equal volume of water before use.

b. **Alkaline copper reagent :**

Reagent A : Copper sulfate (1% w/v) was mixed with an equal volume of sodium potassium tartarate (2% w/v) before use.

Reagent B : Sodium carbonate (2% w/v) into 1N NaOH solution.

The alkaline copper reagent was prepared by mixing 1 ml of reagent A with 50 ml of reagent B immediately before use.

c. Bovine serum ablumin (BSA) solution 250µg/ml: 25mg BSA was dissolved in 0.1N NaoH to make solution of 100ml.

3.8.2.2 Standard curve of bovine serum albumin(BSA) :

Six test tubes were set in a series and 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of standard BSA solution were added. Final volume was made 1.0 ml by addition of water. Alkaline copper reagent (4 ml) was added to each tube; followed by incubation of 15 minutes at 40^0 C water bath. Followed by the addition of F.C.R. (0.5 ml). The tubes were shaken vigoroulsy and allowed to stand for 30 minutes at room temperature to develop the colour. The absorbance was then read at 660 mm in a spectrophotometer against the blank (first tube). To prepare the standard curve the O.D values plotted against corresponding concentration of protein.

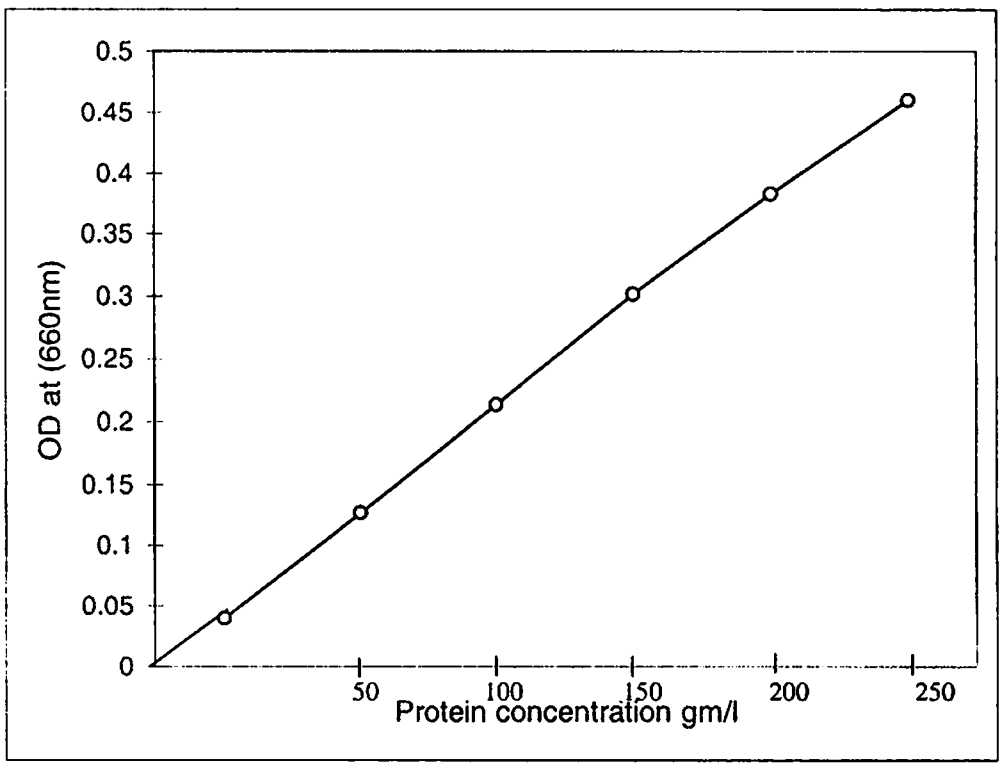


Fig: 3 Standard curve for total protein

3.8.2.3 Assay of serum protein :

0.1ml of serum was taken in 100 ml volumetric flask and volume was made up to 100 ml with distilled water. Take 1 ml of diluted serum in test tubes (triplicte). In above all tubes add water and make the final volume to 4 ml, and then mix well. To each 5.5 ml of Alkaline mix (reagent) and mixed well. The mixture was allowed to stand at room temperature for 10-15 minutes. 0.5 ml of F.C.R was then added to each tube, mix rapidly. The tubes were left for 30 minutes. The absorbance was measured at 650 nm. The amount of protein in the sample was determined form the standard curve.

3.9 Determination of serum Albumin :

3.9.1 Principl[82]

The measurement of serum albumin is based on its quantitative binding to the indicator 3, 3′, 5,5′-tetrabromo-m-cresol sulphonphthalein (bromocresol green, BCG). The albumin-BCG-complex absorbs maximally at 578 nm.

3.9.2 Sample

Serum, heparinized plasma or EDTA plasma.

3.9.3 Reagents

	Contents	Concentration of reagents
1.	BCG concentrate	
	Succinate buffer	75 mmol/l; pH 4.2
	Bromocresol green	0.15 mmol/l
	Brij 35	
	Preservative	
	Standard	
	Human serum albumin	45 g/l (4.5 g/dl)

3.9.4 Preparation of solution

1. BCG Concentrate

   Dilute the contents of one bottle with the appropriate volume of distilled water:

3.9.5 Standard

Contents ready for use. Stable up to expiry date when stored at + 15 to 25^{0}C.

3.9.6 Procedure

Wavelength	Hg 578 nm or Hg 623 nm
Spectrophotometer:	630 nm (600-650 nm)
Cuvette:	1 cm light path
Incubation Temperature :	20 to 25^{0}C
Measurement:	against reagent blank

Pipette into test tubes:

	Reagent Blank	Standard	Sample
Distilled H_2O	0.01 ml	--	--
Standard	--	0.01 ml	--
Serum or Plasma	--	--	0.01 ml
BCG reagent	3.00 ml	3.00 ml	3.00 ml

Mix and incubate for 5 minutes at + 20 to 25^{0}C. Measure the absorbance of the sample (A_{sample}) and of the standard ($A_{standard}$) against the reagent blank.

3.9.7 Calculation

The albumin concentration in the sample may be calculated from the following formula:

$$\text{Albumin Concentration (g/l)} = \frac{A_{sample}}{A_{standard}} \times \text{Concentration of standard}$$

3.10 Determination of blood UREA[83]

The blood urea was determined by the Spinreact method, which is an adaptation of the reaction, proposed by Jung and colleagues.

3.10.1 Materials

i. Reagent 1: Orthophataladehide 4.8 mmol/l.
ii. Reagent 2: Borate solution 87 mmol/l.
iii. Standard urea solution 50 mg/dl.

3.10.2 Procedure

In a test tube take 25 µl of serum and add 1 ml of reagent1. In another test tube take 25 µl of the standard solution and add 1ml of reagent 1. In a third test tube take 1 ml of reagent 1 ml for blank. Shake the tubes and mix the contents well. Add 1 ml of reagent 2 to each tube, mix well and incubate for 15 minutes at 37^0C. Measure absorbance against blank at 510 nm.

3.10.3 Calculation

$$\text{Urea (mg/dl)} = \frac{\textit{Absorbance of sample}}{\textit{Absorbance of standard}} \times \text{Conc. of standard solution.}$$

3.11 Determination of blood creatinine[84]

Blood creatinine is ordinarily determined by reaction in a protein free filtrate with alkaline picrate to form a colour, which is then compared with a standard.

3.11.1 Materials :

3.11.1.1 Preparation of reagents:

i. **Standard creatinine solution :**

Prepare a stock standard by dissolving 1g of pure dry creatinine in 0.1N hydrochloric acid and diluting to 1 litre with the acid. This solution is stable indefinitely and contains 1.0 mg of creatinine per ml.

ii. Working standard :

Transfer 3 ml of stock standard solution containing 3 mg of creatinine to a 500 volumetric flask, add 50 ml of 0.1N hydrochloric acid and dilute with water to 500 ml. The standard contains 0.03 mg in 5 ml.

iii. Alkaline picrate reagent:

Transfer 25 ml saturated picric acid solution to a flask. Add 5 ml of 10% NaOH and mix.

3.11.1.2 Procedure

Transfer 10 ml of 1:10 tungstic acid filtrate of serum to a test-tube. In a second container place 5 ml of standard creatinine solution containing 0.03 mg of creatinine and add 15 ml of water. Add 5 ml of freshly prepared alkaline picrate reagent to the blood filtrate and 10 ml to the diluted creatinine standard. Mix and allow standing for 15 minutes for complete color development. Read absorbance in spectrophotometer at 520 nm. The blank was prepared by 10 ml of distilled water with 5 ml of alkaline picrate

3.11.1.3 Calculation:

$$\text{Creatinine (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100 \times 15/30$$

3.12 Mearugement of Alanine Aminotransferase activity[85]

The quantitative determination of alanine aminotransferase in serum was carried out on the automated clinical chemistry analyzers (Hitachi System 704). The method applied here was recommended by the international Federation of Clinical Chemistry (IFCC) which was based on the method of Bergmeyer, H.U, et.al. (1986).

The Roche supplied all the chemicals and enzymes employed here.

3.12.1 Reagents :

Bottle l: Containing TRIS buffer [Tris (hydroxymethyl)-aminomethane]. 12X50 ml.

Bottle la: containng enzme/coenzymes [-alanine/NADH/LDH]. 1×24 reagent tablets.

Bottle 2: containing α-Ketoglutarate. 3×44ml.

3.12.2 Preparation of working solution:

i. **Solution Ri:** To prepare 50 ml of solution R_1, two reagent tablets form bottle 1a to bottle 1 were added. Swirling the bottle gently dissolved the reagent tablets. After it was done, the concentration of the components became.

TRIS buffer	:	125-mmol/l; pH 7.3
L-alanine	:	625 mmol/l
NADH (yeast)	:	0.23 mmol/l
Lactate dehydrogenase (LDH)	:	> 1.5 U/ml.

ii. Solution R_2: Bottle 2 containing α-Ketoglutarate (44ml in each bottle) was directly used as solution R_2. It was ready to use. Concentration of α-Ketoglutarate was 94 mmol/l.

3.12.3 Assay calibration:

For accuracy of the test a calibration assay was done using the following:

S_1: 0.9% NaCl that was used as blank calibrator.

S_2: C.f.a.s. (Calibrator for automated systems)

3.12.4 Assay procedure:

The following assay procedure were followed to carryout the reaction.

The reagent R_1 and R_2 were placed into the assigned Reagent disk. Approximately 100 µl of serum sample was taken into the reaction cuvette and placed into the Sample disk. Then 500 ml of calibrator (C.f.a.s.) and 600 ml of two different control materials were taken into three different reaction cuvette and placed into the assigned Sample disk. Finally the following data was entered into the operating panel.

Sample volume (µl)	: [15.0]
R_1 volume (µl)	:[350]
R_2 volume (µl)	:[70]
Wavel Length (nm)	:[700]-[340]
Calibration method	:[Linear]

3.12.5 Calculation of results:

Hitachi 704 system automatically calculates the ALT activity of each sample in U/L.

3.13 Measurement of Aspartate Aminotransferase activity[85]:

The quantitative determination of aspartate aminotransferase in serum was done on the automated clinical chemistry analyzers (Hitachi system 704). The method applied in this study was recommended by IFCC, which was based on the method of Bergmeyer, H.U. et,al. (1986)

The Boehringer Mannheim suplied all the chemicals and enzymes employed here.

3.13.1 Reagents:

Bottle 1: Containing TRIS buffer 12×50 ml

Bottle 1a: containing enzymes/coenzymes. (L-aspartate/NADH/ NAD+) 1×24 reagent tablets.

Bottle 2: containing α-Ketoglutarate. 3×44 ml.

3.13.2 Preparation of working solution:

i. Solution R_1: To prepare 50 ml of solution R_1, two reagent tablets form bottle 1a to bottle 1 were added. Swirling the bottle gently dissolved the reagent tablets. After it was done, the concentration of the components became,

TRIS buffer	:	100 mmol/l; pH 7.8
L-aspartate	:	300 mmol/l
NADH	:	0.23 mmol/l
NAD	:	> 0.53 U/ml.

ii. Solution R_2: Bottle 2 containing α-Ketoglutarate (44ml in each bottle) was directly used as solution R_2. It was ready to use. Concentration of α-Ketoglutarate was 75 mmol/l.

3.13.3 Assay calibration:

For accuracy for the test a calibration assay was done using calibrator for auitomated systems (C.f.a.s.).

3.13.4 Assay procedure:

The serum loaded for 'ALT estimation was also used for AST by giving instruction to the operator control panel. Calibration and quality control method was the same as that of ALT.

3.13.5 Calculation of results:

Hitachi 704 system automatically calculates the AST activity of each sample in U/l.

3.14 Determination of serum Alkaline phosphates activity:

3.14.1 Colorimetric Method[86]:

This is an optimized standard method according to the recommendations of the Deutsche Gesellschaft fur Klinische Chemie.

3.14.2 Principle:

$$\text{p-nitrophenylphosphate} + H_2O \xrightarrow{ALP} \text{phosphate} + \text{p-nitrophenol}$$

3.14.3 Sample:

Serum or heparinized plasma.

3.14.4 Reagents

	Contents	Concentration of reagents
1.	Buffer	
	Diethanolamine buffer	100 mmol/l, pH 10.10
	$MgCl_2$	0.5 mmol/l
2.	Substrate	
	P-nitrophenylphosphate	10 mmol/l

3.14.5 Preparation of solutions

1. Buffer

   Contents ready for use. Stable up to the expiry date when stored at+2 +8^{0}C.

2. Substrate

   Ready for use

3.14.6 Procedure

Wavelength:	Hg 405 nm
Cuvette:	1 cm light path
Temperature:	25^0C, 30^0C, 37^0C
Measurement:	against air

Pipette into cuvette:	Macro	Semi-Macro	Micro
Sample	0.05 ml	0.02 ml	0.01 ml
Reagent (25^0C, 30^0C, 37^0C)	3.00 ml	1.00 ml	0.50 ml

Mix, read initial absorbance and start timer simultaneously. Read again after 1,2 and 3 min.

3.14.7 Calculation

To calculate the ALP activity use the following formulae:

U/l = 2760× A 405 nm/min SEMI-MICRO

3.15 Determination of serum Bilirubin

3.15.1 Principle[87]

Total Bilirubin is determined in the presence of caffeine by the reaction with diazotised sulphanillic acid. Direct (conjugated) bilirubin is determined in the absence of caffeine.

3.15.2 Sample

Serum, heparinized plasma or EDTA –plasma.

3.15.3 Reagents

	Contents	Initial Concentration of reagents
1.	Sulphanilic acid	29 mmol/l
	Hydrochloric acid	0.17 N
2.	Sodium Nitrite	25 mmol/l
3.	Caffeine	0.26 mol/l
	Sodium benzoate	0.52 mol/l

4.	Tartrate	0.93 mol/l
	Sodium Hydroxide	1.9 N

3.15.4 Preparation of Solutions

All reagents are ready to use. Stable up to the expiry data when stored at + 15 to + 25^{0}C.

3.15.5 Procedure

Weavelength:	Total bilirubin: Hg 578 nm (560-600 nm)
	Direct bilirubin: Hg 546 nm (530-560 nm)
Cuvette:	I cm light path
Reaction Temperature:	20-25^{0}C
Measurement:	against sample blank

3.15.6 Total Bilrubin (TB)

Pipette into cuvette:

	Sample Blank (ml)	Sample (ml)
Reagent 1	0.20	0.20
Reagent 2	--	1 drop (0.05 ml)
Reagent 3	1.00	1.00
Sample	0.20	0.20

Mix, and allow to stand for 10 min at 20-25^{0}C.

Reagent 4	1.00	1.00

Mix, and allow to stand for 5.30 min at 20-25^{0}C and then read the absorbance of the sample against the sample blank (A_{TB}).

3.16 Determination of serum Cholesterol by liebermann-Burchard method[88]:

3.16.1 Materials :

(a) E. Merck, West Germany

I. Sulfuric acid

II. Acetic anhydride

(b) Sigma Chemical Co., St. Louis, Mo 63178

i. Choloroform

(c) British drug house, U.K.

i. Ether

ii. Ethanol

iii. Cholesterol

3.16.2 Method:

3.16.2.1 Preparation of Reagents:

1. Chloroform.

2. Acetic anhydride-sulfuric acid mixture: 40 ml acetic anhydride was taken in a beaker and kept in an ice bucket. To this 2 ml conc. H_2SO_4 was carefully added with gentle stirring. The mixture was kept in cold all the time. The mixture should be colourless but if a tinge of blue color appears, it was discarded and a fresh batch of acetic anhydride was used. The reagent was prepared freshly before use.

3. Bloor's mixture : 100 ml ether and 50ml ether and 50ml ethanol was taken in a bottle and mixed well.

4. Standard cholesterol solution : 50 mg pure cholesterol was dissolved with chloroform in a 100 ml volumetric flask and the volume was made upto 100 ml with chloroform.

3.16.2.2 Preparation of the standard curve :

0.2, 0.4, 0.6, 0.8 and 1.0 ml of standard cholesterol solutions were taken into different test tubes and volume was made upto 1ml with chloroform. To each tube 5ml of the reagent-2 was added carefully and mixed well. The tubes were left covered with a dark cloth for 15 minutes and then absorbance was was measured at 640 nm. A standard curve was prepared by plotting the optical density data against the cholesterol concentration.

3.16.2.3 Assay of serum cholesterol:

0.2 ml serum was taken in a test tube and 2 ml of bloor's mixture was added and mixed well. Kept it at room temperature for 10 min, the solution was then centrifuged in a clinical centrifuge at 2000 r.p.m. for 10 minutes. The supernatant was decanted into a dry flask. The residue was extracted once more with the same mixture and the supernatant was pooled. The extracts were evaporated to dryness over a stem bath. The residue was dissolved in 1 ml chloroform. The reagent-2 was added carefully and mixed well, and followed the similar procedure as described for preparation of the standard curve. The amount of cholesterol present in serum can be calculated by using the standard curve.

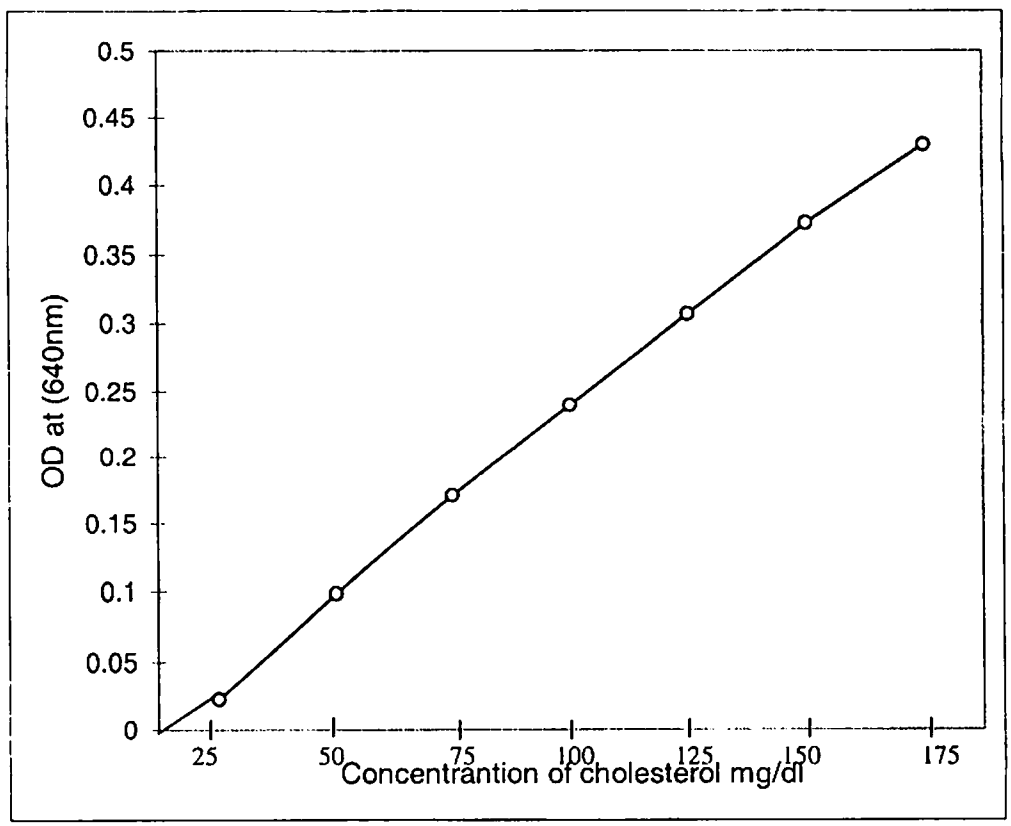


Fig: 4 Standard curve of cholesterol

3.17 Determination of serum Triglycerides

3.17.1 Colorimetric Method

The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen peroxide, 4-aminophenazone and 4-cholorophenol under the catalytic influence of peroxidase.

3.17.2 Principle[89]

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipases}} \text{glycerol} + \text{fatty acids}$$

$$\text{Glycerol} + \text{APT} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{4 cholorophenol} \xrightarrow{\text{POD}} \text{quinoneimine} + HCl + 4H_2O$$

3.17.3 Sample

Serum, heparinized plasma or EDTA-plasma.

3.17.4 Reagents

	Contents	Concentration of reagents
1.	**Buffer**	
	Pipes Buffer	40 mmol/l, pH 7.6
	4-chooro-phenol	5.5 mmol/l
	Magnesium-ions	17.5 mmol/l
2.	**Enzyme Reagents**	
	4-aminophenazone	0.5 mmol/l
	ATP	1.0 mmol/l
	Lipases	≥150 U/ml
	Glycerol-kinase	≥ 0.4 U/ml
	Glycerol-3-phosphate oxidase	≥ 1.5 U/ml
	Peroxidase	≥0.5 U/ml
3.	Standard	2.29 mmol/ (200 mg/dl)

3.17.5 Preparation of Solutions

1. **Buffer**

   Contents ready for use. Stable up to the expiry date when stored at+2 +8^{0}C.

2. **Enzyme Reagent**

   Reconstitute one vial of Enzyme Reagent 2 with 15 ml of Buffer 1. Stable for 21 days at + 2 to + 8^{0}C or 3 days at + 15 to + 25^{0}C.

3. **Standard**

   Contents ready for use. Stable up to the expiry date when stored at + 2 to + 8^{0}C.

3.17.6 Procedure

Wavelength:	500 nm; Hg 546 nm
Cuvette:	1 cm
Temperature:	20-25^{0}C or 37^{0}C
Measurement:	against reagent blank only one reagent blank per series is required

Pipette into test tubes:

	Reagent Blank	Standard	Sample
	µl	µl	µl
Sample	--	--	10
Standard	--	10	--
Reagent	1000	1000	1000

Mix, incubate for 10 minutes at 20-25^{0}C or 5 minutesw at 37^{0}C. Measure the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) against the reagent blank within 60 minutes.

3.17.7 Calculation

When using a standard:

$$\text{Triglyceride concentration} = \left(\frac{A_{sample}}{A_{standard}} \times 200\right) \text{ mg /dl}$$

3.18 Determination of HDL-Cholesterol

3.18.1 Principle[90,91]

Low density lipoproteins (LDL and VLDL) and chylomicron fractions are preecipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density liporprotein) fraction, which remains in the supernatant, is determined.

3.18.2 Sample

Serum, heparinized plasma or EDTA plasma.

3.18.3 Reagent

Contents	Concentration of reagents
Phoshotungstic Acid	0.55 mmol/l
Magnesium Chloride	25 mmol/l

3.18.4 Preparation of Solutions

1. Macro assays
   Contents ready for use undiluted. Stable up to the expiry data specified when stored at 15 to +25^{0}C.

Additional Reagents required

Reagent Solution for Cholesterol CHOD-PAD Assay

3.18.5 Procedure

1. Precipitation

Pipette into centrifuge tubes:

	Macro	Semi Micro
Sample	500µl	200µl
Precipitant	1000 µl	--
Diluted Precipitant	--	500 µl

Mix and allow to sit for 10 minutes at room temperature. Then centrifuge for 10 minutes at 4000 rpm, or 2 minutes at 12,000 rpm.

