بسم الله الرحمن الرحيم

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Liver Function Tests among Mercury Exposure of Gold Mining Workers

A dissertation Submitted in Partial Fulfillment of the Requirement for MSc. Degree in Medical Laboratory Science (Clinical Chemistry)

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الآية

قال الله تعالي:

﴿ قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ﴾

صدق الله العظيم

البقرة الآية (32)

Dedication

To my mother……

To my father soul……..

To my wife……..

To my brothers…

To my friends……
To my colleagues…

I dedicate this work with my best wishes to all

Acknowledgements

All my thanks are in the name of Allah, the most Gracious and the most Merciful.

In this instance, I extended my thanks, deep sincere gratitude and honest appreciation to my supervisor ***Dr.AbdelwahabAbdien****Department of, Clinical Chemistry, Shendi University, for his kindness, good guidance, valuable direction and generous advice that has kept me on the right track. I am indebted to his kind cooperation.*

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I feel indebted to many people who participated and helped me in this work.

List of abbreviations

Abbreviation	Meaning
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
Cl	Chlore
CNS	Central Nervous System
CVAA	Cold Vapor Atomic Absorption
DMA	Direct Mercury Analyzer
FDA	Food and Drug Administration
G/dl	Gram per Deciliter
GGT	Gama-Glutamyl Transpeptidase
Hg	Mercury (hydrargyrum)
ITS	International Temperature Scale
LFTs	Liver Functions Tests
Ng/dl	Nano Gram per Deciliter
SPSS	Statistical Package for Social Scinces

Abstract

The aim of this study is to measure the liver function of the gold prospectors by mercury and compare them with a group of healthy subjects as a control sample. This cross-sectional study was conducted in Abu HAMAD during 2018.

The study included 80 people, the gold prospectors by mercury as a test group (50 people) and healthy (30 people) as control group. The venous blood samples were collected from the two groups to measure the rates of Hg LFTs.

Statistical analysis of the results showed that there was a significant increase in serum GGT and that the average GGT in the experimental group was (38.0 IU/L) when compared with control group (18.9 IU/L) with P.value at 0.000, At the level of the serum ALP in the test group was (82.8 IU/L) when compared to the control group (63.1 IU/L) with P.value at 0.000 and there was a significant decrease in the structural function of the liver because the mean blood albumin in the test group was (3.9 g/dL) when compared with the control group (4.7 g/dL) with P.value at 0.000, there were no significant differences In the other liver parameters between the control group and the control group. The average serum mercury level was in the experimental group (63.6) and in the control group (3.0) with a value of 0.002 The current study found that there was a significant effect of mercury level on serum GGT because the average GGT in a group of normal mercury level was (19.8 IU/L) when compared to the mercury level (43.8 IU/L) with the p.value value of 0.000, That mercury has a significant impact on the level of GGT .

There was a negative correlation between serum albumin and serum mercury level because r = -0.23, p.value was 0.04, and a positive correlation with S.GGT r = 0.526, with p.value value of 0.000. The results of the current study confirmed that

there was no significant effect on the age and duration of exposure to mercury on the liver parameters, because p <0.05.

ملخص البحث

تهدف هذه الدراسة إلي قياس وظائف الكبد للمنقبين عن الذهب المستخدمين للذئيق ومقارنتها بمجموعة من الأصحاء كعينة ضابطة.وقد أجريت هذه الدراسة المقطعية بمدينة أبوحمد خلال عام 2018.

وشملت الدراسة 80 شخص, عبارة عن المنقبين المستخدمينن للذئيق كمجموعة اختبار(50شخص) وأصحاء(30شخص) كمجموعة ضابطة. وقد تم اخذ عينات دم وريدية من المجمعتين لقياس معدلات الزئبق ووظائف الكبد.