2. Cholesterol CHOD-PAP Assay

Wavelength:	500 nm, Hg 546 nm
Cuvette:	1 cm light path
Temperature :	20-25^{0}C or 37^{0}C
Measurement:	against reagent blank

Only one reagent blank per series is required.

Pipette into test tubes

	Reagent Blank	Standard	Sample
Distilled Water	100 µl	--	--
Supernatant	--	--	100 µl
Standard	--	100µl	--
Reagent	1000µl	1000 µl	1000µl

Mix, incubate for 10 minutes at 20-25° C or 5 minutes 37° C. Measure the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) against the reagent blank within 60 minutes.

3.18.6 Calculation

1. HDL Cholesterol

When using a factor:

	MACRO		SEMI-MICRO	
Wavelength	mmol/l	mg/dl	mmol/l	mg/dl
500 nm	4.65	180	5.43	210
Hg 546 nm	7.09	274	8.27	320

3.19 Determination of LDL-Cholesterol

3.19.1 Principle[92]

Low-density lipoproteins (LDL) are precipitated by heparin at their isoelectric point (pH 5.04). After centrifugation the high-de3nsity lipoproteins (HDL) and the very low-density lipoproteins (VLDL) remain in the supernatant. These can then be determined by enzymatic methods.

LDL-cholesterol=Total cholesterol-Cholesterol in the supernatant.

3.19.2 Sample

Serum

3.19.3 Reagents

		Concentrations of Solutions
1.	Precipitation Reagent	
	Heparin	50,000IU/L
	Sodium citrate	0.064 mol/l, pH 5.04

3.19.4 Preparation of Reagents

1. Precipitation Reagent

   Ready for use.

   Stable up to expiry date when stored at + 2 to +8^{0}C.

2. Reagent Solution for Cholesterol Determination

   Cholesterol CHOD-PAP

3.19.5 Procedure

Wavelength:	500 mm; Hg 546
Cuvette:	1 cm light path
Temperature:	+ 20 to + 25^{0}C, 37^{0}C
Pipette into centrifuge tube:	
Serum	100µl
Precipitation reagent (1)	1000µl

Mix well, leave to stand for 10 min at + 15^{0}C to +25^{0}C and centrifuge for 15 min at approx. 4000rpm. Determine the cholesterol concentration of the supernatant within 1 hour after centrifugation.

Pipette into test tubes:

	Reagent Blank	Standard	Sample
Distilled water	50µl	--	--
Standard	--	50µl	--
Supernatant	--	--	50µl

Reagent (2)	1000µl	1000µl	1000µl

Mix well, incubate for 10 min at + 20^{0}C to 25^{0}C or for 5 min at 37^{0}C and measured the absorbance of the sample (A_{sample}) against the reagent blank.

3.19.6 Calculation

Using a standard:

Conc. of cholesterol in the supernatant :

$$= \frac{A_{sample}}{A_{standard}} \times \text{conc. of standard.}$$

Calculation of the LDL- cholesterol:

LDL-Cholesterol = Total cholesterol – cholesterol in the supernatant.

3.20 Determination of serum CK-MB

3.20.1 Principle[93]

Immunoinhibition Assay: An antibody is incorporated in the CK reagent. This antibody will bind to and inhibit the activity of the M subunit of CK-MB. This means that only the activity of the B subunit in serum is measured. If the activity is multiplied by a factor of 2, it will give the activity of CK-MB in serum.

3.20.2 Sample

Serum, heparinized or EDTA plasma.

3.20.3 Reagents

	Contents	Concentration of regents
1.	**Buffer/Glucose**	
	Imidazole Buffer	0.1 mol/l, pH 6.7
	Glucose	20 mmol/l
	Mg-Acetate	10 mmol/l
	EDTA	20 mmol/l
2.	**Enzymes/Coenzymes/Substrate/Antibody**	
	ADP	2.0 mmol/l

AMP	5.0 mmol/l
Diadenosine Pentaphosphate	10 µmol/l
MADP	2.0 mmol/l
HK	≥2.5 U/ml
G6P-DH	≥1.5 U/ml
N-Acetylcysteine	20 mmol/l
Antibody to CK-MB	30 mmol/l

3. **Control Serum**

3.20.4 Preparation of Solutions

1. **Buffer/Glucose**

   Contents ready for use. Stable up to the expiry date when stored at + 2^0C to + 8^0C.

2. **Enzymes/Coenzymes/Substrate/Antibody**

   Reconstitute one vial of enzymes/Coenzymes/Substrate /Antibody 2 with 2.5 ml of buffer. Stable for 5 days at + 2^0C to 8^0C.

3. **Control Serum**

   Open one vial of control serum very carefully, avoiding any loss of material, and reconstitute in exactly 2 ml of distilled water. Close vial and leave to stand for 15 minutes, dissolving contents completely by swirling or rotating gently. CK-MB is stable in the serum for 5 days at + 2^0C to +8^0C, 2 days at 25^0C and 4 weeks at 20^0 C when frozen once.

3.20.5 Procedure

Before carrying out the assay to measure the activity of CK-MB in serum, it is necessary to measure total CK activity using the NAC-activated method.

Wavelength:	340 nm Hg 334 nm or Hg 365 nm
Cuvette:	1 cm light path
Temperature:	25^0C, 30^0C, 37^0C
Measurement:	against air

Pipette into cuvette:

	Semi-Macro	Micro
Sample	0.04 ml	0.1 ml
Enzymes/Coenzymes/Substrate/Antibody	1.0 ml	2.5 ml

Mix, and let stand at the appropriate temperature for 10 minutes. Then add to a cuvette, and read absorbance A_1. Read absorbance A_2 exactly 5 minutes later.

$\Delta A = A_2 - A_1$

3.20.5 Calculation

Wavelength	CK-MB (U/l)	CK-MB (U/l)
340 nm	$825 \times \Delta A$	$1651 \times \Delta A$
Hg 334 nm	$841 \times \Delta A$	$1683 \times \Delta A$
Hg 365 nm	$1486 \times \Delta A$	$2972 \times \Delta A$

3.21 Determination of serum phosphate ion

The quantitative determination of inorganic phosphate ion in serum was carried out on the automated clinical chemistry analyzers (Hitachi system 704). The method applied here was recommended by the international federation of clinical chemistry (IFCC)

The Roche supplied all the chemicals and enzymes employed here.

3.21.1 Principle

Endpoint method with sample blanking

- Sample and addition of R1 (reagent blank)
- Addition of R2 (phosphate reagent) and start of reaction:
  Inorganic phosphate forms an ammonium phosphomolybdate complex having the formula $(NH_4)_3\ [PO_4(MoO_3)_{12}]$ with ammonium molybdate in the presence of sulfuric acid. The complex is determined photometrically in the ultraviolet region (340 nm).

3.21.2 Reagents

Bottle 1: Reagent buffer, 6×63 ml

Bottle 2: Phosphate reagent, 6×31 ml

3.21.3 Preparation of working solution

i. Solution R_1: Bottle 1 containing sulfuric acid containing 0.36 mol/l, detergent.

ii. Solution R_2: Bottle 2 phosphate reagent containing,
Ammonium molybdate----------> 3.5 mmol/l;
Sulfuric acid: ----------> 0.36 mol/l
sodium chloride: ----------> 150 mmol/l

3.21.4 Assay calibration :

For accuracy of the test a calibration assay was done using the following:

S_1: 0.9% NaCl that was used as blank calibrator.
S_2: C.f.a.s. (Calibrator for automated systems)

3.21.5 Assay procedure

The following assay procedure were followed to carryout the reaction.'

The reagent R_1 and R_2 were placed into the assigned Reagent disk. Approximately 100 µl of serum sample was taken into the reaction cuvette and placed into the sample disk. then 500 ml of calibrator (C.f.a.s) and 600 ml of two different control materials were taken into three different reaction cuvette and placed into the assigned sample disk. Finally the following data was entered into the operating panel.

Sample volume (µl)	: [7.0]
R_1 volume (µl)	: [350]
R_2 volume (µl)	: [150]
Wavel Length (nm)	: [660]-[340]
Calibration method	: [Linear]

3.21.6 Calculation of Results:

Hitachi 704 system automatically calculates the $PO_4^{\equiv}$ activity of each sample in mg/dl

3.22 Determination of serum sodium ion

3.22.1 Principle[94]

Sodium is determined enzymatically via sodium dependent β-galactosidase activity with ONPG as substrate. The absorbance at 405 nm of the product O-nitrophenyl is proportional to the sodium concentration.

$$\text{ONPG} \xrightarrow[\beta\text{-galactosidase}]{Na^+} \text{o-nitrophenol} + \text{galactose}$$

ONPG = o-nitrophenyl-β-D-galactopyranose

3.22.2 Sample

Serum, plasma treated with lithium heparinate

3.22.3 Reagents

Contents	Concentration of reagents
1. Buffer/Enzymes	
Tris buffer	450 mmol/l, pH 9.0
Cryptand	5.4 mmol/l
β-galactosidase	≥0.8 U/ml
2. Diluent/Substrate	
Tris buffer	10.0 mmol/l, pH 9.0
O-nitrophenyl galactoside	5.5 mmol/l
Standard low	90 mmol/l (207 mg/dl)
Standard high	175 mmol/l (402 mg/dl)

3.22.4 Preparation of Reagents

1. Buffer / Enzymes

Using the enclosed funnel, transfer the contents of one bottle Enzyme 1a to one bottle buffer 1 and dissolve by swirling gently ensuring that entire contents are completely dissolved. Avoid the formation of foam. Stable for 2 weeks at + 2^0C to + 8^0C or 5 days at + 15^0C 25^0C.

2. Diluent/Substrate

Using the enclosed funnel, transfer the contents of one bottle Substrate 2a to one bottle Diluent 2 and dissolve by swirling gently ensuring that the entire contents are completely dissolved. Stable for 4 weeks at + 2^0C to 8^0C or 2 weeks at + 15^0C to + 25^0C.

3.22.5 Standard low and high

Standard supplied ready for use. Stable up to expiry date when stored at + 2^0C to +8^0C.

3.22.6 Procedure

Wavelength:	405 nm
Cuvette:	1 cm path length
Temperature:	30/37^{0}C
Measurement:	against reagent blank

Pipette into cuvette:

	Blank	Standard	Sample
DDH2O	40µl	--	--
Standard	--	40µl	--
Sample	--	--	40µl
1 Buffer/Enzyme	1000µl	1000µl	1000µl
Mix and incubate for 5 minutes at 30/37^{0}C, then add:			
2 Substrate/Diluent	400µl	400µl	400µl

Mix, and incubate at 30/37^{0}C for 1 minute. Read Absorbance A_1. Incubate for a further 2 minutes and read Absorbance A_2.

ΔA of standards or samples = A_2- A_1

3.22.7 Calculation

Sodium concentration

$$\text{mmol/l} = \frac{A_{sample}}{A_{standard}} \times \text{Conc. of standard}$$

The advantage of he computerized analyzer was that the standard and the sample could be inserted together and the machine was programmed to provide the concentration on the sample. There was no need for a standard curve with this machine.

3.23 Determination of serum Potassium

3.23.1 Principle[95]

Sodium tetraphenylboron reacts with potassium ions in a protein free alkaline medium to produce a turbid suspension of potassium tetraphenylboron. The amount of trubidity produced is proportional to the potassium concentration.

3.23.2 Sample

Serum or lithium-heparin plasma.

3.23.3 Reagents

	Contents	Concentration of solutions
1.	Precipitating Reagent	
	Trichloro Acetic Acid	0.3 mol/l
2.	Sodium Tetraphenylboron	0.2 mol/l
3.	Sodium Hydroxide	2.0 mol/l
	Standard	5.0 mol/l

3.23.4 Reagent Preparation

Use all solutions undiluted. table up to the expiry date when stored at + 15^0C to + 25^0C.

3.23.5 Working Reagent

Mix equal volumes of solutions 2 and 3 to give enough reagent for the number of samples being run. Stable for 30 days at + 15^0C to + 25^0C and 60 days at + 2^0C to +8^0C.

3.23.6 Procedure

1. Precipitation

Pipette into centrifuge tubes:

	Macro	Micro
Sample	100 µl	50µl
Precipitating Reagent	1000µl	500µl

Mix, and centrifuge at high speed for 5-10 mins.

Wavelength:	578 nm, Hg 578 nm
Cuvette:	I cm light path
Measurement:	against reagent blank
Temperature:	+20^{0}C-+25^{0}C

Pipette into test tubes:

	Macro		Micro	
	Standard	Sample	Standard	Sample
Working Reagent	2000µl	2000µl	1000µl	1000µl
Standard	200µl	--	100µl	--
Supernatant	--	200µl	--	100µl

The standard and the clear supernatant must be added to the middle of the working reagent surface to produce a homogeneous turbidity. Mix carefully and allow to stand for at least 5 minutes. Measure the absorbance of standard and sample against reagent blank (ΔA).

3.23.7 Calculation

Potassium Concentration

$$\text{mmol/l} = \frac{A_{sample}}{A_{standard}} \times 5$$

The advantage of the computerized analyzer was that the standard and the sample could be inserted together and the machine was programmed to provide the concentration of the sample. There was no need for a standard curve with this machine.

3.24 Determination of serum calcium ion

3.24.1 Colorimetric Method[96]

O-Cresolphthalein complexone, without deproteinization.

3.24.2 Reaction Principle

Calcium ions from a violet complex with O-Cresolphthalein complexone in an alkaline medium.

3.24.3 Sample Material

Serum, heparinized plasma or urine. Dilute urine 1 +1 in 0.9% NaCl, multiply result by 2.

3.24.4 Reagents

	Contents	Concentration of reagents
1.	**Standard**	
	Calcium	2.5 mmol/l (10 mg/dl)
2.	**Buffer**	
	2-amino-2-methyl-propan-1-o1	3.5 mol/l, pH 10.7
3.	**Chromogen**	0.16 mmol/l
	O-Cresolphthalein complexone	6.89 mmol/l
	8-Hydroxyquinoline	60 mmol/l
	Hydrochloric acid	150 mmol/l
4.	**EDTA**	

3.24.5 Preparation of Reagents

1. For Single Assays

   all solutions are ready for use. Keep bottles closed after use. Stable up to the expiry date when stored at + 15^0C to + 25^0C.

2. Assay Series

   All solutions are ready for use. Keep bottles closed after use. Stable up to the expiry date when stored at + 15^0C to + 25^0C.

3.24.6 Working Reagent

Mix equal volumes of solutions 2 and 3 to give enough reagent for the number of samples being run. Stable for 7 days at + 2^0C to + 8^0C or 3 days at + 15^0C to +25^0C.

3.24.7 Procedure

Wavelength:	Hg 578 nm (550-590 nm)
Spectrophotometer:	570 nm
Cuvette:	1 cm light path
Measurement:	against reagent blank only one blank required per series
Temperature:	20^0C-25^0C/37^0C

1. For Single Assays:

Pipette into test tubes:

	Reagent Blank	Standard	Sample
Sample	--	--	25µl
Distilled Water	25 µl	--	--
Solution 1	--	25 µl	--
Solution 2	0.5 ml	0.5 ml	0.5 ml
Solution 3	0.5 ml	0.5 ml	0.5 ml

Mix, read absorbance of the sample (A_{sample}) and standard ($A_{standard}$) against the reagent blank after 5 to 50 minutes.

2. For Assay Series :

Pipette into test tubes :

	Reagent Blank	Standard	Sample
Sample	--	--	25 µl
Distilled Water	25 µl	--	--
Standard	--	25 µl	--
Working Reagent	1.0 ml	1.0 ml	1.0 ml

Mix, read absorbance of the sample (A_{sample}) and standard ($A_{standard}$) against the reagent blank after 5 to 50 minutes.

3.24.8 Calculation

$$\text{Concentration} = \left(\frac{A_{sample}}{A_{standard}} \times 10.0\right) \text{mg/dl})$$

3.25 Determination of serum Uric Acid

3.25.1 Colorimetric Method

Uric Acid is converted by uricase ot allantoin and hydrogen peroxide, which under the catalytic influence of peroxidase, oxidizes 3,5-Dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red-violet quinoneimine compound.

3.25.2 Principle[97]

Uric Acid + O_2+2H_2O $\xrightarrow{\text{uricase}}$ Allantoin + CO_2 + H_2O_2

2H_2O_2+ 3,5-dichloro-2-hydroxybenzenesulfonic acid + aminophenazone.

$\xrightarrow{\text{peroxidase}}$ N-(4-antipyryl)-3chloro-5-sulfonate-pbenzo-quinoneimine

3.25.3 Sample

Serum, heparinized plasma or EDTA-plasma, urine. Dilute urine 1 + 10 with distilled water.

3.25.4 Reagents

		Concentration of reagents
1.	Buffer	
	Hepes Buffer	50 mmol/l, pH 7.0
	3,5-Dichloro-2-hydroxy-benzenesulfonic acid	4 mmol/l
2.	Enzyme Reagent	0.9 mmol/l
	4-Aminophenazone	0.25 mmol/l
	Peroxidase	≥1000 U/I
	Uricase	≥ 200 U/l
	Standard	595 µmol/l (10 mg/dl)

3.25.5 Preparation of Solutions

1. **Buffer**

   Contents ready for use. Stable up to the expiry date when stored at + 2^0C to 8^0C.

2. **Enzyme Reagent**

   UA 230 6 × 15 ml

Reconstitute one vial of Enzyme Reagent 2 with 15 ml of Buffer 1. Stable for 21 days at + 2^0C + 8^0C or 5 days at + 15^0C + 25^0C stored protected from light.

3.25.6 Standard

Contents ready for use. Stable up to expiry date when stored at + 2^0C to 8^0C.

3.25.7 Procedure

Wavelength:	520 nm; Hg 546 nm
Cuvette:	1 cm light path
Reaction Temperature:	20^0C-25^0C/37^0C
Measurement:	against reagent blank only one reagent blank per series is required

Pipette into cuvette:

	Reagent Blank (µl)	Standard (µl)	Sample (µl)
Sample	--	20	--
Standard	--	--	20
Reagent	1000	1000	1000

Mix, incubate for 15 minuites at 20^0C –25^0C, or for 5 minutes at 37^0C. Measure absorbance of sample (A_{sample}) against reagent blank within 30 minutes.

3.25.8 Calculation of Uric Acid Concentration

Using a standard:

Serum or plasma

$$\text{Uric acid concentration} = 10 \times \frac{A_{smaple}}{A_{standard}} \text{ (mg/dl)}$$

3.26 Statistical Analysis

All data were analyzed using SPSS 7.5 program (SPSS, Inc., Chicago, IL). All data are expressed in frequencies and mean ± SD unless mentioned otherwise. Comparison between groups were done by One-Way ANOVA. P values ≤ 0.05 were considered as significant.

CHAPTER-4

RESULTS

4.1 Dopamine β-Hydroxylase (DBH) activity in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

DBH Levels were measured by a very sensitive method proposed by Nagatsu et. al in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.[

In 15-26 yrs age group the DBH activity was 20.9±4.3 units (12.4-29.5 nmoles/ min/ ml) in the serum of taxi-drivers, 22.5±4.0 units (14.4-30.6 nmoles/ min/ ml) in the serum rickshaw-pullers and 29.5±3.4 units (22.3-36.6 nmoles/min/ml) in the serum of corresponding rural individuals.

In 27-40 yrs age group the DBH activity was 24.5±3.9 units (16.2-32.8 nmoles/min/ml) in the serum of taxi-drivers, 25.0±3.9 units (18.2-31.8 nmoles/min/ml) in the serum of rickshaw-pullers and 39.4±4.8 units (30.3-48.5 nmoles/min/ml) in the serum of corresponding rural individuals.

In 40-65 yrs age group the DBH activity was 21.9±3.3 units (16.3-28.2 nmoles/min/ml) in the serum of taxi-drivers, 22.9±3.3 units (16.3-30.2 nmoles/min/ml) in the serum of rickshaw-pullers and 34.2±4.3 units (27.5-40.8 nmoles/min/ml) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-1(a) &1(b) and the graphical representations of these results are also shown in figures-1(a) & 1(b).

Table 1(a) : Dopamine β-Hydroxylase (DBH) activity in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	DBH activity (nmoles /min/ ml in Serum)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	22.3-36.6	29.5±3.4	12.4-29.5	20.9±4.3	P< 0.001
Group-2 (27-40)	40	30.3-48.5	39.4±4.8	16.2-32.8	24.5±3.9	P< 0.001
Group-3 (41-65)	30	27.5-40.8	34.2±4.3	16.3-28.2	21.9±3.3	P< 0.001

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Table 1(b) : Dopamine β-Hydroxylase (DBH) activity in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	DBH activity (nmoles /min/ ml in Serum)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	22.3-36.6	29.5±3.4	14.4-30.6	22.5±4.0	P< 0.001
Group-2 (27-40)	40	30.3-48.5	39.4±4.8	18.2-31.8	25.0±3.9	P< 0.001
Group-3 (41-65)	30	27.5-40.8	34.2±4.3	16.3-30.2	22.9±3.3	P< 0.001

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

4.2 Ascorbic acid (Vit-C) levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Ascorbic acid was measured by Di-nitrophenyl hydrazine method with modification by lowery et.al. 1945[84], in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group ascorbic acid content was 1.05 ± 0.15 mg/dl (0.83-1.25 mg/dl) in the serum of taxi-drivers, 1.09±0.20 mg/dl (0.88-1.30 mg/dl) in the serum of rickshaw-pullers and 1.32±0.16 mg/dl (1.05-1.60 mg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group ascorbic acid content was 1.24±0.21 mg/dl (0.88-1.60 mg/dl) in the serum of taxi-drivers, 1.32±0.23 mg/dl (0.98-1.66 mg/dl) in the serum of rickshaw-pullers and 1.65±0.22 mg/dl (1.30-2.00 mg/dl) in the serum corresponding rural individuals.

In 41-65 yrs age group ascorbic acid content was 1.03±0.20 mg/dl (0.70-1.35 mg/dl) in the serum of taxi-drivers, 1.13±0.20 mg/dl (0.80-1.45 mg/dl) in the serum of rickshaw-pullers and 1.45±0.26 mg/dl (1.00-1.90 mg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-2(a) &2(b) and the graphical representations of these results are also shown in figures-2(a) & 2(b).

Table-2 (a) Ascorbic acid (Vit-C) levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Ascorbic acid mg/dl in serum)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	1.05-1.60	1.32±0.16	0.83-1.25	1.05± 0.15	P< 0.001
Group-2 (27-40)	40	1.30-2.00	1.65±0.22	0.88-1.60	1.24±0.21	P< 0.001
Group-3 (41-65)	30	1.00-1.90	1.45±0.26	0.70-1.35	1.03±0.20	P< 0.001

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Table-2 (b) Ascorbic acid (Vit-C) levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Ascorbic acid mg/dl in serum)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	1.05-1.60	1.32±0.16	0.88-1.30	1.09± 0.20	P< 0.001
Group-2 (27-40)	40	1.30-2.00	1.65±0.22	0.98-1.66	1.32±0.23	P< 0.001
Group-3 (41-65)	30	1.00-1.90	1.45±0.26	0.80-1.45	1.13±0.20	P< 0.001

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Figure 2(a) : The graphical representations of Ascorbic acid (Vit-C) between rural individuals and taxi-drivers.

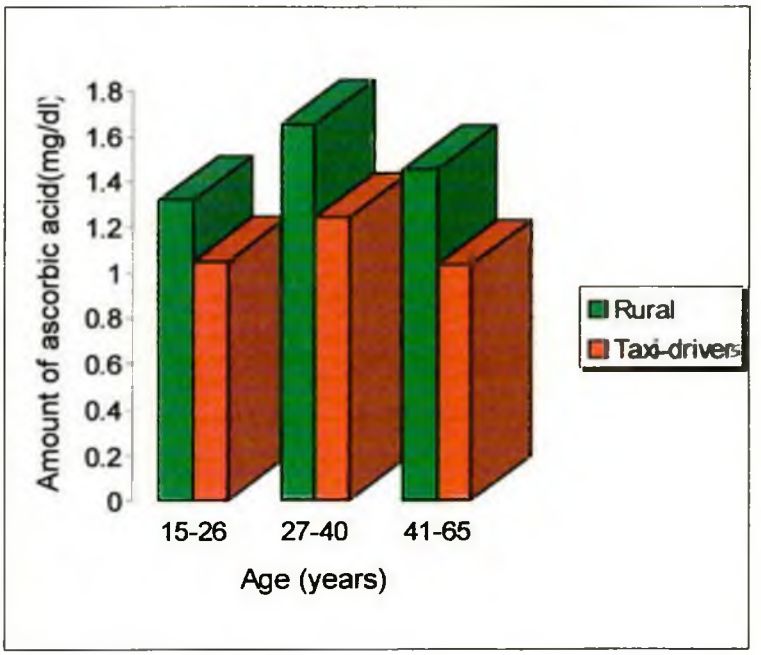
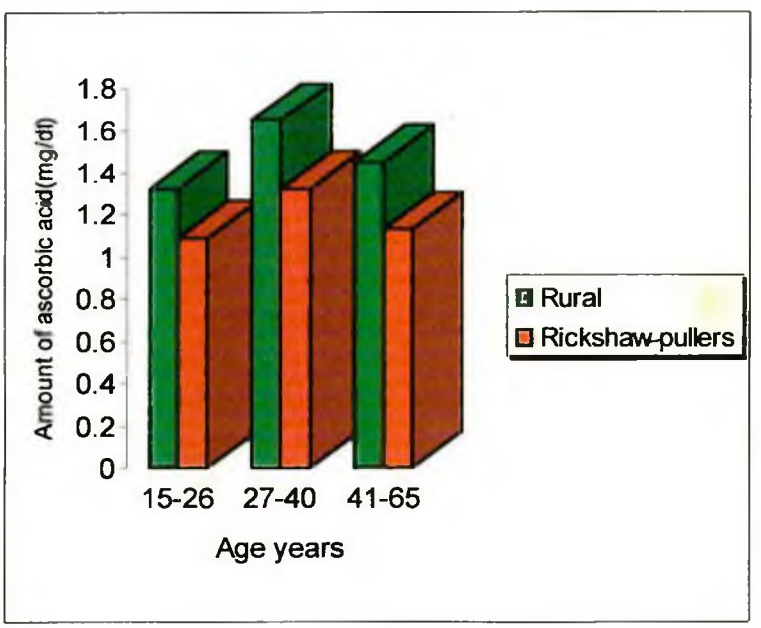


Figure 2(b) : The graphical representations of Ascorbic acid (Vit-C) between rural individuals and rickshaw-pullers.



4.3 Copper levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Copper content was measured by atomic absorption spectrophotometer, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group copper content was 102.7±17.9 μg/dl (72.6-132.8 μg/dl) in the serum of taxi-drivers, 98.7±16.9 μg/dl (68.6-128.8 μg/dl) in the serum of rickshaw-pullers and 97.7±19.2 μg/dl (65.4-124.0 μg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group copper content was 154.4±21.2 μg/dl (112.3-196.5 μg/dl) in the serum of taxi-drivers, 148.4±20.2μg/dl (110.3-186.5μg/dl) in the serum of rickshaw-pullers and 122.5±15.1 μg/dl (96.2-148.6 μg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group copper content was 178.6±16.9 μg/dl (148.5-208.7 μg/dl) in the serum of taxi-drivers, 166.6±16.9μg/dl (144.5-188.7 μg/dl) in the serum of rickshaw-pullers and 115.2±24.6 μg/dl (78.0-152.4 μg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-3(a) &3(b) and the graphical representations of these results are also shown in figures-3(a) & 3(b).

Table-3 (a) Copper levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of copper (μg/dl)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	65.4-124.0	97.7±19.2	72.6-132.8	102.7±17.9	Not Significant
Group-2 (27-40)	40	96.2-148.6	122.5±15.1	112.3-196.5	154.4±21.2	P< 0.001
Group-3 (41-65)	30	78.0-152.4	115.2±24.6	148.5-208.7	178.6±16.9	P< 0.001

$P \leq 0.05$ (significant)

$P < 0.05$ (Moderate significant)

$P < 0.001$ (highly significant)

Table-3 (b) Copper levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of copper (μg/dl)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	65.4-124.0	97.7±19.2	68.6-128.8	98.7±16.9	Not Significant
Group-2 (27-40)	40	96.2-148.6	122.5±15.1	110.3-186.5	148.4±20.2	P< 0.001
Group-3 (41-65)	30	78.0-152.4	115.2±24.6	144.5-188.7	166.6±16.9	P< 0.001

$P \leq 0.05$ (significant)

$P < 0.05$ (Moderate significant)

$P < 0.001$ (highly significant)

4.4 Zinc levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Zinc content was measured by atomic absorption Spectrophotometer, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group zinc content was 107.5±17.2 μg/dl (80-135 μg/dl) in the serum of taxi-drivers, 102.5±16.2μg/dl (75-130μg/dl) in the serum of rickshaw-pullers and 104.7±18.8 μg/dl (70-125μg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group zinc content was 125.0±14.0 μg/dl (100-150 μg/dl) in the serum of taxi-drivers, 117.0±14.0μg/dl (90-144 μg/dl) in the serum of ricksahw-pullers and 108.8±13.8 μg/dl (88-130 μg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group zinc content was 115.0±17.0 μg/dl (90-140 μg/dl) in the serum of taxi-drivers, 110.0±15.0 μg/dl (86-134 μg/dl) in the serum of rickshaw-pullers and 94.5±13.3 μg/dl (75-120 μg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-4(a) &4(b) and the graphical representations of these results are also shown in figures-4(a) & 4(b).

Table-4 (a) Zinc levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of zinc (μg/dl of serum)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	70-125	104.7±18.8	80-135	107.5±17.2	Not significant
Group-2 (27-40)	40	88-130	108.8±13.8	100-150	125.0±14.0	$P < 0.02$
Group-3 (41-65)	30	75-120	94.5±13.3	90-140	115.0±17.0	$P < 0.001$

$P \leq 0.05$ (significant)

$P < 0.05$ (Moderate significant)

$P < 0.001$ (highly significant)

Table-4 (b) Zinc levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of zinc (μg/dl of serum)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	70-125	104.7±18.8	75-130	102.5±16.2	Not significant
Group-2 (27-40)	40	88-130	108.8±13.8	90-144	117.0±14.0	$P < 0.02$
Group-3 (41-65)	30	75-120	94.5±13.3	86-134	110.0±15.0	$P < 0.001$

$P \leq 0.05$ (significant)

$P < 0.05$ (Moderate significant)

$P < 0.001$ (highly significant)

Figure 4(a) : The graphical representations of Zinc levels between rural individuals and taxi-drivers.

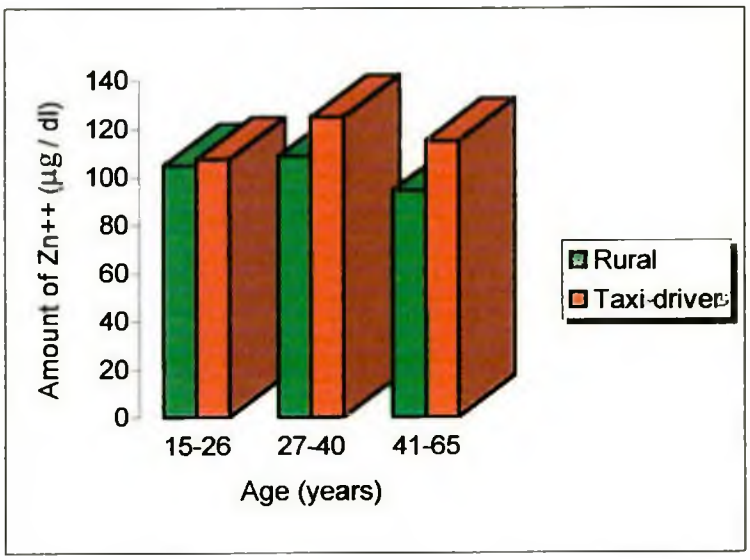
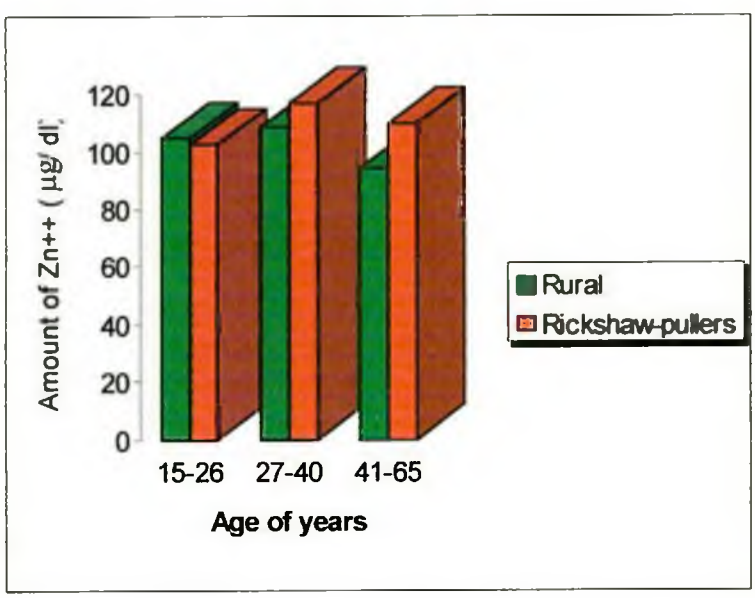


Figure 4(b) : The graphical representations of Zinc levels between rural individuals and rickshaw-pullers.



4.5 Vit-A levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Vit-A was measured by the method of Buri and Tollivers with adaptation from Horustt et.al, in the serum of three different age groups (15-26, 27-41 and 41-65 years of age) and each group contained 30,40,and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group Vit-A content was 38.6±7.9 μg/dl (24.6-52.8 μg/dl) in the serum of taxi-drivers, 40.1±7.7 μg/dl (25.6-54.6 μg/dl) in the serum of rickshaw-pullers and 48.3±9.4 μg/dl (32.4-64.2 μg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group Vit-A content was 46.4±8.4 μg/dl (32.2-60.6 μg/dl) in the serum of taxi-drivers, 43.3±8.6 μg/dl (34.2-62.4 μg/dl) in the serum of rickshaw-pullers and 62.2±8.2 μg/dl (48.2-76.5 μg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group Vit-A content was 48.6±9.2 μg/dl (34.2-62.0 μg/dl) in the serum of taxi-drivers, 50.2±9.4 μg/dl (36.2-64.2 μg/dl) in the serum of rickshaw-pullers and 60.5±9.7 μg/dl (42.4-78.6 μg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-5(a) &5(b) and the graphical representations of these results are also shown in figures-5(a) & 5(b).

Table-5 (a) Vit-A levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Vit-A (μg/dl of serum)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	32.4-64.2	48.3±9.4	24.6-52.8	38.6±7.9	$P < 0.05$
Group-2 (27-40)	40	48.2-76.5	62.2±8.2	32.2-60.6	46.4±8.4	$P < 0.001$
Group-3 (41-65)	30	42.4-78.6	60.5±9.7	34.2-62.0	48.6±9.2	$P < 0.02$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Table-5 (b) Vit-A levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Vit-A (μg/dl of serum)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	32.4-64.2	48.3±9.4	25.6-54.6	40.1±7.7	$P < 0.04$
Group-2 (27-40)	40	48.2-76.5	62.2±8.2	34.2-62.4	43.3±8.6	$P < 0.001$
Group-3 (41-65)	30	42.4-78.6	60.5±9.7	36.2-64.2	50.2±9.4	$P < 0.02$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Figure 5(a) : The graphical representations of Vit-A levels between rural individuals and taxi-drivers.

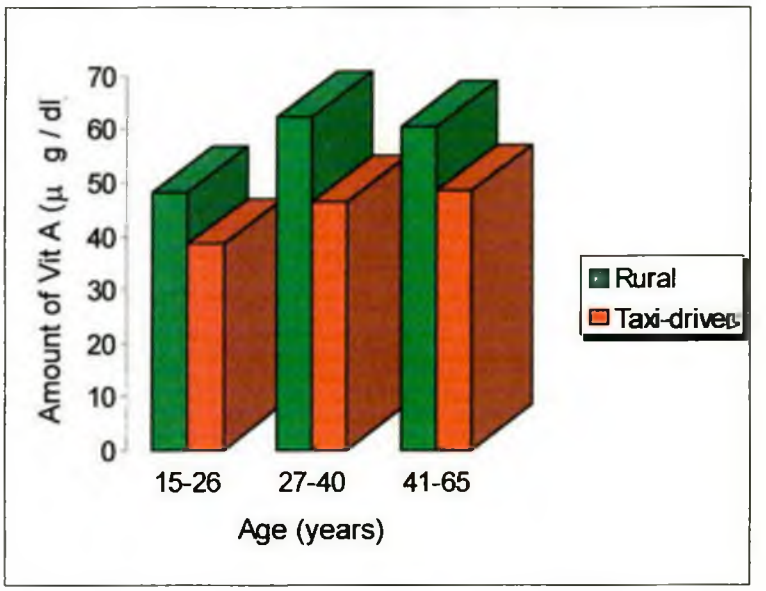
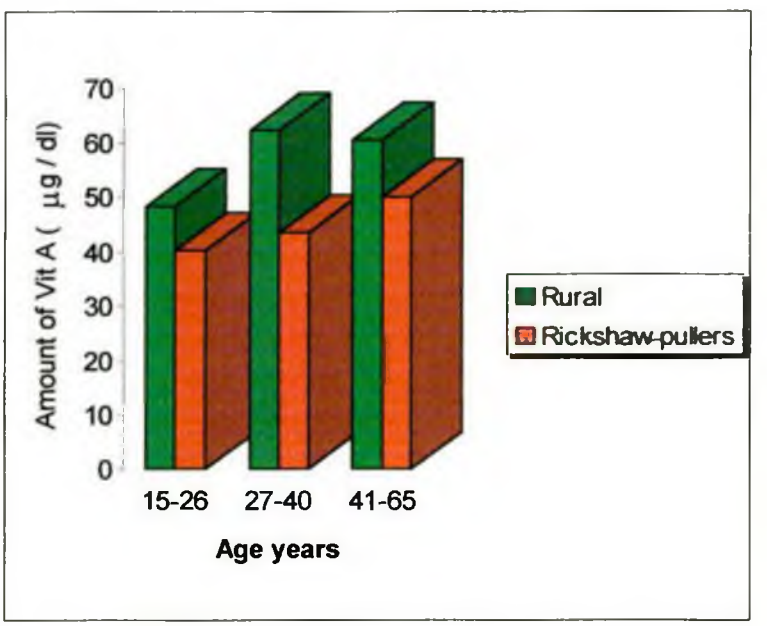


Figure 5(b) : The graphical representations of Vit-A levels between rural individuals and rickshaw-pullers.



4.6 Glucose levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Glucose content was measured by Nelson-Somogy method, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group glucose content was 4.4±0.4 mmol/L (3.8-5.0 mmol/L) in the serum of taxi-drivers, 4.5±0.4 mmol/L (3.8-5.2 mmol/L) int the serum of rickshaw-pullers and 4.1±0.3 mmol/L (3.6-4.6 mmol/L) in the serum of corresponding rural individuals.

In 27-40 yrs age group glucose content was 5.4±0.4 mmol/L (4.8-6.0 mmol/L) in the serum of taxi-drivers, 5.3±0.3 mmol/L (4.7-6.0 mmol/L) in the serum of rickshaw-pullers and 4.8±0.5 mmol/L (4.0-5.6 mmol/L) in the serum of corresponding rural individuals.

In 40-65 yrs age group glucose content was 6.8±0.5 mmol/L (6.0-7.6 mmol/L) in the serum of taxi-drivers, 6.8±0.5 mmol/L (6.2-7.4 mmo/L) in the serum of rickshaw-pullers and 5.5±0.3 mmol/L (5.0-6.0 mmol/L) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-6(a) &6(b) and the graphical representations of these results are also shown in figures-6(a) & 6(b).

Table-6 (a) Glucose levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of glucose (mmol/L of serum)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	3.6-4.6	4.1±0.3	3.8-5.0	4.4±0.4	P < 0.03
Group-2 (27-40)	40	4.0-5.6	4.8±0.5	4.8-6.0	5.4±0.4	P < 0.02
Group-3 (41-65)	30	5.0-6.0	5.5±0.3	6.0-7.6	6.8±0.5	P < 0.001

P ≤ 0.05 (significant)

P < 0.05 (Moderate significant)

P < 0.001 (highly significant)

Table-6 (b) Glucose levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of glucose (mmol/L of serum)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	3.6-4.6	4.1±0.3	3.8-5.2	4.5±0.4	P < 0.03
Group-2 (27-40)	40	4.0-5.6	4.8±0.5	4.7-6.0	5.3±0.3	P < 0.02
Group-3 (41-65)	30	5.0-6.0	5.5±0.3	6.2-7.4	6.8±0.5	P < 0.001

P ≤ 0.05 (significant)

P < 0.05 (Moderate significant)

P < 0.001 (highly significant)

Figure 6(a) : The graphical representations of Glucose levels between rural individuals and taxi-drivers.

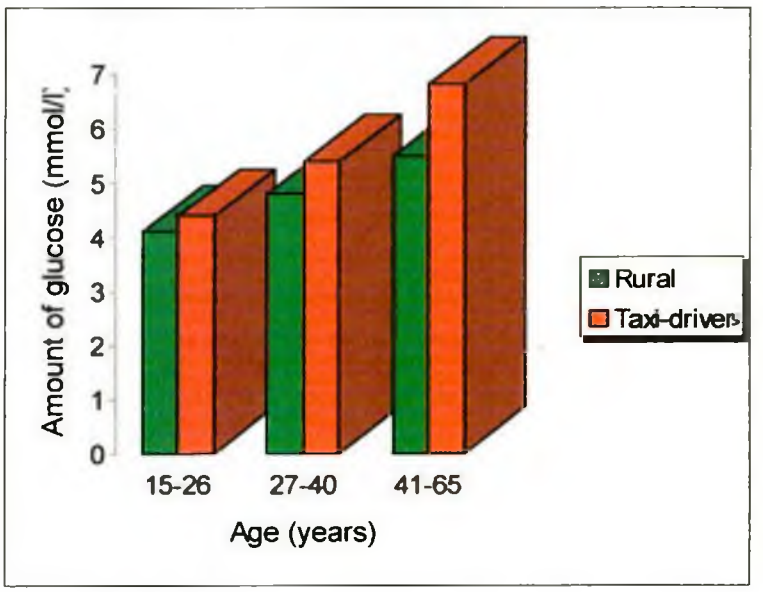


Figure 6(b) : The graphical representations of Glucose levels between rural individuals and rickshaw-pullers.

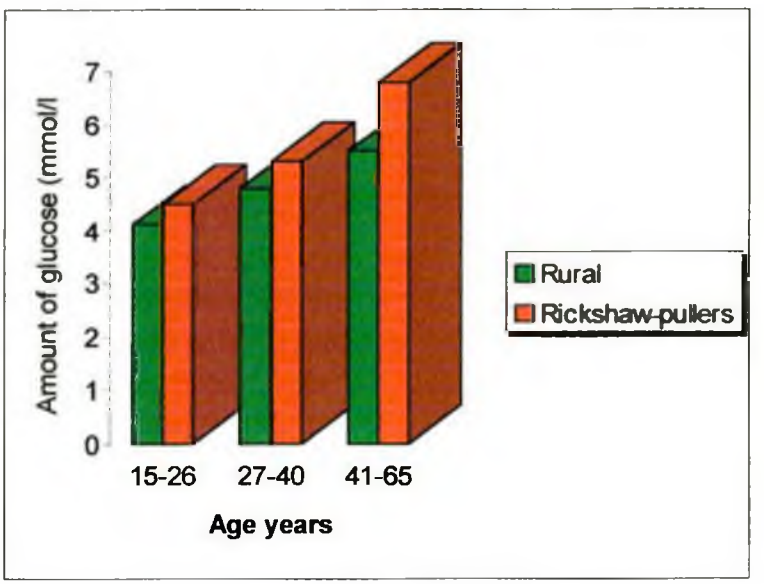


Table-7 (a) Protein Levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Total Protein (gm/L				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	66.0-82.0	74.0±5.4	58.4-68.0	63.2±3.4	$P < 0.001$
Group-2 (27-40)	40	64.2-80.4	72.3±5.4	56.0-64.2	60.1±2.3	$P < 0.02$
Group-3 (41-65)	30	64.3-72.6	68.4±2.7	44.0-62.4	53.2±5.7	$P < 0.001$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Table-7 (b) Protein Levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Total Protein (gm/L				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	66.0-82.0	74.0±5.4	58.4-66.0	62.2±3.4	$P < 0.001$
Group-2 (27-40)	40	64.2-80.4	72.3±5.4	57.0-64.9	60.9±2.3	$P < 0.02$
Group-3 (41-65)	30	64.3-72.6	68.4±2.7	46.0-62.4	54.2±5.5	$P < 0.001$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Figure 7(a) : The graphical representations of Protein Levels between rural individuals and taxi-drivers.

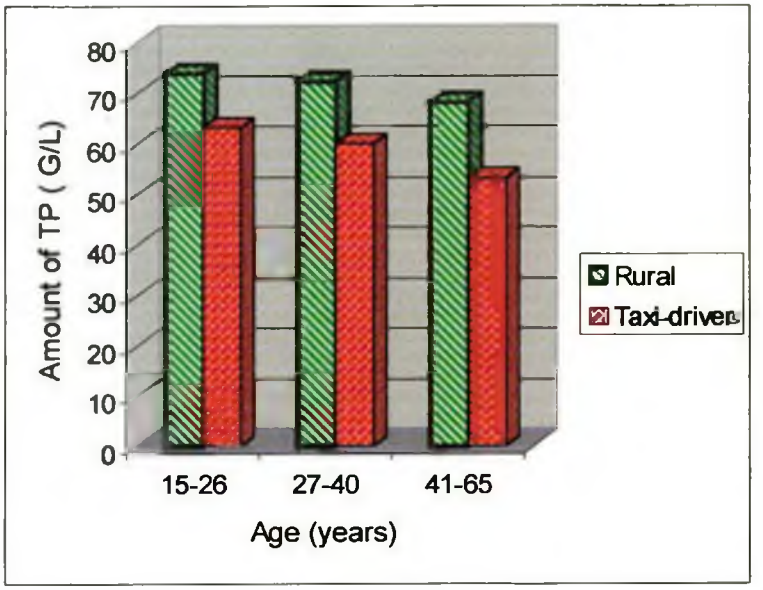
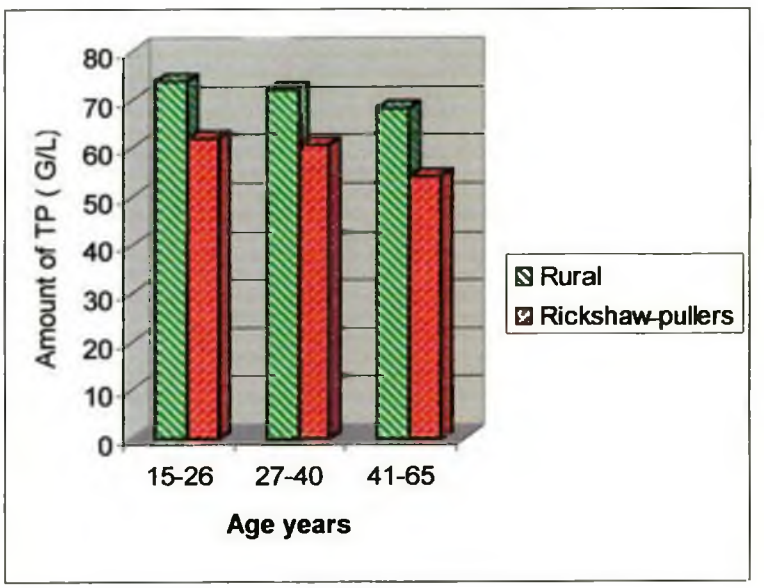


Figure 7(b) : The graphical representations of Protein Levels between rural individuals and rickshaw-pullers.