ظهر التحليل الإحصائي للنتائج أن هناك زيادة معنوية في GGT في المصل ، وأن متوسط GGT في الدم في المجموعة التجريبية كان (38.0 وحدة دولية / لتر) عند المقارنة مع المجموعة الضابطة (18.9 وحدة دولية / لتر) مع P.value بقيمة 0.000 ، وزيادة كبيرة في مستوى المصل ALP في مجموعة الاختبار كان (82.8 وحدة دولية / لتر) عند مقارنتها بمجموعة التحكم (63.1 وحدة دولية / لتر) مع P.value بقيمة 0.000 ، وكان هناك انخفاض معنوي في الوظيفة التركيبية للكبد لأن متوسط الزلال في الدم في مجموعة الاختبار كانت (3.9 جم / ديسيلتر) عند المقارنة مع المجموعة الضابطة (4.7 جم / ديسيلتر) مع P.value بقيمة 0.000 ، لم يكن هناك فروق ذات دلالة إحصائية في معاملات الكبد الأخرى بين مجموعة الاختبار والمجموعة الضابطة. وكان متوسط مستوى الزئبق في المصل في المجموعة التجريبية (63.6) وفي المجموعة الضابطة (3.0) مع قيمة 0.002.

خلصت الدراسة الحالية إلى وجود تأثير معنوي لمستوى الزئبق على GGT في المصل لأن متوسط GGT في مجموعة من مستوى الزئبق العادي كان (19.8 وحدة دولية / لتر) عند مقارنته بمستوى الزئبق (43.8 IU / L) مع القيمة p.value من 0.000 ، مما يعني أن للزئبق تأثيرًا كبيرًا على مستوى GGT في الدم..

كان هناك ارتباط سلبي بين ألبيومين المصل ومستوى الزئبق في المصل لأن r = -0.23 ، وكان p.value 0.04 ، وارتباط إيجابي مع S.GGT r = 0.526 ، مع قيمة p.value من 0.000. أكدت نتائج الدراسة الحالية أنه لم يكن هناك تأثير مهم لعمر ومدة التعرض للزئبق على بارامترات الكبد ، لأن القيمة p> كانت 0.05.

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Chapter One

Introducti, objectives and rationale

1. Introduction

The liveris an organ only found in vertebrates, detoxifies, various metabolites, synthesizes proteins, and produces biochemical necessary for digestion.[1][2][3] In humans, it is located in the right upper quadrant of the abdomen, below the diaphragm. Its other roles in metabolism include the regulation of glycogen storage, decomposition of red blood cells and the production of hormones.[3]

The liver is an accessory digestive gland that produces bile, an alkaline compound which helps the breakdown of fat .Bile acids in digestion via the emulsification of lipids. The gallbladder, a small pouch that sits just under the liver, stores bile produced by the liver.[4] The liver's highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions.[5] Estimates regarding the organ's total number of functions vary, but textbooks generally cite it being around 500.[6]

Terminology related to the liver often starts in hepat- from ἡπατο-, the Greek word for liver.[7]

Mercury (Hg) exists naturally as elemental Hg, in inorganic mercurous and mercuric compounds, and in organic Hg compounds. These 3 types of Hg are known to have different toxicity and health effects [8][9] Elemental and inorganic forms of Hg are predominantly absorbed through the respiratory tract, while organic Hg is mainly absorbed and bioaccumulated through the gastrointestinal tract because of its highly lipophilic nature. [8].Hg is removed from the human body through urine or feces, and the half-life of total Hg in the blood was found to be 57 days on average in a Japanese study. [10]

The toxicity of high-level Hg exposure is well known from Minamata disease in Japan outbreaks of which occurred in 1956 and 1965 due to the consumption of Hg-contaminated seafood, [11] and in Iraq.[12]

In addition to neurological toxicity, high levels of Hg exposure affect various human organs, including the cardiovascular, endocrine, reproductive, and immune systems. [13] The mechanisms of its toxicity have been suggested to involve degeneration, oxidative stress, and changes in the energy metabolism of the cell, but are not fully understood.[14]

1.2 Rationale

The rates of random prospecting for gold have increased, prospectors using the mercury element, In the absence of necessary safety precautions, Exposure to mercury is the cause of many diseases like cancer .