4.8 Albumin (ALB) levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Serum albumin was measured by spectrophotometric method, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group albumin content was 32.0±1.4 gm/L (30.0-34.0 gm/L) in the serum of taxi-drivers, 33.0±1.6 gm/L (30.0-36.0 gm/L) in the serum of rickshaw-pullers and 35.0±2.0 gm/L (32.0-38.0 gm/L) in the serum of corresponding rural individuals.

In 27-40 yrs age group albumin content was 31.0±3.1 gm/L (26.0-36.0 gm/L) in the serum of taxi-drivers, 32.0±2.2 gm/L (28.0-36.0 gm/L) in the serum of rickshaw-pullers and 37.0±2.2 gm/L (34.0-40.0 gm/L) in the serum of corresponding rural individuals.

In 41-65 yrs age group albumin content was 30.0±1.5 gm/L (28.0-34.0 gm/L) in the serum of taxi-drivers, 31.0±2.8 gm/L (29.0-35.0 gm/L) in the serum of rickshaw-pullers and 38.8±2.1 gm/L (36.0-40.0 gm/L) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-8(a) &8(b) and the graphical representations of these results are also shown in figures-8(a) & 8(b).

Table-8 (a) Albumin (ALB) levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Albumin (gm/l))				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	32.0-38.0	35.0±2.0	30.0-34.0	32.0±1.4	P < 0.03
Group-2 (27-40)	40	34.0-40.0	37.0±2.2	26.0-36.0	31.0±3.1	P < 0.02
Group-3 (41-65)	30	36.0-40.0	38.8±2.1	28.0-34.0	30.0±1.5	P < 0.001

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Table-8 (b) Albumin (ALB) levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Albumin (gm/l))				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	32.0-38.0	35.0±2.0	30.0-36.0	33.0±1.6	P < 0.03
Group-2 (27-40)	40	34.0-40.0	37.0±2.2	28.0-36.0	32.0±2.2	P < 0.02
Group-3 (41-65)	30	36.0-40.0	38.8±2.1	29.0-35.0	31.0±2.8	P < 0.001

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Figure 8(a) : The graphical representations of Albumin (ALB) levels between rural individuals and taxi-drivers.

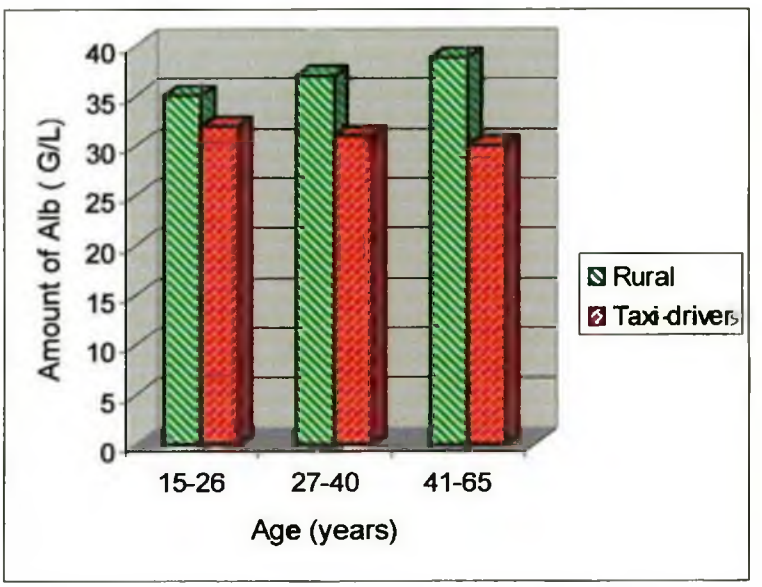
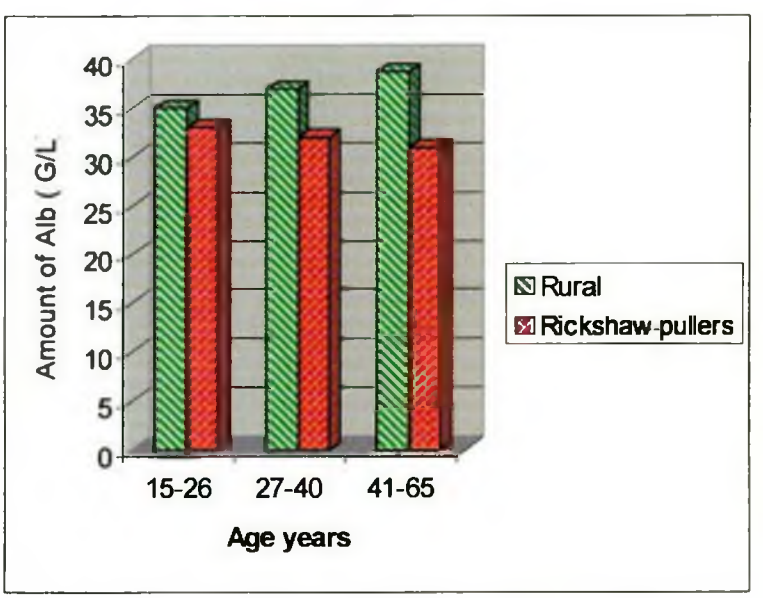


Figure 8(b) : The graphical representations of Albumin (ALB) levels between rural individuals and rickshaw-pullers.



4.9 Urea Levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Urea was measured by the spineact method, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group urea content was 36±7.4 mg/dl (25-48 mg/dl) in the serum of taxi-drivers, 34±7.2 mg/dl (25-44 mg/dl) in the serum of rickshaw-pullers and 16±2.5 mg/dl (12-20 mg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group urea content was 42±5.8 mg/dl (30-54 mg/dl) in the serum of taxi-drivers, 40±5.8 mg/dl (28-52 mg/dl) in the serum of rickshaw-pullers and 24±4.0 mg/dl (18-30 mg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group urea content was 52±6.1 mg/dl (40-60 mg/dl) in the serum of taxi-drivers, 50±6.1 mg/dl (40-56 mg/dl) in the serum of rickshaw-pullers and 39±5.5 mg/dl (30-48 mg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-9(a) &9(b) and the graphical representations of these results are also shown in figures-9(a) & 9(b).

Table-9 (a) Urea Levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of urea (mg/ dl of serum)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	12-20	16±2.5	25-48	36±7.4	P < 0.02
Group-2 (27-40)	40	18-30	24±4.0	30-54	42±5.8	P < 0.03
Group-3 (41-65)	30	30-48.0	39±5.5	40-60	52±6.1	P < 0.02

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Table-9 (b) Urea Levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of urea (mg/ dl of serum)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	12-20	16±2.5	25-44	34±7.2	P < 0.02
Group-2 (27-40)	40	18-30	24±4.0	28-52	40±5.8	P < 0.04
Group-3 (41-65)	30	30-48.0	39±5.5	40-56	50±6.1	P < 0.02

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Figure 9(a) : The graphical representations of Urea Levels between rural individuals and taxi-drivers.

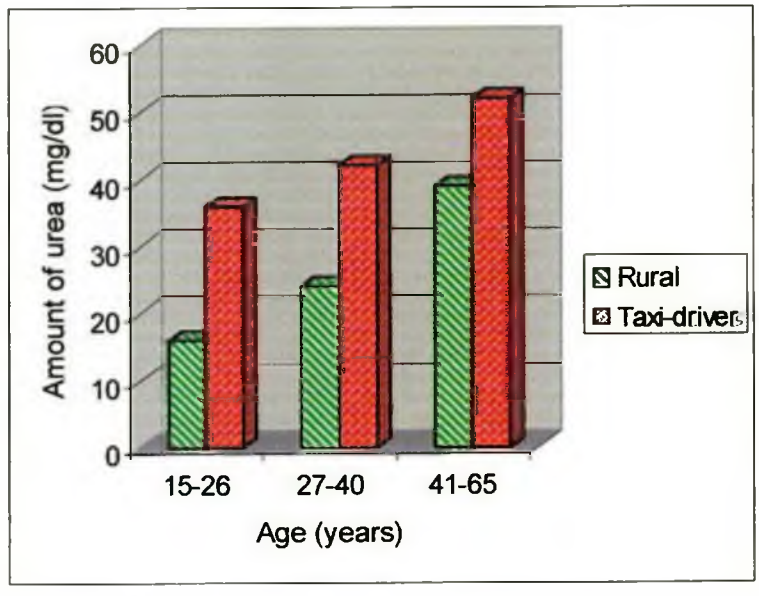
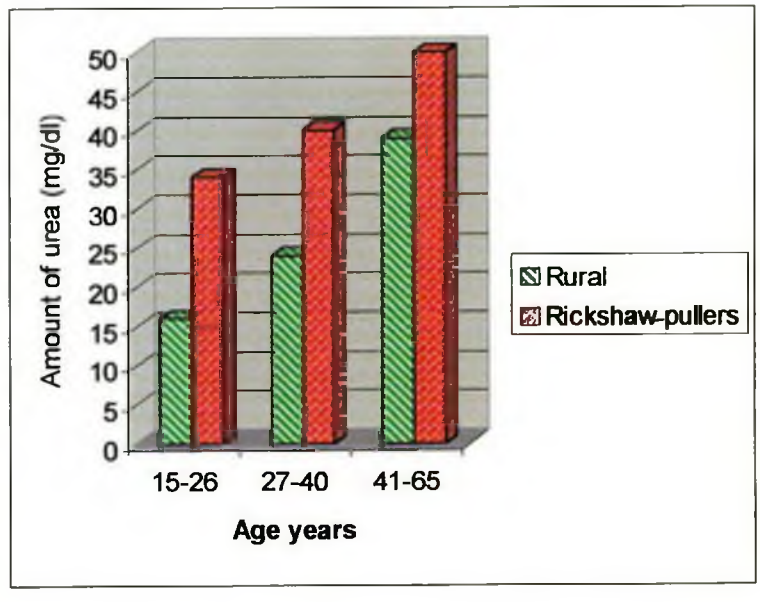


Figure 9(b) : The graphical representations of Urea Levels between rural individuals and rickshaw-pullers.



4.10 Creatinine levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Creatinine was measured by using the reaction with alkaline picrate with spectrophotometric method, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group creatinine content was 0.95±0.17 mg/dl (0.7-1.2 mg/dl) in the serum of taxi-drivers, 0.95±0.17 mg/dl (0.7-1.2 mg/dl) in the serum of rickshaw-pullers and 0.85±0.15 mg/dl (0.7-1.0 mg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group creatinine content was 1.2±0.2 mg/dl (0.8-1.4 mg/dl) in the serum of taxi-drivers, 1.1±0.2 mg/dl (0.8-1.3 mg/dl) in the serum of rickshaw-pullers and 1.0±0.14 mg/dl (0.8-1.2 mg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group creatinine content was 1.2±0.2 mg/dl (1.0-1.4 mg/dl) in the serum of taxi-drivers, 1.15±0.2 mg/dl (1.0-1.2 mg/dl) in the serum of rickshaw-pullers and 1.1±0.15 mg/dl (0.9-1.3 mg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-10(a) &10(b) and the graphical representations of these results are also shown in figures-10(a) & 10(b).

Table-10 (a) Creatinine levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of creatinine (mg/ dl of serum)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	0.7-1.0	0.85±0.15	0.7-1.2	0.95±0.17	Not significant
Group-2 (27-40)	40	0.8-1.2	1.0±0.14	0.8-1.4	1.2±0.2	$P < 0.04$
Group-3 (41-65)	30	0.9-1.3	1.1±0.15	1.0-1.4	1.2±0.2	Not significant

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Table-10 (b) Creatinine levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of creatinine (mg/ dl of serum)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	0.7-1.0	0.85±0.15	0.7-1.2	0.95±0.17	Not significant
Group-2 (27-40)	40	0.8-1.2	1.0±0.14	0.8-1.3	1.1±0.2	$P < 0.04$
Group-3 (41-65)	30	0.9-1.3	1.1±0.15	1.0-1.2	1.15±0.2	Not significant

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Figure 10(a) : The graphical representations of Creatinine levels between rural individuals and taxi-drivers.

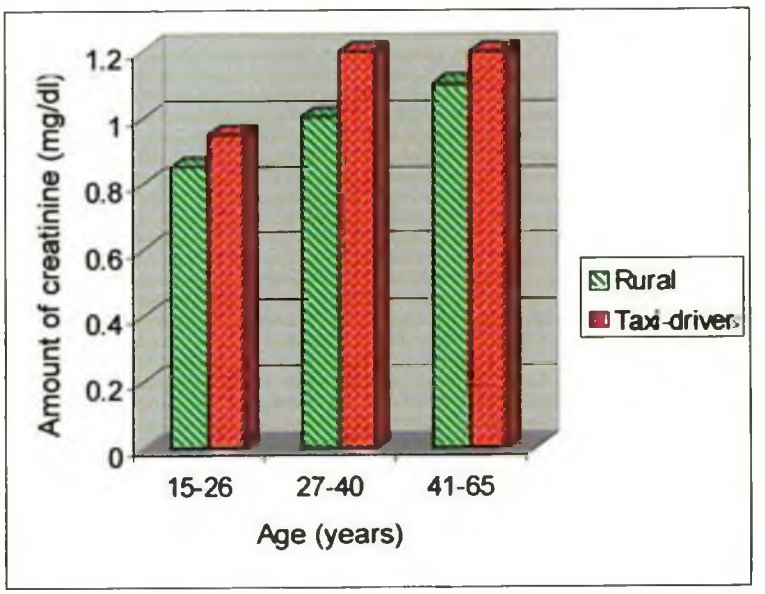
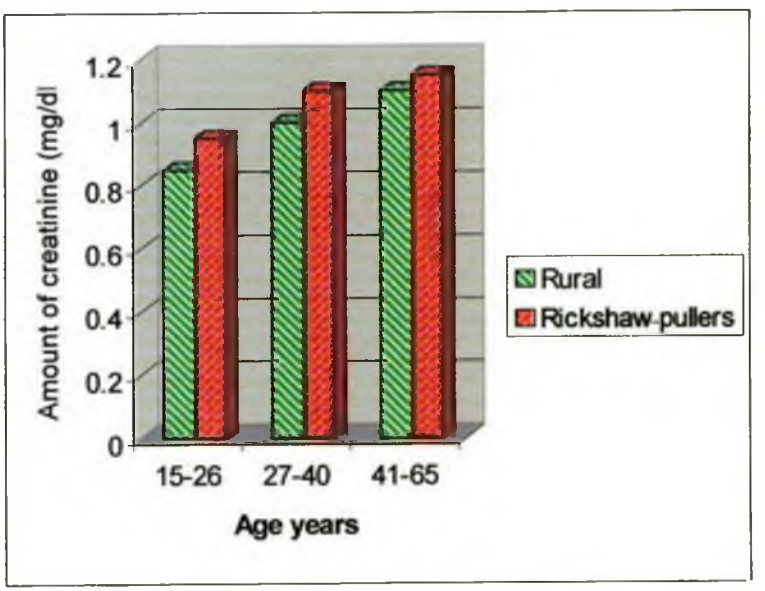


Figure 10(b) : The graphical representations of Creatinine levels between rural individuals and rickshaw-pullers.



4.11 sGPT activities in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Amino transferase glutamate pyruvate transaminase (GPT) was measured by the method of Retiman and Frankel, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group GPT content was 30.4±3.4 U/L (25.3-35.5 U/L) in the serum of taxi-drivers, 32.4±3.4 U/L (26.3-36.5 U/L) in the serum of rickshaw-pullers and 19.3±4.5 U/L (12.2-26.4 U/L) in the serum of corresponding rural individuals.

In 27-40 yrs age group GPT content was 40.6±5.8 U/L (30.4-50.8 U/L) in the serum of taxi-drivers, 41.1±5.8 U/L (30.4-51.8 U/L) in the serum of rickshaw-pullers and 22.5±4.7 U/L (14.4-30.6 U/L) in the serum of corresponding rural individuals.

In 41-65 yrs age group GPT content was 49.7±7.2 U/L (38.6-60.8 U/L) in the serum of taxi-drivers, 48.7±7.2 U/L (38.6-58.8 U/L) in the serum of rickshaw-pullers and 29.5±4.2 U/L (22.4-36.6 U/L) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-11(a) &11(b) and the graphical representations of these results are also shown in figures-11(a) & 11(b).

Table-11 (a) sGPT activities in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	sGPT activity (U/L of serum)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	12.2-26.4	19.3±4.5	25.3-35.5	30.4±3.4	$P < 0.001$
Group-2 (27-40)	40	14.4-30.6	22.5±4.7	30.4-50.8	40.6±5.8	$P < 0.001$
Group-3 (41-65)	30	22.4-36.6	29.5±4.2	38.6-60.8	49.7±7.2	$P < 0.001$

$P \leq 0.05$ (significant)

$P < 0.05$ (Moderate significant)

$P < 0.001$ (highly significant)

Table-11 (b) sGPT activities in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	sGPT activity (U/L of serum)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	12.2-26.4	19.3±4.5	26.3-36.5	32.4±3.4	$P < 0.001$
Group-2 (27-40)	40	14.4-30.6	22.5±4.7	30.4-51.8	41.1±5.8	$P < 0.001$
Group-3 (41-65)	30	22.4-36.6	29.5±4.2	38.6-58.8	48.7±7.2	$P < 0.001$

$P \leq 0.05$ (significant)

$P < 0.05$ (Moderate significant)

$P < 0.001$ (highly significant)

Figure 11(a) : The graphical representations of sGPT activities between rural individuals and taxi-drivers.

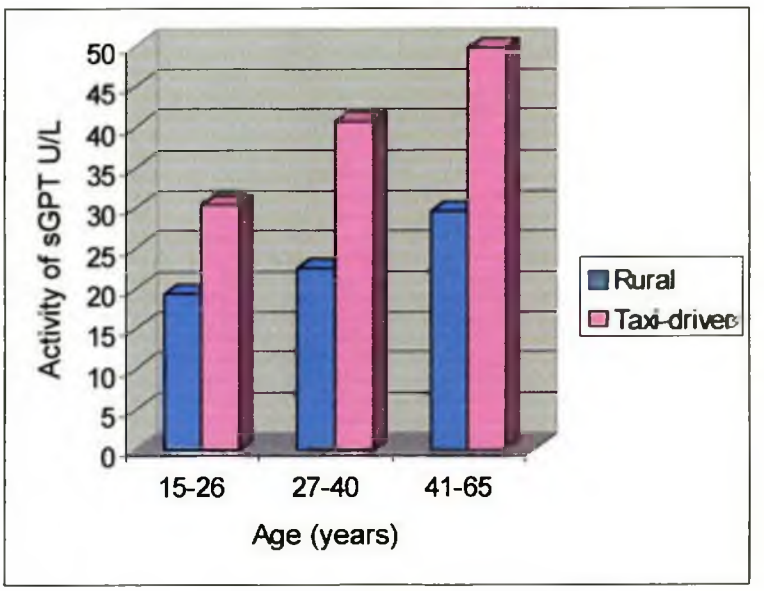
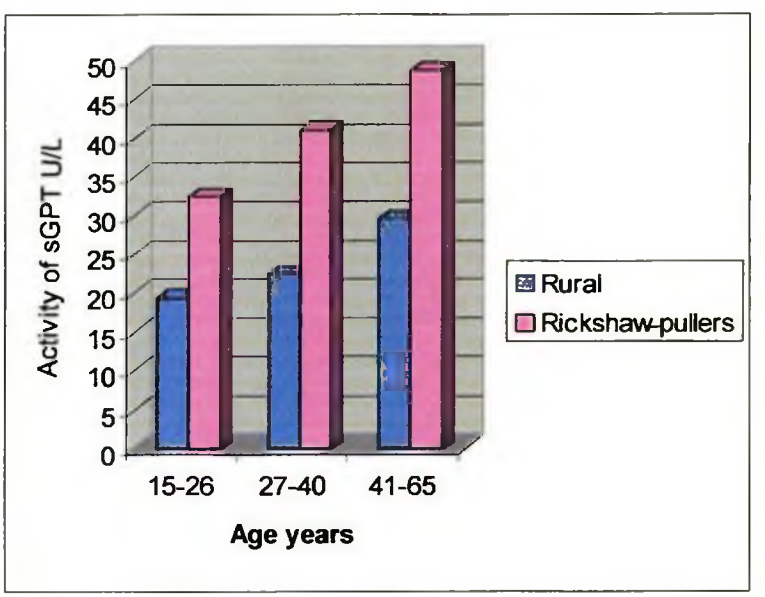


Figure 11(b) : The graphical representations of sGPT activities between rural individuals and rickshaw-pullers.



4.12 sGOT activitiy in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Amino transferase glutamate Oxaloacetate transaminase (GOT) was measured by the method of Retiman and Frankel, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group GOT content was 28.3±6.4 U/L (18.4-38.3 U/L) in the serum of taxi-drivers, 30.3±6.4 U/L (20.4-38.3 U/L) in the serum of rickshaw-pullers and 15.4±4.9 U/L (8.2-22.6 U/L) in the serum of corresponding rural individuals.

In 27-40 yrs age group GOT content was 40.3±9.8 U/L (24.1-56.2 U/L) in the serum of taxi-drivers, 44.3±8.8 U/L (26.1-58.2 U/L) in the serum of rickshaw-pullers and 30.3±8.5 U/L (12.4-42.3 U/L) in the serum of corresponding rural individuals.

In 41-65 yrs age group GOT content was 47.5±13.1 U/L (28.3-66.6 U/L) in the serum of taxi-drivers, 47.5±10.1 U/L (30.3-62.6 U/L) in the serum of rickshaw-pullers and 37.6±10.5 U/L (14.5-48.7 U/L) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-12(a) &12(b) and the graphical representations of these results are also shown in figures-12(a) & 12(b).

Table-12 (a) sGOT activitiy in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	sGOT activity (U/L of serum)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	8.2-22.6	15.4±4.9	18.4-38.3	28.3±6.4	P < 0.001
Group-2 (27-40)	40	12.4-42.3	30.3±8.5	24.1-56.2	40.3±9.8	P < 0.03
Group-3 (41-65)	30	14.5-48.7	37.6±10.5	28.3-66.6	47.5±13.1	P < 0.001

P ≤ 0.05 (significant)

P < 0.05 (Moderate significant)

P < 0.001 (highly significant)

Table-12 (b) sGOT activitiy in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	sGOT activity (U/L of serum)				Level of Significance
		Rural individuals		Rickshaw-puller		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	8.2-22.6	15.4±4.9	20.4-38.3	30.3±6.4	P < 0.001
Group-2 (27-40)	40	12.4-42.3	30.3±8.5	26.1-58.2	44.3±8.8	P < 0.03
Group-3 (41-65)	30	14.5-48.7	37.6±10.5	30.3-62.6	47.5±10.1	P < 0.001

P ≤ 0.05 (significant)

P < 0.05 (Moderate significant)

P < 0.001 (highly significant)

Figure 12(a) : The graphical representations of sGOT activities between rural individuals and taxi-drivers.

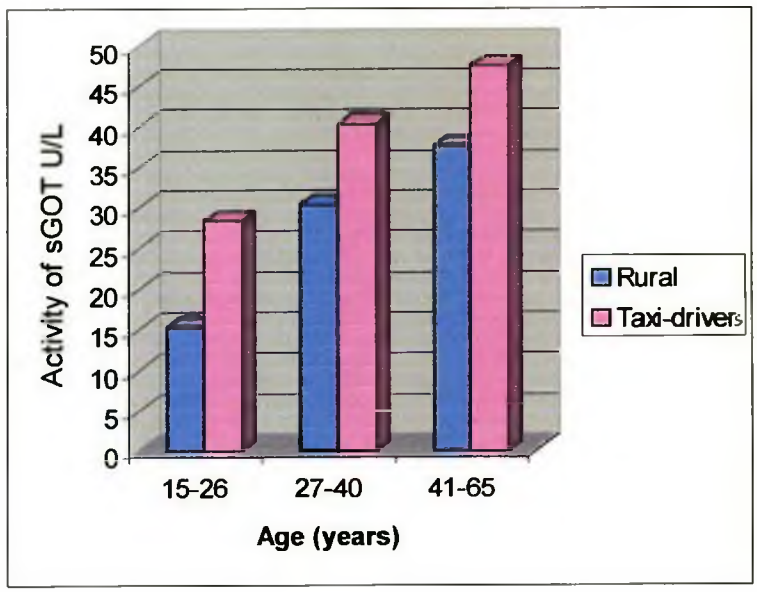


Figure 12(b) : The graphical representations of sGOT activities between rural individuals and rickshaw-pullers.

4.13 Alkaline-phosphatase (ALP) activities in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Alkaline phosphatase was measured by colorimetric method in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group alkaline phosphatase content was 102±16 U/L (86-118 U/L) in the serum of taxi-drivers, 104.±16 U/L (88-118 U/L) in the serum of rickshaw-pullers and 65±9 U/L (56-74 U/L) in the serum of corresponding rural individuals.

In 27-40 yrs age group alkaline phosphatase content was 117±10 U/L (108-126 U/L) in the serum of taxi-drivers, 120±10 U/L (112-130 U/L) in the serum of rickshaw-pullers and 74±10 U/L (64-84 U/L) in the serum of corresponding rural individuals.

In 41-65 yrs age group alkaline phosphatase content was 140±20 U/L (120-160 U/L) in the serum of taxi-drivers, 142±15 U/L (124-160 U/L) in the serum of rickshaw-pullers and 86±10 U/L (76-90 U/L) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-13(a) &13(b) and the graphical representations of these results are also shown in figures-13(a) & 13(b).

Table-13 (a) Alkaline-phosphatase (ALP) activities in the serum of three different age groups of rural individuals and air polluted taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Alkaline-phosphatase activity (U/L of serum)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	56-74	65±9	86-118	102±16	$P < 0.02$
Group-2 (27-40)	40	64-84	74±10	108-126	117±10	$P < 0.03$
Group-3 (41-65)	30	76-96	86±10	120-160	140±20	$P < 0.001$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Table-13 (b) Alkaline-phosphatase (ALP) activities in the serum of three different age groups of rural individuals and air polluted rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Alkaline-phosphatase activity (U/L of serum)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	56-74	65±9	88-118	104±16	$P < 0.02$
Group-2 (27-40)	40	64-84	74±10	112-130	120±10	$P < 0.03$
Group-3 (41-65)	30	76-96	86±10	124-160	142±15	$P < 0.02$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Figure 13(a) : The graphical representations of Alkaline-phosphatase (ALP) activities between rural individuals and taxi-drivers.

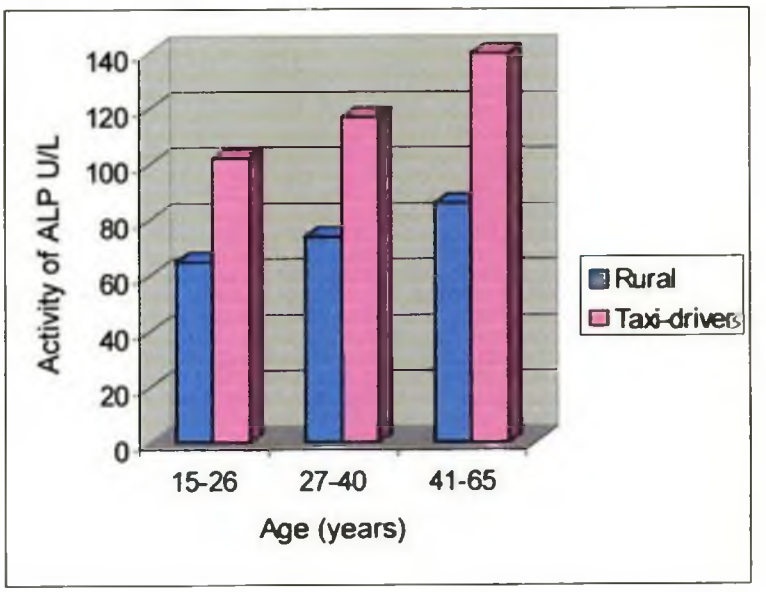
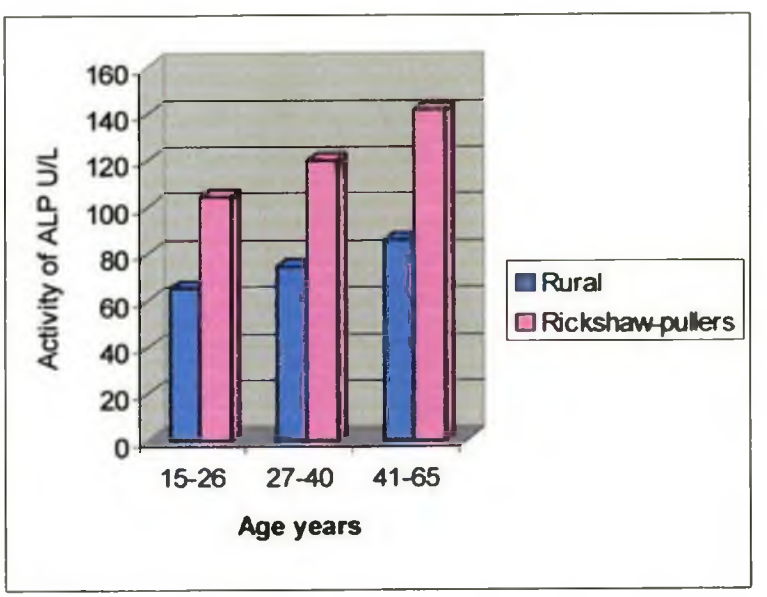


Figure 13(b) : The graphical representations of Alkaline-phosphatase (ALP) activities between rural individuals and rickshaw-pullers.



4.14 Bilirubin levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Total bilirubin was measured in the presence of caffeine by the reaction with diazotised sulphanillic acid, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group bilirubin content was 0.65±0.05 mg/dl (0.6-0.7 mg/dl) in the serum of taxi-drivers, 0.70±0.10 mg/dl (0.6-0.8 mg/dl) in the serum of rickshaw-pullers and 0.5±0.05 mg/dl (0.4-0.6 mg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group bilirubin content was 0.9±0.10 mg/dl (0.8-1.0 mg/dl) in the serum of taxi-drivers, 0.95±0.15 mg/dl (0.8-1.1 mg/dl) in the serum of rickshaw-pullers and 0.8±0.20 mg/dl (0.6-1.0 mg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group bilirubin content was 1.0±0.20 mg/dl (0.8-1.2 mg/dl) in the serum of taxi-drivers, 1.0±0.20 mg/dl (0.8-1.2 mg/dl) in the serum of rickshaw-pullers and 0.8±0.20 mg/dl (0.6-1.0 mg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-14(a) &14(b) and the graphical representations of these results are also shown in figures-14(a) & 14(b).

Table-14 (a) Bilirubin levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Bilirubin (mg/ dl of serum)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	0.4-0.6	0.5±0.05	0.6-0.7	0.65±0.05	$P < 0.02$
Group-2 (27-40)	40	0.6-1.0	0.8±0.20	0.8-1.0	0.9±0.10	$P < 0.05$
Group-3 (41-65)	30	0.6-1.0	0.8±0.20	0.8-1.2	1.0±0.20	$P < 0.02$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Table-14 (b) Bilirubin levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Bilirubin (mg/ dl of serum)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	0.4-0.6	0.5±0.05	0.6-0.8	0.70±0.10	$P < 0.02$
Group-2 (27-40)	40	0.6-1.0	0.8±0.2	0.8-1.1	0.95±0.15	$P < 0.05$
Group-3 (41-65)	30	0.6-1.0	0.8±0.20	0.8-1.2	1.0±0.20	$P < 0.02$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Figure 14(a) : The graphical representations of Bilirubin levels between rural individuals and taxi-drivers.

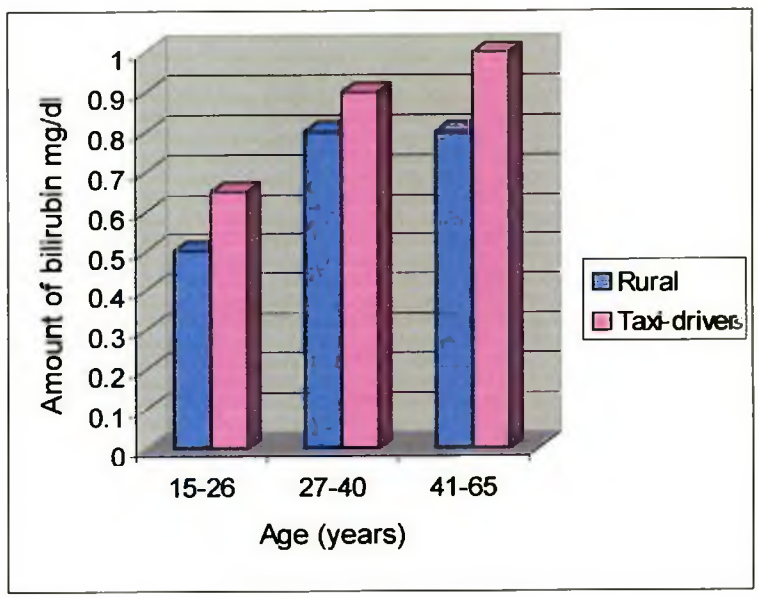
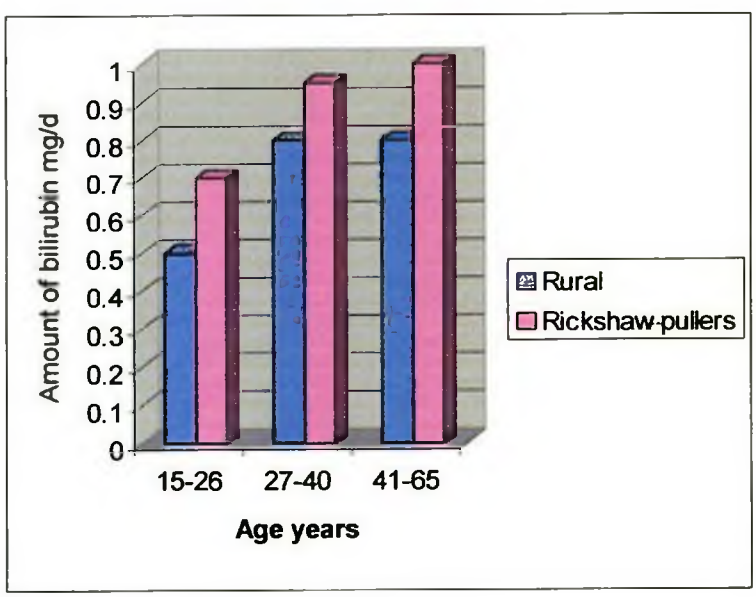


Figure 14(b) : The graphical representations of Bilirubin levels between rural individuals and rickshaw-pullers.



4.15 Cholesterol levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Cholesterol was measured by Liebermann-Burchard method[91], in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group Cholesterol content was 109±18.8 mg/dl (80-138 mg/dl) in the serum of taxi-drivers, 99±16.8 mg/dl (70-128 mg/dl) in the serum of rickshaw-pullers and 164±10.5 mg/dl (148-180 mg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group Cholesterol content was 124±16.6 mg/dl (96-152 mg/dl) in the serum of taxi-drivers, 117±15.4 mg/dl (86-148 mg/dl) in the serum of rickshaw-pullers and 176±14.5 mg/dl (168-184 mg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group Cholesterol content was 130±17.5 mg/dl (100-158 mg/dl) in the serum of taxi-drivers, 124±16.5 mg/dl (98-152 mg/dl) in the serum of rickshaw-pullers and 167±18.1 mg/dl (138-196 mg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-15(a) &15(b) and the graphical representations of these results are also shown in figures-15(a) & 15(b).

Table-15 (a) Cholesterol levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Cholesterol (mg/ dl)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	148-180	164±10.5	80-138	109±18.8	P < 0.001
Group-2 (27-40)	40	168-184	176±14.5	96-152	124±16.6	P < 0.02
Group-3 (41-65)	30	138-196	167±18.1	100-158	130±17.5	P < 0.001

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Table-15 (b) Cholesterol levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Cholesterol (mg/ dl)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	148-180	164±10.5	70-128	99±16.8	P < 0.001
Group-2 (27-40)	40	168-184	176±14.5	86-148	117±15.6	P < 0.001
Group-3 (41-65)	30	138-196	167±18.1	98-152	124±16.5	P < 0.001

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Figure 15(a) : The graphical representations of Cholesterol levels between rural individuals and taxi-drivers.

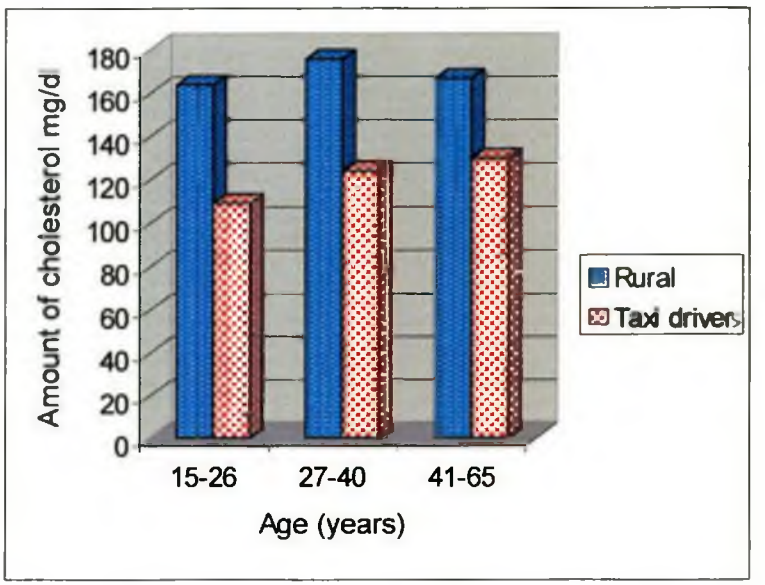
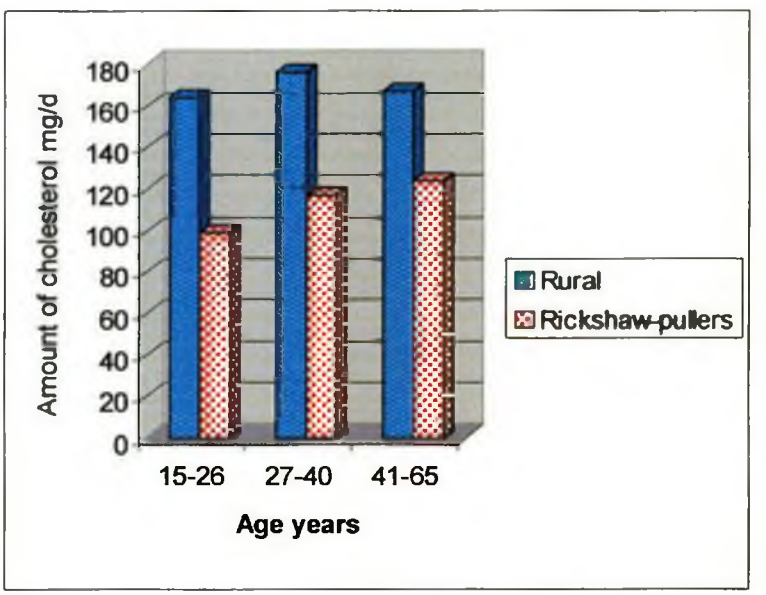


Figure 15(b) : The graphical representations of Cholesterol levels between rural individuals and rickshaw-pullers.



4.16 Triglyceride (Tg) levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Triglyceride (Tg) was measured after enzymetic hydrolysis with lipase by colorimetric method, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group Tg content was 58±7.1 mg/dl (46-70 mg/dl) in the serum of taxi-drivers, 58±7.1 mg/dl (48-58 mg/dl) in the serum of rickshaw-pullers and 69 ±7.2 mg/dl (58-80 mg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group Tg content was 86±15.7 mg/dl (60-112 mg/dl) in the serum of taxi-drivers, 79±12.7 mg/dl (56-102 mg/dl) in the serum of rickshaw-pullers and 93±19.1 mg/dl (65-120 mg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group Tg content was 94±19.3 mg/dl (62-124 mg/dl) in the serum of taxi-drivers, 90±17.3 mg/dl (62-114 mg/dl) in the serum of rickshaw-pullers and 102±28.0 mg/dl (56-148 mg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-16(a) &16(b) and the graphical representations of these results are also shown in figures-16(a) & 16(b).

Table-16 (a) Triglyceride (Tg) levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Tg (mg/ dl)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	58-80	69 ±7.2	46-70	58±7.1	P < 0.001
Group-2 (27-40)	40	65-120	93±19.1	60-112	86±15.7	Not significant
Group-3 (41-65)	30	56-148	102±28.0	62-124	94±19.3	Not significant

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Table-16 (b) Triglyceride (Tg) levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Tg (mg/ dl)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	58-80	69 ±7.2	48-68	58±7.1	P < 0.001
Group-2 (27-40)	40	65-120	93±19.1	56-102	79±12.7	P < 0.02
Group-3 (41-65)	30	56-148	102±28.0	62-114	90±17.3	P < 0.001

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Figure 16(a) : The graphical representations of Triglyceride (Tg) levels between rural individuals and taxi-drivers.

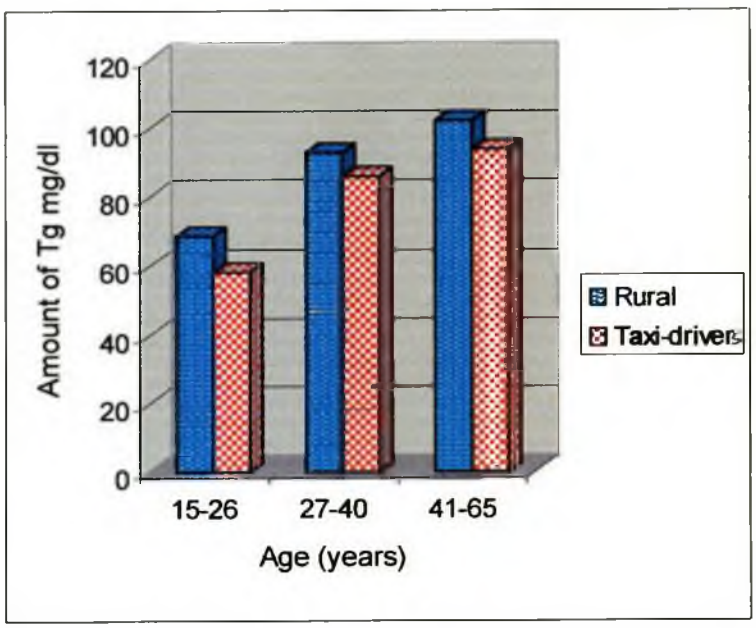
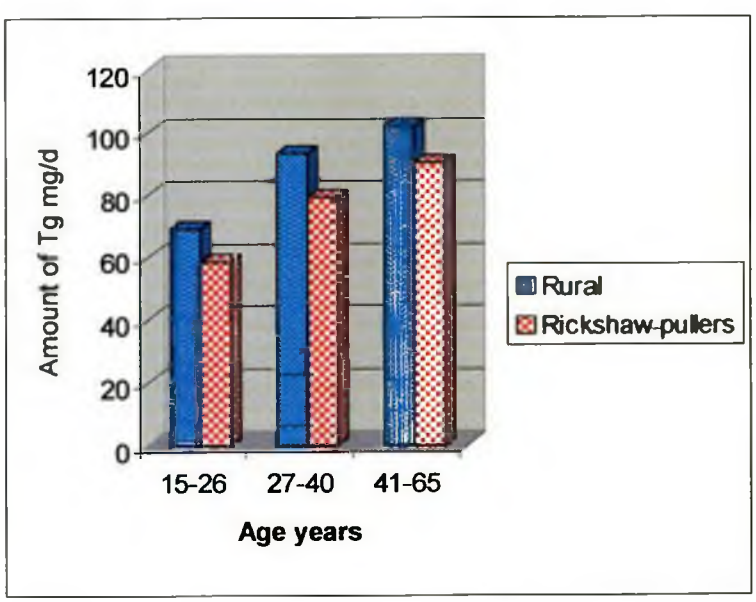


Figure 16(b) : The graphical representations of Triglyceride (Tg) levels between rural individuals and rickshaw-pullers.



4.17 HDL- cholesterol levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

HDL (high density lipoprotein) cholesterol was measured by colorimetric method by the addition of phosphotungstic acid in the presences of magnesium ions, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group HDL cholesterol content was 46±4.2 mg/dl (40-52 mg/dl) in the serum of taxi-drivers, 45±4.2 mg/dl (40-50 mg/dl) in the serum of rickshaw-pullers and 43±3.1 mg/dl (38-48 mg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group HDL cholesterol content was 47±5.2 mg/dl (38-54 mg/dl) in the serum of taxi-drivers, 48±5.2 mg/dl (40-54 mg/dl) in the serum of rickshaw-pullers and 39±3.1 mg/dl (34-44 mg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group HDL cholesterol content was 50±5.8 mg/dl (42-58 mg/dl) in the serum of taxi-drivers, 50±5.8 mg/dl (42-56 mg/dl) in the serum of rickshaw-pullers and 42±3.7 mg/dl (36-48 mg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-17(a) &17(b) and the graphical representations of these results are also shown in figures-17(a) & 17(b).

Table-17 (a) HDL- cholesterol levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of HDL-cholesterol (mg/ dl)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	38-48	43±3.1	40-52	46±4.2	P < 0.04
Group-2 (27-40)	40	34-44	39±3.1	38-54	47±5.2	P < 0.001
Group-3 (41-65)	30	36-48	42±3.7	42-58	50±5.8	P < 0.001

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Table-17 (b) HDL- cholesterol levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of HDL-cholesterol (mg/ dl)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	38-48	43±3.1	40-50	45±4.2	P < 0.04
Group-2 (27-40)	40	34-44	39±3.1	40-54	48±5.2	P < 0.02
Group-3 (41-65)	30	36-48	42±3.7	42-56	50±5.8	P < 0.02

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Figure 17(a) : The graphical representations of HDL- cholesterol levels between rural individuals and taxi-drivers.