In this research I want to know the effect of the element of mercury on liver function in the gold mining workers in the area of Abu Hamad in northern Sudan

1.3 objectives

1.3.1 General objective

Measure liver function tests among mercury exposures of gold mining workers

1.3.2 Specific objectives

1. Evaluate liver proteins and enzymes in affected (exposed) individuals (Total protein - albumin - AST-ALT-ALP - GGT).
2. Evaluate liver proteins and enzymes in non affected (nonexposed) individuals (Total protein - albumin - AST-ALT-ALP - GGT).
3. Measure mercury (Hg) concentration in affected individual.
4. Measure mercury (Hg) concentration in non affected individual.
5. Compare the results between the exposed and non exposed groups.
6. Compare the results with previous studies.
7. Compare the results between different age groups.

Chapter two

Literature review

2. Literature review

2.1 Liver:

The liver is an organism only found in vertebrates, detoxifies various metabolites, synthesizes proteins, and produces biochemicals necessary for digestion.[1][2][3]

2.1.1 Liver structure:

The liver is a reddish-brown, wedge-shaped organ with four lobes of unequal size and shape. A human liver normally weighs 1.44–1.66 kg (3.2–3.7 lb),[15] It is both the heaviest internal organ and the largest gland in the human body. Located in the right upper quadrant of the abdominal cavity, it rests just below the diaphragm, to the right of the stomach and overlies the gallbladder.[16]

The liver is connected to two large blood vessels: the hepatic artery and the portal vein. The hepatic artery carries oxygen-rich blood from the aorta, whereas The hepatic portal vein delivers around 75% of the liver's blood supply, carries blood rich in digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas, and its associated organs.[17-18]

Oxygen is provided from both sources; about half of the liver's oxygen demand is met by the hepatic portal vein, and half is met by the hepatic arteries.[18]

The central vein joins to the hepatic vein to carry blood out from the liver. A distinctive component of a lobule is the portal triad, which can be found running along each of the lobule's corners. The portal triad, misleadingly named, consists of five structures: a branch of the hepatic artery, a branch of the hepatic portal vein, and a bile duct, as well as lymphatic vessels and a branch of the vagus nerve.[19]

These blood vessels subdivide into small capillaries known as liver sinusoids, which then lead to lobules, lobules are the functional units of the liver. Each lobule is made up of millions of hepatic cells (hepatocytes), which are the basic metabolic cells. The lobules are held together by a fine, dense, irregular, fibroelastic

connective tissue layer which extends from the fibrous capsule covering the entire liver known as Glisson's capsule.[20]

2.1.2 Liver gross anatomy

2.1.2.1 Lobes

The liver is grossly divided into two parts when viewed from above – a right and a left lobe, and four parts when viewed from below (left, right, caudate, and quadrate lobes).(21)

The falciform ligament, divides the liver into a left and right lobe. From below, the two additional lobes are located between the right and left lobes, one in front of the other. A line can be imagined running from the left of the vena cava and all the way forward to divide the liver and gallbladder into two halves.(22) This line is called "Cantlie's line".(23)

Other anatomical landmarks include the ligamentumvenosum and the round ligament of the liver (ligamentumteres), which further divide the left side of the liver in two sections. An important anatomical landmark, the portahepatis, divides this left portion into four segments, which can be numbered starting at the caudate lobe as I in an anticlockwise manner. From this parietal view, seven segments can be seen, because the eighth segment is only visible in the visceral view.(24)

2.1.2.2 Surface:

On the diaphragmatic surface, apart from a triangular bare area where it connects to the diaphragm, the liver is covered by a thin, double-layered membrane, the peritoneum, that helps to reduce friction against other organs.[25] This surface covers the convex shape of the two lobes where it accommodates the shape of the diaphragm. The peritoneum folds back on itself to form the falciform ligament and the right and left triangular ligaments.[26]

These peritoneal ligaments are not related to the anatomic ligaments in joints, and the right and left triangular ligaments have no known functional importance,

though they serve as surface landmarks. The falciform ligament functions to attach the liver to the posterior portion of the anterior body wall.[26]

The visceral surface or inferior surface is uneven and concave. It is covered in peritoneum apart from where it attaches the gallbladder and the portahepatis.[25]

2.1.3 Microscopic anatomy

The liver is divided into microscopic units called lobules. The lobules are the functional units of the liver; they are responsible for all metabolic and excretory functions performed by the liver. Each lobule is roughly a six-sided structure with a centrally located vein (called the central vein) with portal triads at each of the corners. Each portal triad contains a hepatic artery, a portal vein, and a bile duct surrounded by connective tissue. (27)