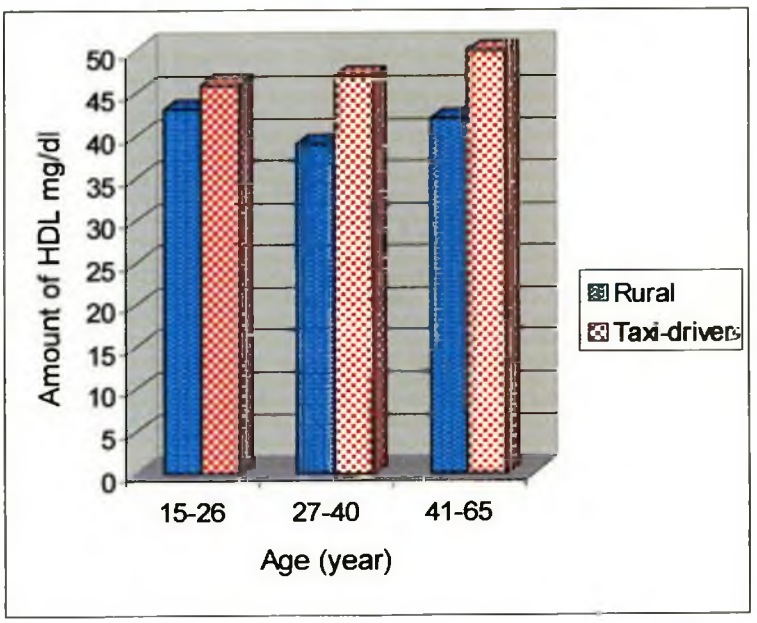
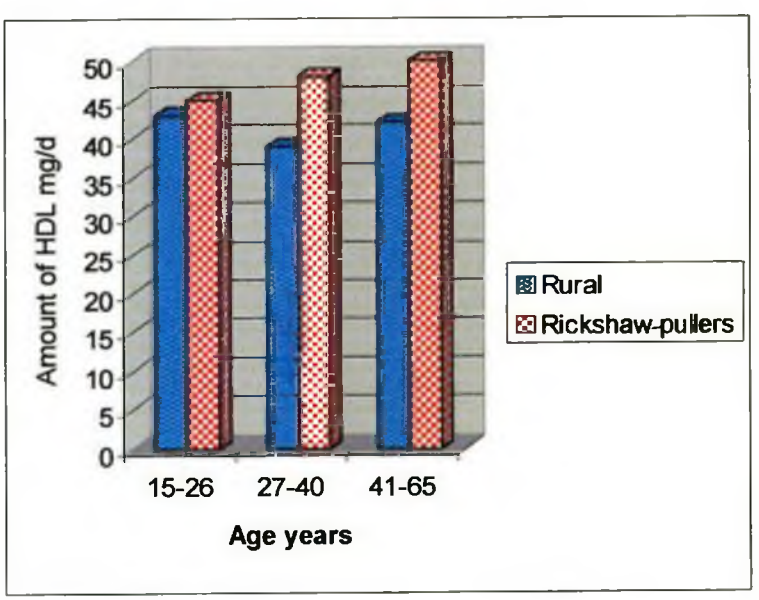


Figure 17(b) : The graphical representations of HDL- cholesterol levels between rural individuals and rickshaw-pullers.



4.18 LDL- cholesterol levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

LDL-(low density lipoprotein) cholesterol was measured by enzymatic method, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group LDL- cholesterol content was 72±2.5 mg/dl (68-76 mg/dl) in the serum of taxi-drivers, 70±2.5 mg/dl (66-76 mg/dl) in the serum of rickshaw-pullers and 74±2.6 mg/dl (70-78 mg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group LDL- cholesterol content was 74±2.8 mg/dl (70-78 mg/dl) in the serum of taxi-drivers, 76±2.8 mg/dl (70-80 mg/dl) in the serum of rickshaw-pullers and 77±2.1 mg/dl (74-80 mg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group LDL- cholesterol content was 76±2.6 mg/dl (72-80 mg/dl) in the serum of taxi-drivers, 77±2.6 mg/dl (72-82 mg/dl) in the serum of rickshaw-pullers and 82±2.6 mg/dl (78-86 mg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-18(a) &18(b) and the graphical representations of these results are also shown in figures-18(a) & 18(b).

Table-18 (a) LDL- cholesterol levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of LDL-cholesterol (mg/ dl)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	70-78	74±2.6	68-76	72±2.5	$P < 0.05$
Group-2 (27-40)	40	74-80	77±2.1	70-78	74±2.8	$P < 0.02$
Group-3 (41-65)	30	78-86	82±2.6	72-80	76±2.6	$P < 0.001$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Table-18 (b) LDL- cholesterol levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of LDL-cholesterol (mg/ dl)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	70-78	74±2.6	66-76	70±2.5	$P < 0.05$
Group-2 (27-40)	40	74-80	77±2.1	70-80	76±2.8	$P < 0.02$
Group-3 (41-65)	30	78-86	82±2.6	72-82	77±2.6	$P < 0.02$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Figure 18(a) : The graphical representations of LDL- cholesterol levels between rural individuals and taxi-drivers.

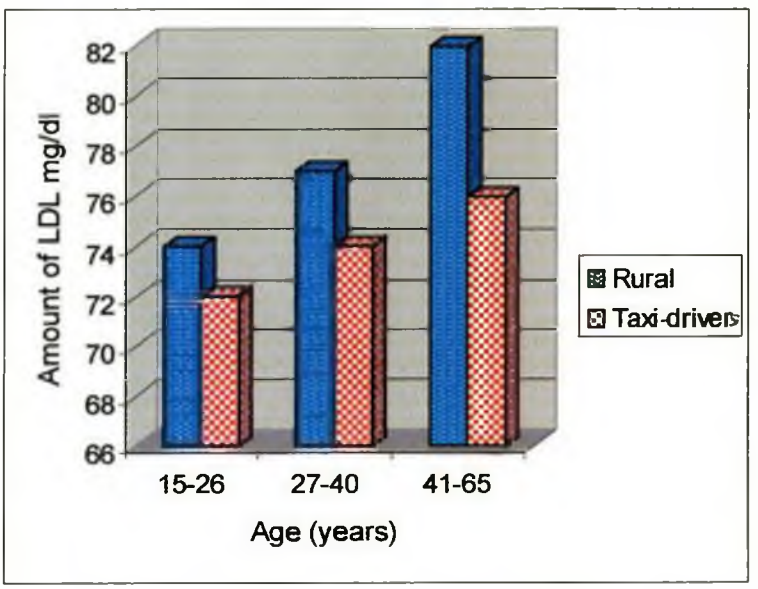
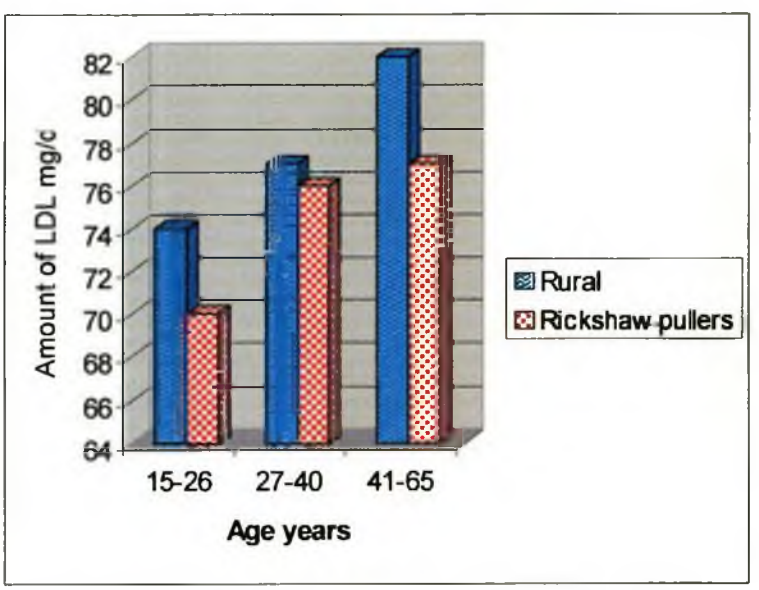


Figure 18(b) : The graphical representations of LDL- cholesterol levels between rural individuals and rickshaw-pullers.



4.19 CKMB activities in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

CKMB was measured by an Immuno assay procedure with colorimetric method, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group CKMB content was 11.9±0.5 U/L (11.0-12.8 U/L) in the serum of taxi-drivers, 11.7±0.5 U/L (11.0-12.4 U/L) in the serum of rickshaw-pullers and 13.2±0.7 U/L (12.2-14.2 U/L) in the serum of corresponding rural individuals.

In 27-40 yrs age group CKMB content was 12.1±1.0 U/L (10.5-13.7 U/L) in the serum of taxi-drivers, 11.9±1.0 U/L (10.3-13.4 U/L) in the serum of rickshaw-pullers and 13.05±1.2 U/L (11.0-15.1 U/L) in the serum of corresponding rural individuals.

In 41-65 yrs age group CKMB content was 13.6±0.7 U/L (12.3-14.8 U/L) in the serum of taxi-drivers, 13.1±0.7 U/L (12.3-13.8 U/L) in the serum of rickshaw-pullers and 13.8±0.4 U/L (13.2-14.4 U/L) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-19(a) &19(b) and the graphical representations of these results are also shown in figures-19(a) & 19(b).

Table-19 (a) CKMB activities in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of CKMB (U/ L)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	12.2-14.2	13.2±0.7	11.0-12.8	11.9±0.5	Not significant
Group-2 (27-40)	40	11.0-15.1	13.05±1.2	10.5-13.7	12.1±1.0	Not significant
Group-3 (41-65)	30	13.2-14.4	13.8±0.4	12.3-14.8	13.6±0.7	Not significant

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Table-19 (b) CKMB activities in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of CKMB (U/ L)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	12.2-14.2	13.2±0.7	11.0-12.4	11.7±0.5	Not significant
Group-2 (27-40)	40	11.0-15.1	13.05±1.2	10.3-13.4	11.9±1.0	Not significant
Group-3 (41-65)	30	13.2-14.4	13.8±0.4	12.3-13.8	13.1±0.7	Not significant

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Figure 19(a) : The graphical representations of CKMB activities between rural individuals and taxi-drivers.

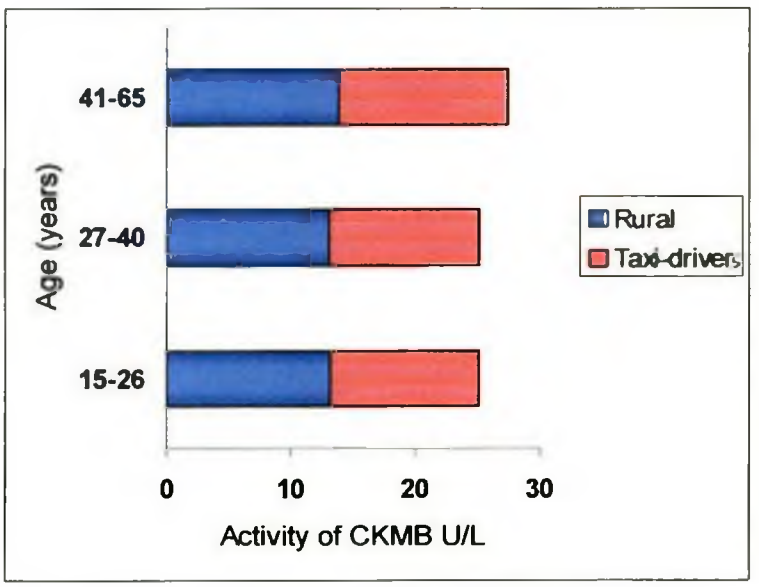
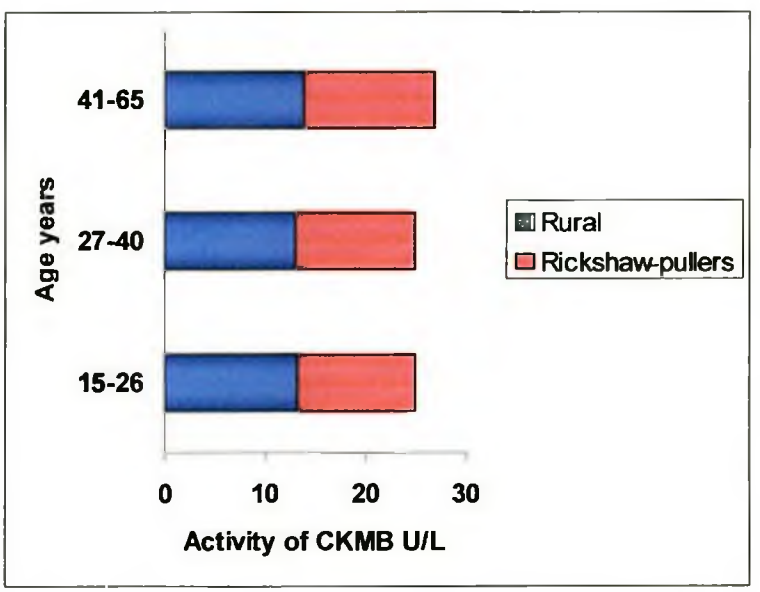


Figure 19(b) : The graphical representations of CKMB activities between rural individuals and rickshaw-pullers.



4.20 Phosphate ($PO_4^{\equiv}$) ion levels in the serum of three different age groups of normal individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Phosphate ion was measured by colorimetric method with an antoanalyzer Hitachi 704 model in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group phosphate content was 3.05±0.45 mg/dl (2.6-3.5 mg/dl) in the serum of taxi-drivers, 3.55±0.45 mg/dl (2.8-3.5 mg/dl) in the serum of rickshaw-pullers and 3.3±0.5 mg/dl (2.8-3.8 mg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group phosphate content was 3.4±0.4 mg/dl (3.0-3.8 mg/dl) in the serum of taxi-drivers, 3.6±0.4 mg/dl (3.2-4.0 mg/dl) in the serum of rickshaw-pullers and 3.6±0.4 mg/dl (3.2-4.0 mg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group phosphate content was 3.6±0.4 mg/dl (3.2-4.0 mg/dl) in the serum of taxi-drivers, 3.8±0.4 mg/dl (3.4-4.2 mg/dl) in the serum of rickshaw-pullers and 4.0±0.4 mg/dl (3.6-4.4 mg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-20(a) &20(b) and the graphical representations of these results are also shown in figures-20(a) & 20(b).

Table-20 (a) Phosphate ($PO_4\equiv$) ion levels in the serum of three different age groups of normal individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of $PO_4\equiv$ (mg/dl)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	2.8-3.8	3.3±0.5	2.6-3.5	3.05±0.45	$P < 0.04$
Group-2 (27-40)	40	3.2-4.0	3.6±0.4	3.0-3.8	3.4±0.4	$P < 0.001$
Group-3 (41-65)	30	3.6-4.4	4.0±0.4	3.2-4.0	3.6±0.4	$P < 0.05$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Table-20 (b) Phosphate ($PO_4\equiv$) ion levels in the serum of three different age groups of normal individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of $PO_4\equiv$ (mg/dl)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	2.8-3.8	3.3±0.5	2.8-3.5	3.55±0.45	$P < 0.04$
Group-2 (27-40)	40	3.2-4.0	3.6±0.4	3.2-4.0	3.6±0.4	$P < 0.02$
Group-3 (41-65)	30	3.6-4.4	4.0±0.4	3.4-4.2	3.8±0.4	$P < 0.05$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Figure 20(a) : The graphical representations of Phosphate ($PO_4^{\equiv}$) ion levels between rural individuals and taxi-drivers.

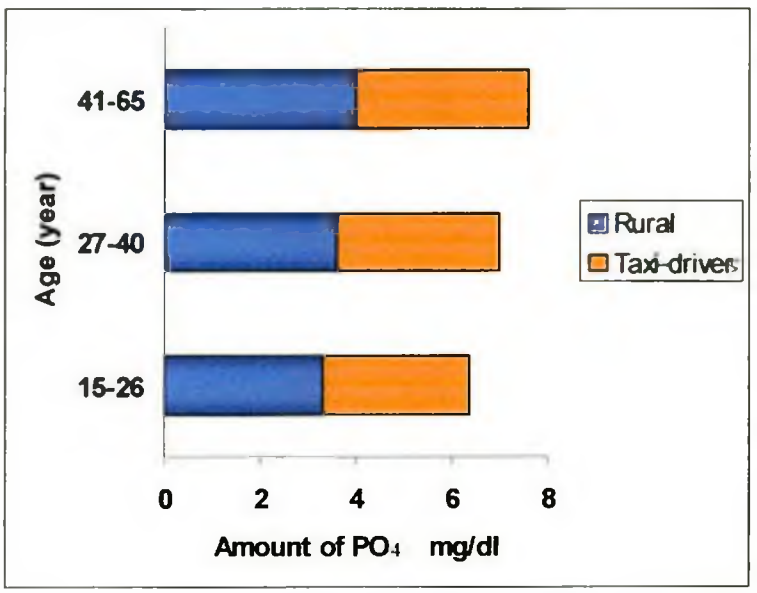
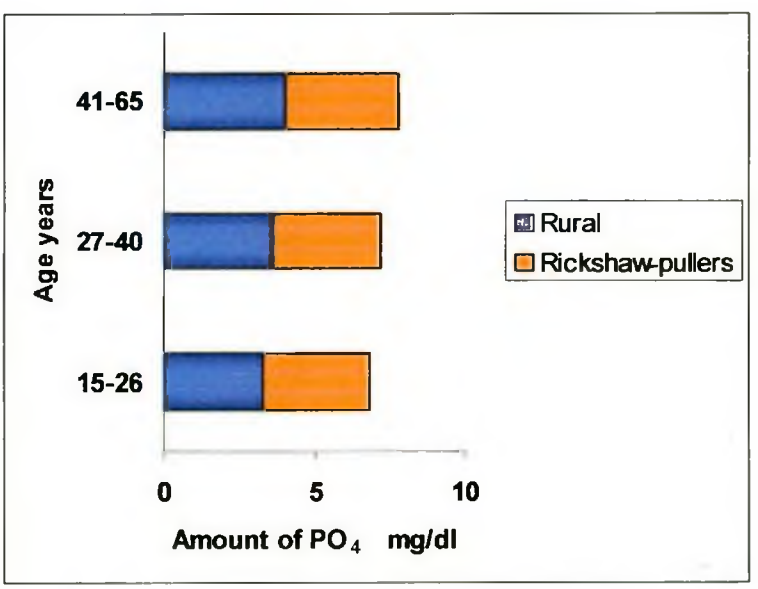


Figure 20(b) : The graphical representations of Phosphate ($PO_4^{\equiv}$) ion levels between rural individuals and rickshaw-pullers.



4.21 Sodium (Na+) ion levels in the serum of three different age groups of normal individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Sodium (Na+) ions were measured enzymatically by sodium dependent β-galactosidase activity with ONPG as substrate, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group sodium content was 136±1.4 mmol/L (134-138 mmol/L) in the serum of taxi-drivers, 135±1.4 mmol/L (133-137 mmol/L) in the serum of rickshaw-pullers and 134±1.5 mmol/L (132-137 mmol/L) in the serum of corresponding rural individuals.

In 27-40 yrs age group sodium content was 138±1.6 mmol/L (135-140 mmol/L) in the serum of taxi-drivers, 138±1.6 mmol/L (136-140 mmol/L) in the serum of rickshaw-pullers and 136±2.1 mmol/L (134-139 mmol/L) in the serum of corresponding rural individuals.

In 41-65 yrs age group sodium content was 139±1.5 mmol/L (136-141 mmol/L) in the serum of taxi-drivers, 140±1.5 mmol/L (137-142 mmol/L) in the serum of rickshaw-pullers and 138±1.0 mmol/L (137-139 mmol/L) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-21(a) &21(b) and the graphical representations of these results are also shown in figures-21(a) & 21(b).

Table-21 (a) Sodium (Na+) ion levels in the serum of three different age groups of normal individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Na+ (mmol /L)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	132-137	134±1.5	134-138	136±1.4	P < 0.001
Group-2 (27-40)	40	134-139	136±2.1	135-140	138±1.6	P < 0.07
Group-3 (41-65)	30	137-139	138±1.0	136-141	139±1.5	P < 0.03

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Table-21 (b) Sodium (Na+) ion levels in the serum of three different age groups of normal individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Na+ (mmol /L)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	132-137	134±1.5	133-137	135±1.4	P < 0.001
Group-2 (27-40)	40	134-139	136±2.1	136-140	138±1.6	P < 0.06
Group-3 (41-65)	30	137-139	138±1.0	137-142	140±1.5	P < 0.03

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Figure 21(a) : The graphical representations of Sodium (Na+) ion levels between rural individuals and taxi-drivers.

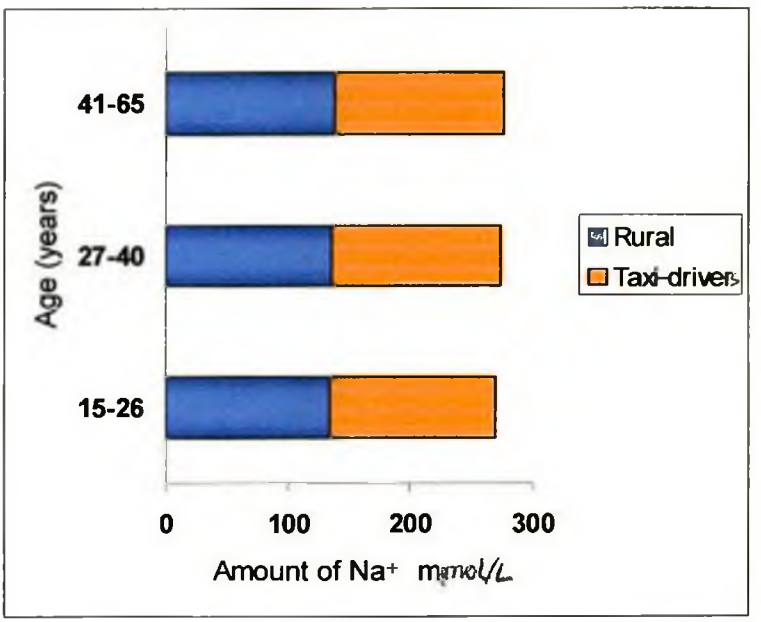
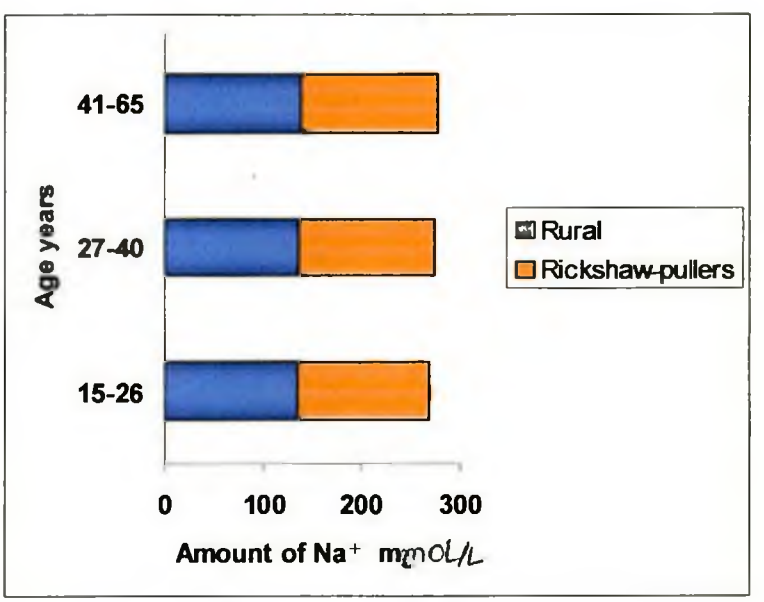


Figure 21(b) : The graphical representations of Sodium (Na+) ion levels between rural individuals and rickshaw-pullers.



4.22 Potassium (K^+) ion levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Sodium tetraphenylboron reacts with potassium ions in a protein free alkaline medium to produce a turbid suspension of potassium tetraphenylboron. The amount of turbidity produced is proportional to the potassium concentration, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-puller respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group potassium content was 3.65±0.16 mmol/L (3.4-3.9 mmol/L) in the serum of taxi-drivers, 3.7±0.16 mmol/L (3.4-4.0 mmol/L) in the serum of rickshaw-pullers and 3.5±0.21 mmol/L (3.2-3.8 mmol/L) in the serum of corresponding rural individuals.

In 27-40 yrs age group potassium content was 4.0±0.13 mmol/L (3.8-4.1 mmol/L) in the serum of taxi-drivers, 4.1±0.13 mmol/L (4.0-4.2 mmol/L) in the serum of rickshaw-pullers and 3.7±0.20 mmol/L (3.4-4.0 mmol/L) in the serum of corresponding rural individuals.

In 41-65 yrs age group potassium content was 4.1±0.16 mmol/L (3.9-4.3 mmol/L) in the serum of taxi-drivers, 4.2±0.16 mmol/L (4.0-4.4 mmol/L) in the serum of rickshaw-pullers and 3.9±0.20 mmol/L (3.6-4.2 mmol/L) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-22(a) &22(b) and the graphical representations of these results are also shown in figures-22(a) & 22(b).