The liver contains two major cell types: hepatocytes and kupffer cells. The hepatocytes, making up approximately 80% of the volume of the organ, are large cells that radiate outward from the central vein in plates to the periphery of the lobule. These cells perform the major functions associated with the liver and are responsible for the regenerative properties of the liver. Kupffer cells are macrophages that line the sinusoids of the liver and act as active phagocytes capable of engulfing bacteria, debris, toxins, and other substances flowing through the sinusoids. (27)

2.1.4 Biochemical function:

The liver performs four major functions: excretion/secretion, synthesis, detoxification, and storage. The liver is so important that if the liver becomes nonfunctional, death will occur with 24 hours due to hypoglycemia. Althoughthe liver is responsible for a number of functions, this hapter focuses on the four major functions mentioned previously.(27)

2.1.4.1 Excretory and Secretory function

One of the most important functions of the liver is the processing and excretion of endogenous and exogenous substances into the bile or urine such as the major heme waste product, bilirubin.[27]

2.1.4.2 Synthetic function

The liver has extensive synthetic capacity; it is responsible for synthesizing many biological compounds including carbohydrates, lipids, and proteins.[27]The metabolism of carbohydrates is one of the most important functions of the liver.[27]

When carbohydrates are ingested and absorbed, the liver can do three things:

Firstly use the glucose for its own cellular energy requirements,secondly circulate the glucose for use at the peripheral tissues, or thirdly store glucose as glycogen (principal storage form of glucose) within the liver itself or within other tissues.[27]

2.1.4.3 Detoxification and Drug Metabolism

The liver serves as a gatekeeper between substances absorbed by the gastrointestinal tract and those released into systemic circulation. Every substance that is absorbed in the gastrointestinal tract must first pass through the liver; this is referred to as first pass. This is an important function of the liver because it can allow important substances to reach the systemic circulation and can serve as a barrier to prevent toxic or harmful substances from reaching the systemic circulation. The body has two mechanisms for detoxification of foreign materials (drugs and poisons) and metabolic products (bilirubin and ammonia). It may either bind the material reversibly so as to inactivate the compound, or it may chemically modify the compound so it can be excreted. The most important mechanism is the drug-metabolizing system of the liver. This system is responsible for the detoxification of many drugs through oxidation, reduction, hydrolysis, hydroxylation, carboxylation, and demethylation. [27]

2.1.5 Liver function tests

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs. The most common LFTs include the serum aminotransferases, alkaline phosphatase, bilirubin, albumin, and prothrombin time. Aminotransferases, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. Alkaline phosphatase (ALP), gama-glutamyltranspeptidase (GGT), and bilirubin act as markers of biliary function and cholestasis. Albumin and prothrombin reflect liver synthetic function. The aminotransferases AST and ALT are normally < 30–40 units/l. Elevations of aminotransferases greater than eight times the upper limit of normal reflect either acute viral hepatitis, ischemic hepatitis, or drug- or toxin-induced liver injury. Much more common than patients with acute hepatitis, however, are patients with chronic mild elevation of aminotransferases, or AST and ALT < 250 units/l for > 6 months.[28]

2.2 Mercury

Mercury (Hg), also called quicksilver, is a heavy, silvery metal. Mercury is one of two (the other is bromine) elements that are liquid at room temperature and pressure. Extremely toxic mercury compound, dimethyl mercury, looks like water but is three times as dense. There are three naturally occurring oxidation states of mercury: Hg(0), Hg(I), and Hg(II). Organic mercury refers to forms of mercury bound to a carbon atom, with mercury usually in Hg (II) oxidation state. (27)

Mercury is a chemical element with symbol Hg and atomic number 80. It is commonly known as quicksilver and was formerly named hydrargyrum(29)

Mercury (Hg) is a naturally occurring metal, which is widespread and persistent in the environment. It exists in three forms: elemental or metallic mercury, inorganic mercury, and organic mercury. Most of the mercury in the atmosphere is elemental

mercury vapor; most of the mercury in water, soil, plants, and animals is inorganic and organic [30].