Table-22 (a) The Potassium (K^+) ion levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of K^+ (mmol /L)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	3.2-3.8	3.5±0.21	3.4-3.9	3.65±0.16	P < 0.04
Group-2 (27-40)	40	3.4-4.0	3.7±0.20	3.8-4.1	4.0±0.13	P < 0.001
Group-3 (41-65)	30	3.6-4.2	3.9±0.20	3.9-4.3	4.1±0.16	P < 0.05

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Table-22 (b) The Potassium (K^+) ion levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of K^+ (mmol /L)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	3.2-3.8	3.5±0.21	3.4-4.0	3.7±0.16	P < 0.04
Group-2 (27-40)	40	3.4-4.0	3.7±0.20	4.0-4.2	4.1±0.13	P < 0.001
Group-3 (41-65)	30	3.6-4.2	3.9±0.20	4.0-4.4	4.2±0.16	P < 0.05

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Figure 22(a) : The graphical representations of The Potassium (K^+) ion levels between rural individuals and taxi-drivers.

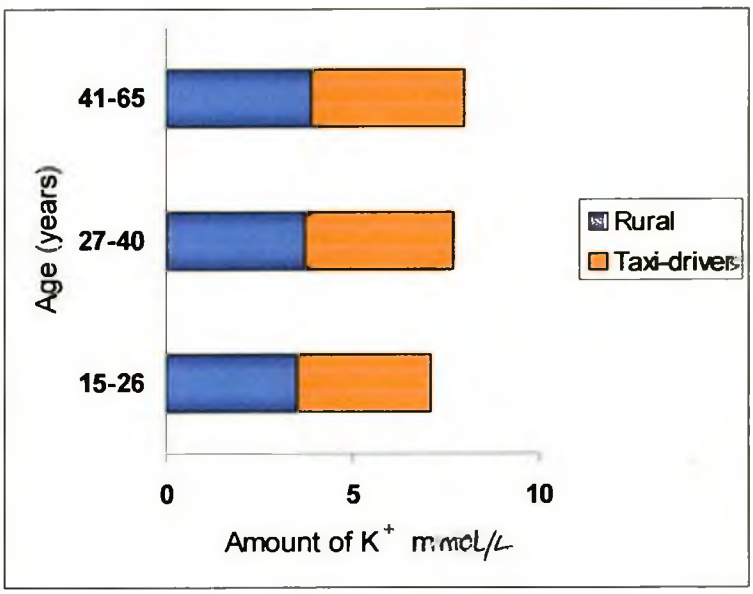
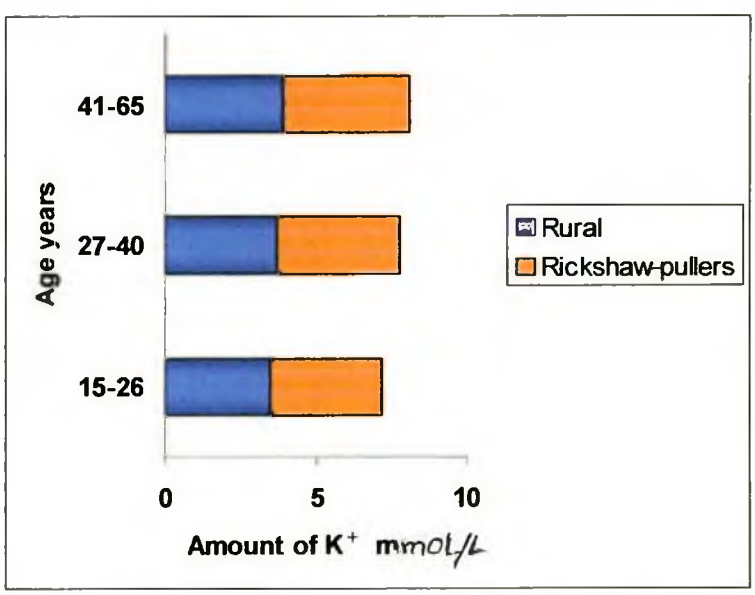


Figure 22(b) : The graphical representations of The Potassium (K^+) ion levels between rural individuals and rickshaw-pullers.



4.23 Calcium (Ca++) ion levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Calcium (Ca++) ion was measured by colorimetric method, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and ricksaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group calcium content was 8.75±0.3 mg/dl (8.3-9.2 mg/dl) in the serum of taxi-drivers, 8.9±0.4 mg/dl (8.4-9.4 mg/dl) in the serum of rickshaw-pullers and 9.50±0.2 mg/dl (9.2-9.8 mg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group calcium content was 9.2±0.4 mg/dl (8.6-9.8 mg/dl) in the serum of taxi-drivers, 9.4±0.4 mg/dl (8.8-9.8 mg/dl) in the serum of rickshaw-pullers and 9.95±0.3 mg/dl (9.5-10.4 mg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group calcium content was 9.45±0.3 mg/dl (8.9-10.0 mg/dl) in the serum of taxi-drivers, 9.6±0.5 mg/dl (9.0-10.2 mg/dl) in the serum of rickshaw-pullers and 10.30±0.2 mg/dl (9.8-10.8 mg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-23(a) &23(b) and the graphical representations of these results are also shown in figures-23(a) & 23(b).

Table-23 (a) Calcium (Ca++) levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Ca++ (mg /dl)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	9.2-9.8	9.5±0.2	8.3-9.2	8.75±0.3	P < 0.001
Group-2 (27-40)	40	9.5-10.4	9.95±0.3	8.6-9.8	9.20±0.4	P < 0.001
Group-3 (41-65)	30	9.8-10.8	10.3±0.2	8.9-10.0	9.45±0.3	P < 0.001

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Table-23 (b) Calcium (Ca++) levels in the serum of three different age groups of rural individuals and rickshaw-puiiers in the Dhaka city.

Age groups (years)	No of Samples	Amount of Ca++ (mg /dl)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	9.2-9.8	9.5±0.2	8.4-9.4	8.9±0.4	P < 0.02
Group-2 (27-40)	40	9.5-10.4	9.95±0.3	8.8-9.8	9.4±0.4	P < 0.03
Group-3 (41-65)	30	9.8-10.8	10.3±0.2	9.0-10.2	9.6±0.5	P < 0.02

P ≤ 0.05 (significant)
P < 0.05 (Moderate significant)
P < 0.001 (highly significant)

Figure 23(a) : The graphical representations of Calcium (Ca++) levels between rural individuals and taxi-drivers.

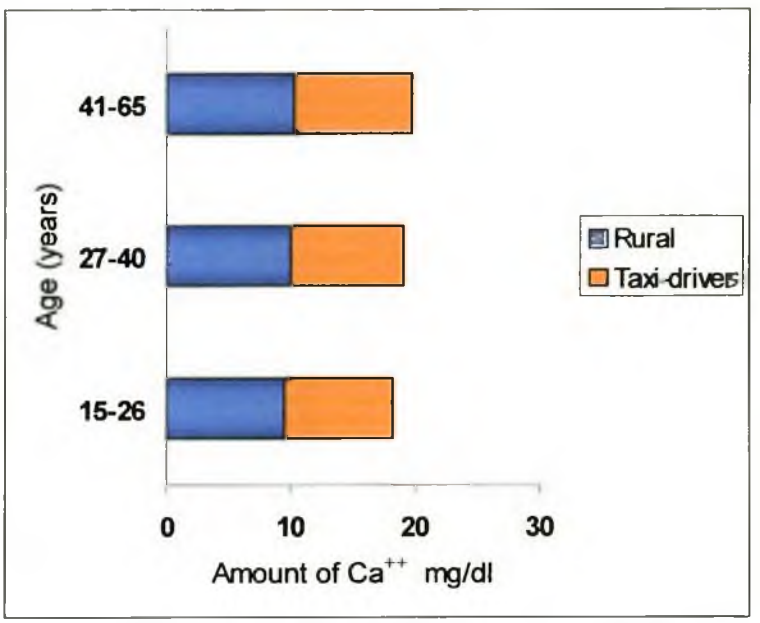
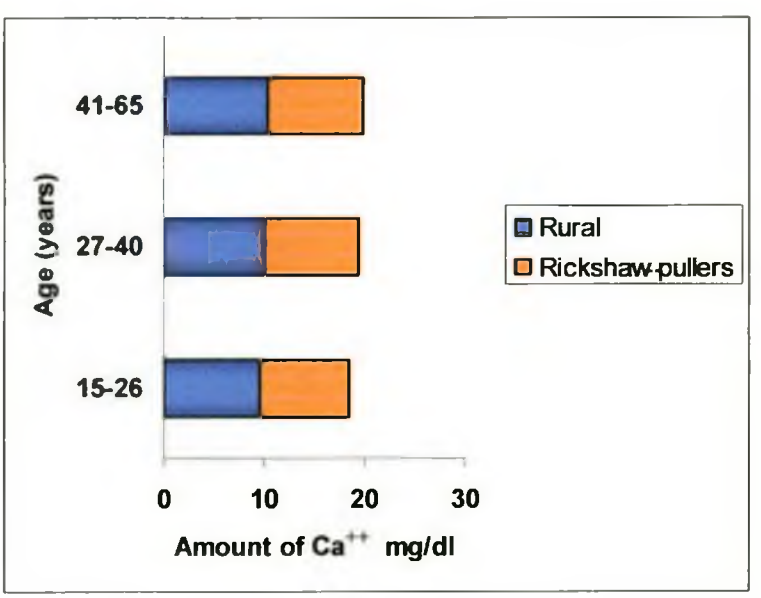


Figure 23(a) : The graphical representations of Calcium (Ca++) levels between rural individuals and rickshaw-pullers.



4.24 Uric acid levels in the serum of three different age groups of rural individuals, taxi-drivers and rickshaw-pullers in the Dhaka city.

Uric acid was measured by colorimetric method, in the serum of three different age groups (15-26, 27-40 and 41-65 years of age) and each group contained 30, 40 and 30 samples of taxi-drivers and rickshaw-pullers respectively. Equal number of rural human samples were used as control for each corresponding age group.

In 15-26 yrs age group uric acid content was 3.0±0.26 mg/dl (2.6-3.4 mg/dl) in the serum of taxi-drivers, 3.2±0.26 mg/dl (2.8-3.6 mg/dl) in the serum of rickshaw-pullers and 2.8±0.25 mg/dl (2.4-3.2 mg/dl) in the serum of corresponding rural individuals.

In 27-40 yrs age group uric acid content was 3.7±0.37 mg/dl (3.0-4.4 mg/dl) in the serum of taxi-drivers, 3.9±0.37 mg/dl (3.4-4.4 mg/dl) in the serum of rickshaw-pullers and 3.4±0.40 mg/dl (2.8-4.0 mg/dl) in the serum of corresponding rural individuals.

In 41-65 yrs age group uric acid content was 5.0±0.30 mg/dl (3.8-6.2 mg/dl) in the serum of taxi-drivers, 5.1±0.30 mg/dl (4.0-6.2 mg/dl) in the serum of rickshaw-pullers and 4.2±0.30 mg/dl (3.4-5.0 mg/dl) in the serum of corresponding rural individuals.

The statistical alalysis of the results are shown in tables-24(a) &24(b) and the graphical representations of these results are also shown in figures-24(a) & 24(b).

Table-24 (a) Uric acid levels in the serum of three different age groups of rural individuals and taxi-drivers in the Dhaka city.

Age groups (years)	No of Samples	Amount of uric (mg /dl)				Level of Significance
		Rural individuals		Taxi-drivers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	2.4-3.2	2.8±0.25	2.6-3.4	3.0±0.26	$p < 0.05$
Group-2 (27-40)	40	2.8-4.0	3.4±0.40	3.0-4.4	3.7±0.37	$p < 0.02$
Group-3 (41-65)	30	3.4-5.0	4.2±0.30	3.8-6.2	5.0±0.30	$p < 0.001$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Table-24 (b) Uric acid levels in the serum of three different age groups of rural individuals and rickshaw-pullers in the Dhaka city.

Age groups (years)	No of Samples	Amount of uric (mg /dl)				Level of Significance
		Rural individuals		Rickshaw-pullers		
		Range	Mean ± SD	Range	Mean ± SD	
Group-1 (15-26)	30	2.4-3.2	2.8±0.25	2.8-3.6	3.2±0.26	$p < 0.05$
Group-2 (27-40)	40	2.8-4.0	3.4±0.40	3.4-4.4	3.9±0.37	$p < 0.02$
Group-3 (41-65)	30	3.4-5.0	4.2±0.30	4.0-6.2	5.1±0.30	$p < 0.02$

$P \leq 0.05$ (significant)
$P < 0.05$ (Moderate significant)
$P < 0.001$ (highly significant)

Figure 24(a) : The graphical representations of Uric acid levels between rural individuals and taxi-drivers.

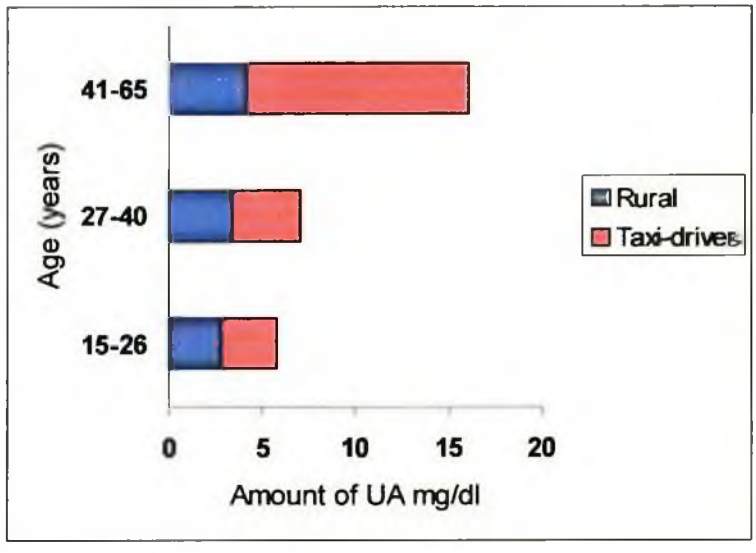
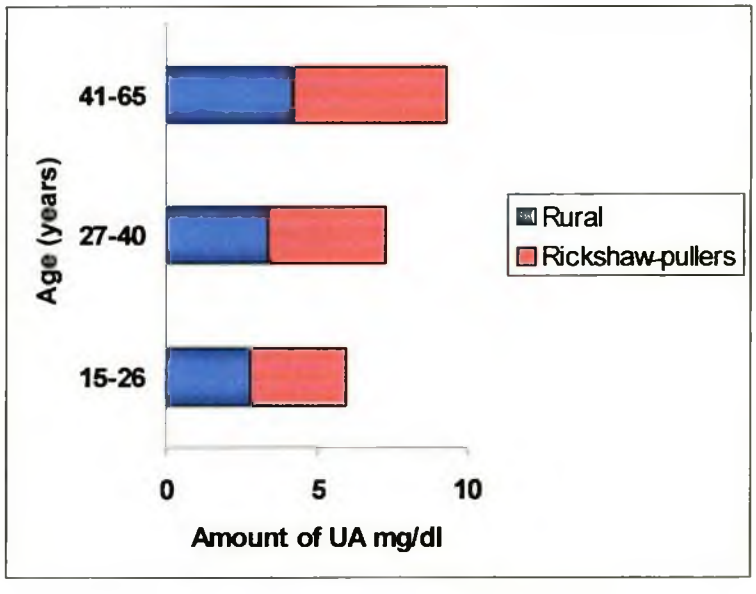


Figure 24(b) : The graphical representations of Uric acid levels between rural individuals and rickshaw-pullers.



CHAPTER-5
DISCUSSION

Discussion

Dopamine β-hydroxylase (DBH) is the most important enzyme in the catecholamine biosynthesis. It is a copper containing glycoprotein, consisting of four identical subunits and catalyzed the oxidation of dopamine to norepinephrine, requiring ascorbic acid as an electron donor. DBH is localized in the nerve terminals of peripheral sympathetic nerves and in the adrenal medulla and secreted into blood by a process of exocytosis, So a very small amount at DBH is found in human serum.

DBH activity in the serum is considered as a possible index of sympathetic nervous functions by the clinical and pharmacological investigators. Previous studies have been shown that the serum DBH activity has been altered in various neurological diseases. T. Nagatsu and Co-workers have reported that a decrease in the DBH levels in the cerebrospinal fluid and brain of patients with Parkinson's disease. The product of DBH is not only a neurotransmitter but also a hormone, which regulates emotion, excitation, behaviour, sex, mood and sleep. So it is very important in biochemical pharmacological as well as neurochemical point of view. But the measurement procedure of DBH activity is very difficult, as it is present in a very tiny amount. So the severity of air pollution from vehicles, various biochemical and nutritional changes in taxi-drivers and rickshaw-pullers tempted us to carryout this study of neuronal activities.

To serve the purposes of our research, 100 blood samples were collected from three different age groups (15 –26yrs, 27-40yrs and 41-65yrs) of taxi-drivers in the Dhaka city and the serum were collected and they were subjected to the assay of DBH activity. 100 blood samples of rickshaw-pullers were also collected from the Dhaka city at three different age groups. Equal number of rural human samples were used as control. Cofactors of DBH (cu^{++} and vitC) and several other biochemical parameters were also determined by using the samples among the taxi-drivers, rickshaw-pullers and rural individuals for comparative studies.

The DBH activity from these serum samples was measured by a very sensitive method proposed by Nagatsu et, al. The DBH activity was found to decrease in the serum of taxi-drivers and rickshaw-pullers as compared to their control groups of rural individuals. A significant decrease ($P < 0.001$) in DBH activity was found in the age group of 15-26 years. The taxi-drivers had a mean DBH activity of 20.9 nmol/min/ml and in the rural individuals it was 29.5 nmol/min/ml. The mean DBH activity of rickshaw-pullers was 22.5 nmol/min/ml.

In 27-40 yrs age group, the mean DBH activity was found 24.5 nmol/min/ml in the serum of taxi-drivers, 25.0 nmol/min/ml in rickshaw-pullers and 39.4 nmol/min/ml in the serum of corresponding rural individuals. A significant decrease ($P < 0.001$) in DBH activity was found.

A significant decrease in DBH activity ($P < 0.001$) was also found in the taxi-drivers and rickshaw-pullers of 41-65 yrs age group. The mean DBH activity was 21.9 nmol/min/ml in the serum of taxi-drivers, 22.9 nmol/min/ml in the rickshaw-pullers and 34.2 mmol/min/ml in the rural individuals.

The profound decrease of DBH activity in three different age groups of taxi-drivers indicates a possible role of DBH to the air polluted condition. Due to lack of DBH there would be a accumulation of dopamine, which may be responsible for causing the symptoms of neurological diseases. From this result, it has been concluded that air pollution affected taxi-drivers and rickshaw-pullers have the possibility to be affected by various neurological disorders.

Copper and ascorbic acid both are cofactors of DBH. Copper contents were increased and ascorbic levels were decreased in different groups of taxi-drivers and rickshaw-pullers in comparison with control groups of rural individuals .

The decrease of ascorbic acid may support the decrease of DBH activity. However it has been found that the enzyme activity is unaffected by severe ascorbic acid deficiency. So the specific role of ascorbic acid as a cofactor of DBH in vivo is not clear .

Copper is a cofactor for the activity of DBH enzyme, one mole of enzyme contains two moles at cupric ion.[98] An excess of copper inhibitory to DBH activity. Launder and austen[99] have speculate that the high levels of soluble copper found in some region at rat brain (Caudate nucleus, hypothalamus) may act to regulate DBH activity.

Decreased DBH activities in three different age groups (15-26 yrs, 27-40 yrs and 41-65 yrs) in the serum of taxi-drivers and rickshaw-pullers may be caused for the decreased levels of ascorbic acid, though copper contents were increased in those groups and copper may have an inhibitory effect. Serum iron level 40 μg/dl is the indicative of iron deficiency. In the present study, the three different age groups of taxi-drivers and rickshaw-pullers showed significant ($P < 0.001$) decrease in ascorbic acid levels as compared to the rural individuals. Ascorbic acid plays a critical role in the conversion of Fe^{+++} to Fe^{++} and hence has a vital role in the absorption of dietary iron. As the ascorbic acid content in the air pollution affected taxi-drivers and rickshaw-pullers decreased significantly, may be a possible cause of poor iron status.

Vitamin-A is essential for growth and also normal function of retina and development of epithelial surfaces. It is also necessary in higher animals to support growth and health and is particularly necessary for vision, reproduction mucus secretion and the maintaince of different epithelia. VitA has an antioxidative effect. In this study, decreased the vit A level was found in the serum of taxi-drivers and rickshaw-pullers as compared to the rural individuals. Vitamin A deficiency reduces the rhodopsin in the rods of the retina and in this way leads to night blindness. It indicates that the taxi-drivers and rickshaw-pullers also affected by night blindness.

As both norepinephrine and epinephrine are the metabolic products of dopamine and neurotransmitter mediating enzyme Dopamine β-hydroxylase is responsible for the conversion of dopamine into norepinephrine, So decreased DBH activities in three different age groups of taxi-drivers and rickshaw-pullers indicate the decreased levels of norepirephrine in the above mentioned age groups.

Zinc is an important micronutrient and also cofactor of many enzymes such as lactate dehydrogenase, arginase etc. Zn content was found to increase in the taxi-drivers and rickshaw-pullers as compared to the rural individuals. This finding is in agreement with the finding of Ivore et.al, that dopamine levels are significantly higher in zinc deficient animals[100-101]. But in this study excess Zn levels acted as inhibitory effect and decreased the enzyme Dopamine β-hydroxylase.

This suggests that behavioral changes may be in part catecholamine related[102]. Further more Caldwell et. al, showed that reduced zinc is an indication of increased emotion (fear) and higher levels of Zn indication of increased excitation.

Among other biochemical parameters glucose levels were increased in three different age groups of taxi-drivers and rickshaw-pullers. Glucose concentration was significantly increased 41-65 yrs age group of taxi-drivers and rickshaw-pullers in comparison with rural individuals. The normal range of glucose is 3.6-5.6 mmol/l. It has been reported that high glucose concentration usually present in the diabetes.

Protein concentration is essential to obtain the specific activity of DBH activity. Protein contents were decreased in three different age groups of taxi-drivers and rickshaw-pullers. But decreased protein contents in group-I and group-III were significantly lower than the normal range of protein level 63-82 gm/l.

Serum total protein is decreased in inadvertent overhydration , in conditions involving protein loss through the kidneys (nephrotic syndrome) from skin (severe burns) or gut (protein lossing interopathies) or in failure at protein synthesis (starvation, protein malnutrition, severe nonviral liver cell damage)[103]. So these complications may be associated with three different age groups of taxi-drivers and rickshaw-pullers.

Among other biochemical parameters serum albumin was also decreased in three different age groups of taxi-drivers and rickshaw-pullers in comparison with rural individuals. The normal range of albumin level is 35-57 gm/l. The same conditions

that produce a lowered total protein concentration also lowered the serum albumin concentration. So serum albumin decreased in inadvertent over hydration, in conditions involving protein loss through the kidneys (nephrotic syndrome). from skin (severe burns) or gut (protein-losing interopathies) or in failure of protein synthesis (starvation, protein malnutrition, severe nonviral liver cell damage)[103]. So decreased albumin levels in three different age groups of taxi-drivers and rickshaw-pullers may be associated with these complications.

Alanine transaminase also called glutamate pyruvate transaminase (GPT) and aspartate transaminase also called glutamate oxaloacetate transaminase are important in the diagnosis of heart and liver damage. Measurement of the concentration of these two transaminases in the serum, sGPT and sGOT tests (s for serum) can give important information regarding the severity of damage to the heart and liver. For clinical purposes the sGPT test is primary used in the diagnosis at liver disease. From the injured liver cell leakage various enzymes occur into the blood. An increase in serum GOT levels can be observed in case of myocardial infraction.

In this experiment serum GPT activities were significantly increased ($P < 0.001$) in three different age groups of taxi-drivers and rickshaw-pullers in comparison with rural individuals. The normal range of sGPT activity is below 40 u/l. Group III of taxi-drivers and rickshaw-pullers sGPT activities were significantly higher than normal range. Our study reported that group III may be suffering from liver disease.

sGOT activities were increased in three different age groups of taxi-drivers and rickshaw-pullers. 15-26 years and 41-65 years age groups of taxi-drivers and rickshaw-pullers GOT activities were significantly higher ($P < 0.001$). The normal range of GOT is below 37u/l. These activities may because for "silent" myocardial infraction, sGOT is also released by damaged red blood cells, kidney, liver, lungs[104]. So increased GOT activities in the taxi-drivers and rickshaw-pullers may also be caused by inhaling polluted air emitted from vehicles and hard work.

Alkaline phosphatase (ALP) were slightly increased three different age groups at taxi-drivers and rickshaw-pullers in comparison with rural individuals. The normal range of ALP activity is 39-117U/l (Adult). ALP activities were significantly increased ($P < 0.001$) in the 41-65 yrs age group of taxi-drivers. This study supported that, suffering from liver cancer may be increased ALP activities.

Serum bilirubin levels were slightly increased in three different age groups of taxi-drivers and rickshaw-pullers in comparison with rural individuals. But increased levels of bilirubin in the above mentioned groups were with in the normal range of 0.4-1.2 mg/dl.

Serum urea levels were slightly increased in three different age groups of taxi-drivers and rickshaw-pullers in comparison with rural individuals. But in group I and II of taxi-drivers the increased levels of urea within normal range, 10-50mg/dl; Urea levels were slightly increased in group III of taxi-drivers and rickshaw-pullers than normal range. High Protein diet, Administration of cortisol like steroids, stressful situations and prerenal, renal and postrenal factors increase the concentration of urea-N[105]. Increased urea level in group III may be caused by administration of cortisol like steroids, stressful situations and prerenal, renal and postrenal factors.

Serum creatinine levels were not significantly changed in three different age groups of taxi-drivers and rickshaw-pullers in comparison with rural individuals. But increased creatinine levels were within the normal range of 0.67-1.58 mg/dl.

Serum cholesterol levels were significantly decreased in group-I and group-III of taxi-drivers than rural individuals. Decreased cholesterol levels were below the normal range (150-200 mg/dl). Serum cholesterol levels were significantly decreased ($P<0.001$) in three different age groups of rickshaw-pullers. It has been reported that hypocholesteremia usually is present in hyperthyroidism, hepatocellular disease, anaemias, starvation and in certain genetic defects[106]. So the hypocholesterimia was caused in the above mentioned groups due to anaemias, starvation or in certain genetic defects. If both norepinephrine and epinephrine are ;the metabolic products of

dopanine and serum cholesterol levels decreased in starvation/poor food having condition in the taxi-drivers and rickshaw-pullers in our experiment, we can assume that norepinephrine may play a role in hypocholesteremin.

Triglyceride has an important role in the metablisom. Serum Tg levels were decreased in different age groups (15-26, 27-40 and 41-65 yrs of age) of taxi-drivers and rickshaw-pullers in comparison with rural individuals. Decreased Tg levels were within the normal range 50-150 mg/dl. Serum Tg levels were significantly decreased in three different age groups of rickshaw-pullers due to intake of poor food and starvation.

Serum HDL (high density lipoproteins) were increased three different age groups (15-26, 27-40 and 41-65 yrs of age) of taxi-drivers and rickshaw-pullers in comparison with rural individuals. But increased levels of HDL were within normal range (41-58 mg/dl) in adult. HDL cholesterol levels has an important role in the cardiovascular disease. The serum HDL cholesterol may be increased by moderate to vigorous exercise, estrogen and the moderate consumption of alcohol[107]. Present evidence suggest that an elevated HDL cholesterol reduces the risk of cardiovascular disease[107]. The normal range of HDL cholesterol in human serum are Men 41-58 mg/dl, women 48-75 mg/dl and children 52-72 mg/dl and its favorable prognosis values are 55 mg/dl for male and 65 mg/dl for women, standard risk level values are 35-55 mg/dl for males and 45-65 mg/dl for female as well as increased risk level values are < 35 mg/dl for male and <45 mg/dl for female[108]. Our experiment findings showed that increased HDL-cholesterol levels in taxi-drivers and rickshaw-pullers were with in the normal range.

LDL (low density lipoproteins) cholesterol levels were decreased in three different age groups of taxi-drivers and rickshaw-pullers in comparison with rural individuals. But the decreased LDL cholesterol levels were within the normal range > 150 mg/dl. Decreased LDL –cholesterol levels may be caused for genetic defects in LDL cholesterol metabolism. An increased LDL cholesterol levels also may be caused for genetic defects in LDL cholesterol metabolism.

CKMB activities were slightly decreased in three different age groups of taxi-drivers and rickshaw-pullers in comparison with rural individuals. But the decreased levels of CKMB activities were within the normal range below 25 U/l. Serum CKMB activity beings to rise 3-6 hours after a myocardial infraction and reaches a maximum range around 24 hours. It is usually back to normal range before 48 hours[104].

Phosphate ion plays a very important role in the human body for formation of bone and teeth and metabolism. Phosphate ion decreased in three different age groups of taxi-drivers and rickshaw-pullers in comparison with rural individuals. But the decreased levels of phosphate in the taxi-drivers and rickshaw-pullers were within the normal range 2.4-5.4 mg/dl.

Na^+ levels increased in three different age groups of (15-26, 27-40 and 41-65 yrs of age) taxi-drivers and rickshaw-pullers in comparison with rural individuals. Increased Na^+ levels were with in normal range 132-145 mmol/L Elevated levels of serum sodium found in (I) Hyper adrenalism in which excessive reabsorption of sodium in renal tubules occurs as a result of over production of adrenal steroids (II) Osmotic diureses (III) Excessive loss of water through skin or lungs and excessive administration of hypertonic Na^+ may also contribute to excessive reliance on 0.9% (150 mmol/l) saline for volume replacement, Administration of drugs with a high Na^+ content, And use of excessive alcohol. Na^+ levels may be decreased in severe dehydration owing to inadequate intake of water irrespective of cause or to excessive water loss.

K^+ levels were increased in three different age groups of taxi-drivers and rickshaw-pullers in comparison with rural individual. But the increased levels were with in the normal range 3.2-5.2 mmol/l. Na^+ and K^+ are excreted into the urine as salts of the keto-acids, with replacement of water because of thirst. Renal disease with malfunction of the tubular ion exchange system of Na^+ and K^+ as well as (I) hypothysodium (II) Severe Potassium depletion (III) Unmeasured Osmotically active substances stimulating osmotic ADH release (a) Glucose (b) Alcohol (c) Sickel cell Syndrome (d) Diarrhoea. Vomiting etc.

The fluid of the gastrointestinal tract contain relatively high concentration of potassium, and their removal or loss can produce serious deficits. (I) Increased secretion of adrenal steroid primarily aldosterone, results in excessive potasium loss through the kidneys and a low serum K^+ concentration as well as (I) Nephritic syndrome (II) Renal tubular damage (III) severe dietary deficiency correction of megaloblastic anaemia (IV) Alkalosm.

Serum Ca^+ levels were decreased in three different age groups of taxi-drivers and rickshaw-pullers in comparison with rural individuals. But the decrease Ca^{++} levels were within normal range 8.0-11.0 mg/dl. Decreased levels of Ca^{++} may be caused tetany, hypoparathyroidism, pseudohypoparathysodium (failure of the target organs to respond PTH) a deficiency of Vit D, gastointestinal disease that interfere with the absorption of vit D or calcium (sprue steatorrhea), nephrosis or other conditions in which the serum protein concentration is low, chronic renal disease acute pancreatitis and magnesium deficiency.

Serum uric acid levels were increased in three different age groups of taxi-drivers and rickshaw-pullers in comparison with rural individuals. But increased uric acid levels were within normal range 2.4-7.2 mg/dl. It has been reported that serum urate concentration is elevated in most patients with gout, in renal disease after increased break down of nucleic acid or nucleoprotein[109]. Some individuals have an elevated concentration of serum urate despite the absence of disease (iodopathic hyperuricemia)[109]. So increased levels of uric acid in our experimental finding are not associated with there complications.

CHAPTER-6

CONCLUSIONS

Conclusions

Dhaka is the most polluted city in Bangladesh overall the world. The Pb concentration in the busy area of the Dhaka city is 463 ngm/m^3 which is unbearable and this is the alarming rate for the health of the people of Dhaka city.

We made systematic comparative studies of neuronal activities (DBH) of the rural individuals between taxi-drivers and rickshaw-pullers of Dhaka city.

From the results of DBH activities and other biochemical parameters, we could make the following conclusions.

(I) The DBH activities were decreased in the serum of taxi-drivers and rickshaw-pullers as compare to the rural individuals.

(II) Cofactors of DBH ascorbic acid was decreased, Cu^{++} levels were increased. Zn^{++} levels were also found to increase in the serum of taxi-drivers and rickshaw-pullers.

(III) Serum protein, ALB, Tg cholesterol levels were decreased and glucose, sGPT and sGOT levels were increased. Other biochemical parameters such as urea, creatinine, bilirubin, CKMB, uric acid, Na+, K^+, PO_4, Ca^{++}, HDL, LDL etc did not change significantly.

Since DBH activities decrease in neurological disorders such as psychoses, mental depression it may also be useful as one of the parameters to diagnose these disorders. In our studies we found that DBH activities were decreased significantly in air pollution affected taxi-drivers and rickshaw-pullers.

CHAPTER-7

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CHAPTER-8

APPENDIX

LIST OF ABBREVIATIONS

α	-	Alpha
β	-	Beta
SD	-	Standard Deviation
CA	-	Catecholamine
cDNA	-	Complementary deoxyribouncleic acid
Conc.	-	Concentration
^0C	-	Degree celsius
DNA	-	Deoxyribonucleic acied
dl		Decilitre
DBH	-	Dopamine-β-Hydroxylase
g	-	Gram
V_{max}	-	Maximum velocity
mRNA	-	Messenger ribonucleic acid
K_m	-	Michaelis constant
μg	-	Micro gram
μl	-	Microliter
μ mole	-	Micromole
mg	-	Miligram
ml	-	Millilitre
TG	-	Triaclglycerol
HDL	-	High density lipoprotein
LDL	-	Low density lipoprotein
VLDL	-	Very low density lipoprotein
yrs	-	Years
Ach	-	Acetylcholine
nm	-	Nanomole
min	-	Minute
M	-	Molar
N		Normal
nm	-	Nanometer
ppm	-	Parts per milion

%	-	Percentage
-SH	-	Sulfhydril radical
ZD	-	Zinc deficient
FFA	-	Free fatty acid
AMI	-	Acute myocardial infarction
CK-MB	-	Creatine kinase-Muscle Brain
GOT	-	Glutamate Oxaloacetate Transaminase
RBC	-	Red Blood Cell
GPT	-	Glutamate Pyruvate Transaminase
ALP	-	Alkaline Phosphatase
Na^+	-	Sodium ion
K^+	-	Potassium ion
CI	-	Chloride ion
Ca^{++}	-	Calcium ion
Zn^{++}	-	Zinc ion
RNA	-	Ribonucleic acide
NE	-	Norepinephrin
PEM	-	Protein Energy Malnutrition
PTH	-	Parathyroid Hormone
NADPH	-	Nicotinamide Adenine Dinucleotide Hydrogen Phosphate
NADH	-	Nicotinamidce Adenine Dinucleotide Hydrogen
NAD^+	-	Nicotinamide Adenine Dinucleotide ion
DOPA	-	Dihydroxyphenylalanine
KDa	-	Kilodalton
BIRDEM	-	Bangladesh Institute of Research and Rehjabilitation in diabetes, Endocrine and Metabilic Disorder
TCA	-	Trichloroacetic acid
1M	-	One molar
DTC	-	Dinitrophenyl-Thiourea-Coppersulfate
DOE	-	Department of Environment
O.D	-	Optical Density
GK	-	Glycerol Kinase
GPO	-	Glycerol-3 phosphate oxidase

EDTA	-	Ethylenediaminotetra-acctic acid
CHOD	-	Cholesterol Oxidase
PAP	-	Phenol Peroxidase
U/L	-	Unit/Litre
ADP	-	Adenosine Di phosphate
AMP	-	Adenosine Mono Phosphate
MADP	-	Nono Adenosine Diphosphate
HK	-	Hexokinase
FCR	-	Folin-ciocalteau Reagent
BSA	-	Bovine serum albumin
Hg	-	Mercury
BCG	-	Bromocresol green
PNP	-	Paranitrophenol
TB	-	Total Bilirubin
ONPG	-	O-nitrophenyl-β-d-galactopyranose
ΔA	-	Absorbance
DDH_2O	-	Deionized distilled water
BATC	-	Bangladesh Road Transport Corporation
CFC	-	Chlorofloro Carbon
O_3	-	Ozone

List of taxi-drivers

Sl. No.	Name	Age (Yeas) (15-26)	Name of the Taxi stands
1	Karim Uddin Varsha	21	Gulishtan
2	Abul Hossain	19	
3	Kabir Hossain	20	
4	Ali ahamed	22	
5	Said Khan	16	
6	Azgar Ali	17	Azimpur Bot Tala
7	Zakir Hossain	15	
8	Mohammed Tapash Khan	19	
9	Jamir uddin	24	
10	Md. Rahim uddin	25	
11	Md. Rasedul Ismal	18	Farmegate
12	Md. Farid Ali	16	
13	Md. Mofizul Islam	19	
14	Malek Hossain	24	
15	Md. Akkus Ali	26	
16	Md. Shirazul Islam	21	Shahbag
17	Md. Yousoof Ali	19	
18	Mizanur Rahman	20	
19	Mukter Hossain	15	
20	Razu Islam	24	
21	Masud Hossain	22	Lalbag
22	Rana	25	
23	Rafiq Hossain	18	
24	Rahman Ali Khan	19	
25	Tareq Hossain	17	
26	Munna Islam	17	Newmarket

27	Mamun Khan	24	
28	Yousoof Ali	26	
29	Azad Hossain	22	
30	Md. Arif	21	
		Age (Years) (27-40)	
31	Md. Sazu Islam	30	Gulishtan
32	Nurul huque	35	
33	Sumsul Islam	40	
34	Ali Ahammed	28	
35	Md. Ali	33	
36	Md. Malek	36	Azimpur Bot Tala
37	Kafil Uddin	27	
38	Monsur Ali	39	
39	Md. Tamis uddin	29	
40	Md. Khalek uddin	38	
41	Md. Faruk Islam	34	Farmegate
42	Md. Helal Uddin	32	
43	Md. Belal uddin	31	
44	Nurul Islam	30	
45	Islam Mia	28	
46	Zaker Hossain	30	Shahbag
47	Mofis uddin	33	
48	Mobaruk Islam	37	
49	Md. Abul Islam	40	
50	Md. Sahidul Islam	37	
51	Atour Rahman	34	Lalbag
52	Mamun-or-rasid	36	
53	Mahfus Hossain	38	
54	Saiful Islam	39	
55	Khairul Islam	40	

56	Badiul hossin	39	Newmarket
57	Anisur Hossain	38	Mogbazar
58	Monto mia	34	
59	Salim Hossain	31	
60	Samad uddin	32	
61	Kashem Ali	37	Gabtali
62	Md. Satter hossani	37	
63	Md. Ballel Hossain	36	
64	Md. Batan Ali	35	
65	Roub Hossain	30	
66	Moziber Hossain	29	Mohammedpur
67	Sumsuddin Hossain	29	
68	Md. Hasan Ali	38	
69	Md. Salim	39	
70	Rabiul mia	40	
		Age years (41-65)	
71	Sekander Ali	45	Gulishtan
72	Abul Kalam	50	
73	Md. Ali Azgar	56	
74	Md. Sukkur Ali	58	
75	Khon mia	59	
76	Mahfus Khan	54	Azimpur Bot Tala
77	Ali Akber	62	
78	Iqbal Hossain	63	
79	Salim uddin	57	
80	Tashu mia	65	
81	waihid Ali	62	Farmegate
82	Md. Zafor Hossain	60	
83	Md. Din Islam	61	

84	Md. Amin	57	
85	Md. Shabuddin	52	
86	Md. Based Ali	54	
87	Md. Alauddin	60	New Market
88	Md. Karim	55	
89	Zahid Hossain	59	
90	Kanchan Ali	60	
91	Reza Khan	48	
92	Md. Sahed Ali	49	Gabtali
93	Md. Kamrul Islam	47	
94	Md. Salauddin	53	
95	Nazrul Islam	63	
96	Rahed Ali	62	Mohammedpur
97	Rafiq Hossain	60	
98	Anowar Hossain	55	
99	Md. Nurul Islam	65	
100	Md. Jamiruddin	47	

List of the rickshaw-pullers

Sl. No.	Name	Age (Yeas) (15-26)	Name of the rickshaw stands
1	Jasim uddin	21	Rokeya Hall
2	Farid Ali	19	
3	Barkatulah	20	
4	Hakim Ali	22	
5	Quddus Ali	16	
6	Mohmmad Jabbar	17	Azimpur
7	Mohin Uddid	15	
8	Abdul Samad	19	
9	Mohammad Malek	24	
10	Awoul Mia	25	
11	Amir Hossain	18	Farmegate
12	Suqur Ali	16	
13	Nurul Islam	19	
14	Rafiq Mia	24	
15	Habib	26	
16	Zalal Hossain	21	Shahbag
17	Ismail	19	
18	Kabir	20	
19	Ali Akbar	15	
20	Salim	24	
21	Iqbal	22	Lalbag
22	Gopal Pal	25	
23	Nuruzzaman	18	
24	Mofiz Uddin	19	
25	Akkel ali	17	
26	Mizan	17	Newmarket
27	Mohammad Mohid	24	
28	Wahid	26	

29	Mohammad Saidul	22	
30	Khokon	21	
		Age (Years) (27-40)	
31	Halim	30	Gulishtan
32	Afzal	35	
33	Anowar Hossain	40	
34	Delowar	28	
35	Atowar	33	
36	Dulal	36	Mohakali
37	Tomser	27	
38	Suzid Kumar	39	
39	Mohonta	29	
40	Abdul Latif	38	
41	Abdul Gafur	34	Farmegate
42	Amzad	32	
43	Badal Hossain	31	
44	Polto Mia	30	
45	Nomir Uddin	28	
46	Badiul Islam	30	Shahbag
47	Akram	33	
48	Jasim	37	
49	Faqruddin	40	
50	Sahinoor	37	
51	Yasin Mollah	34	Lalbag
52	Idris Mollah	36	
53	Dulal	38	
54	Monser Ali	39	
55	Joynal	40	
56	Abdul	39	Newmarket
57	Sahidul Islam	38	

58	Zaker Ali	34	
59	Meraz Uddin	31	
60	Mobarak	32	
61	Forhad	37	Rokeya Hall
62	Nader Ali	37	
63	Nur Islam	36	
64	Rezaul Islam	35	
65	Karim Uddin	30	
66	Sirazul Islam	29	Mohammedpur
67	Safiq Mia	29	
68	Razzab Ali	38	
69	Obaidul Islam	39	
70	Samlim Uddin	40	
		Age years (41-65)	
71	Joynuddin	45	Gulishtan
72	Mukul	50	
73	Mokbul	56	
74	Azizul Islam	58	
75	Saiful Islam	59	
76	Bijoy	54	Shamsunnahar Hall Gate
77	Kunto Mia	62	
78	Kalam	63	
79	Rahim Uddin	57	
80	Mohammad Alam	65	
81	Zakir Uddin	62	Farmegate
82	Mosharrof	60	
83	Monir Hossain	61	
84	Mamun	57	
85	Masum Ali	52	
86	Murad	54	
87	Hanifa	60	
88	Jamal	55	

89	Rashid Mia	59	
90	Hanan	60	
91	Shah Alom	48	
92	Tapan	49	
93	Harun Ali	47	
94	Fazlul Karim	53	
95	Bazlul	63	Elephant Road
96	Habibul Islam	62	
97	Garib Uddin	60	
98	Abdur Razzak	55	
99	Abdul Barek	65	
100	Moshlem Uddin	47	Mohammedpur

List of the rural individuals

Sl. No.	Name of the rural individuals	Age (Yeas) (15-26)	Villages of Manikgoanj District
1	Zahirul Islam	21	Nilua
2	Moinul Islam	19	
3	Azahar Uddin	20	
4	Tofazzal Hossain	22	
5	Abdul Mozid	16	
6	A.K.M. Azad	17	Poila
7	Rafiqul Islam	15	
8	Abu Moain Khan	19	
9	Nondan Kumar Shil	24	
10	Chandan Kumar Shil	25	
11	Sukumar Karmoker	18	Terroshree
12	Abdul Jalil	16	
13	Nurunnabi	19	
14	Abdul Hakim Ali (Shanto)	24	
15	Shah Jalal	26	
16	Mucklesur Rahman	21	Borobila
17	Abdul Hamid Khan	19	
18	Tareq Rahman	20	
19	Ziaur Rahman	15	
20	Rashedul Islam	24	
21	Nabibur Rahman	22	Shreedor Nagoar
22	Mominur Chowdury	25	
23	Anisul Islam (Roky)	18	
24	Mamunur Rashid	19	
25	Shamim Hossain	17	
26	Mozibur Rahman	17	Cholkmirpur

27	Khalekuzzaman Chowdury	24	
28	Ashaduzzaman Khan	26	
29	Tariqul Islam	22	
30	Tapash Roy	21	
		Age (Years) (27-40)	
31	Hadiul Islam	30	Shampur
32	Harun-or-Rashid	35	
33	Ranjoy Kumar Das	40	
34	Saifur Rahman	28	
35	Sobahan Ali	33	
36	Solayman Rahman	36	Mulkandi
37	Masud Rana	27	
38	Abdul Manan	39	
39	Sayad Hadi	29	
40	Rahul Panday	38	
41	Nazrul Islam	34	Akashi
42	Mirza Zafar	32	
43	Tarequl Islam	31	
44	Khan Mirza	30	
45	Rany Kayser	28	
46	Rakibul Islam	30	Ghoriala
47	Sayed Mofazzel	33	
48	Mozammel Hoq	37	
49	Abu Taher	40	
50	Md. Abdur Rob	37	
51	Zashim Uddin	34	Dhamshar
52	Jamir Uddin Sharker	36	
53	Nayeb Uddin Mollah	38	
54	Akber Hossain	39	
55	Titumir Rahi	40	

56	Zamirul Islam	39	Bangala
57	Zahir Rayhan	38	Nilua
58	Uttam Kumar Roy	34	
59	Mirza Akanda	31	
60	Rony Kayser	32	
61	Sukanta Kumar	37	Poila
62	Malek Ruhi	37	
63	Abdur Razzak	36	
64	Shajahan Mollah	35	
65	Abdul Gafur	30	
66	Tariqul Islam	29	Terroshree
67	Rakibul Islam	29	
68	Mohammad Anowar Hossain	38	
69	Abdul Zahid	39	
70	Dilip Kumar Pal	40	
		Age years (41-65)	
71	Mozammel Manik	45	Borobila
72	Badiul Islam Bodi	50	
73	Arafad Rahman	56	
74	Minul Islam	58	
75	Sarafat Ali	59	
76	Nahid Manik	54	Shreedor Nagoar
77	Farad Akshar	62	
78	Anamul Rakib	63	
79	Alamin (Topo)	57	
80	Mohoto komar	65	
81	Rashidul Islam	62	Cholkmirpur
82	Thairfur Uddin	60	
83	Oyaskorony	61	

84	Golam Robbany	57	
85	Shaukat Ali	52	
86	Mosrafijur Rahman	54	
87	Abdur Rashead	60	Ghoriala
88	Mukul Rahman	55	
89	Jonal Abadin	59	
90	Abul kahem	60	
91	Mahabub-bu-bur Rahman	48	
92	Babul Khayar	49	Dhamshar
93	Abdul Bary Rajib	47	
94	Ahanul Kabir	53	
95	Abdul Hashem	63	
96	Dr. Kamal	62	Bangala
97	Habubur Rahman	60	
98	Abdulah-Al-Mamun	55	
99	Paresh Kumer	65	
100	Obaidullah Al- Masum	47	

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