Mercury is released to atmosphere as a product of the natural out gassing of rock (30,000 tons per year) and as a fungicide (6,000 tons per year), and it is incorporated into dental amalgams (90 tons per year). Mercury is also used in electrical switches. [27]

2.2.1 Physical and chemical Properties

Mercury is a heavy, silvery-white liquid metal. Compared to other metals, it is a poor conductor of heat, but a fair conductor of electricity.[31] It has a freezing point of −38.83 °C and a boiling point of 356.73 °C[32][33][34] both the lowest of any stable metal, although preliminary experiments on copernicium and flerovium have indicated that they have even lower boiling points (copernicium being the element below mercury in the periodic table, following the trend of decreasing boiling points down group 12).[35] Upon freezing, the volume of mercury decreases by 3.59% and its density changes from 13.69 g/cm^3 when liquid to 14.184 g/cm^3 when solid. The coefficient of volume expansion is 181.59 × 10^{-6} at 0 °C, 181.71 × 10^{-6} at 20 °C and 182.50 × 10^{-6} at 100 °C (per °C). Solid mercury is malleable and ductile and can be cut with a knife.[36]

Mercury does not react with most acids, such as dilute sulfuric acid, although oxidizing acids such as concentrated sulfuric acid and nitric acid or aqua regia dissolve it to give sulfate, nitrate, and chloride. Like silver, mercury reacts with atmospheric hydrogen sulfide. Mercury reacts with solid sulfur flakes, which are used in mercury spill kits to absorb mercury (spill kits also use activated carbon and powdered zinc).[37]

2.2.2 Mercury Isotopes:

There are seven stable isotopes of mercury with ^{202}Hg being the most abundant (29.86%). The longest-lived radioisotopes are ^{194}Hg with a half-life of 444 years, and ^{203}Hg with a half-life of 46.612 days. Most of the remaining radioisotopes have

half-lives that are less than a day. ^{199}Hg and ^{201}Hg are the most often studied NMR-active nuclei, having spins of $^{1}/_{2}$ and $^{3}/_{2}$ respectively.[38]

2.2.3 Mercury applications:

Mercury is used primarily for the manufacture of industrial chemicals or for electrical and electronic applications. It is used in some thermometers, especially ones which are used to measure high temperatures. A still increasing amount is used as gaseous mercury in fluorescent lamps, while most of the other applications are slowly phased out due to health and safety regulations and is in some applications replaced with less toxic but considerably more expensive Galinstan alloy.[39]

In medicine, mercury and its compounds have been used in medicine, although they are much less common today than they once were, now that the toxic effects of mercury and its compounds are more widely understood(40) .

Mercury is an ingredient in dental amalgams. Thiomersal (called Thimerosal in the United States) is an organic compound used as a preservative in vaccines, though this use is in decline.[41]

Today, the use of mercury in medicine has greatly declined in all respects, especially in developcountries. Thermometers and sphygmomanometers containing mercury were invented in the early 18th and late 19th centuries, respectively.(42)

Mercury compounds are found in some over-the-counter drugs, including topical antiseptics, stimulant laxatives, diaper-rash ointment, eye drops, and nasal sprays. The FDA has "inadequate data to establish general recognition of the safety and effectiveness" of the mercury ingredients in these products.[43]

In laboratorysome transit telescopes use a basin of mercury to form a flat and absolutely horizontal mirror, useful in determining an absolute vertical or perpendicular reference. Concave horizontal parabolic mirrors may be formed by rotating liquid mercury on a disk, the parabolic form of the liquid thus formed reflecting and focusing incident light. Such telescopes are cheaper than

conventional large mirror telescopes by up to a factor of 100, but the mirror cannot be tilted and always points straight up.[44][45][46]

Liquid mercury is a part of popular secondary reference electrode (called the calomel electrode) in electrochemistry as an alternative to the standard hydrogen electrode. The calomel electrode is used to work out the electrode potential of half cells.[47] Last, but not least, the triple point of mercury, −38.8344 °C, is a fixed point used as a temperature standard for the International Temperature Scale (ITS-90).[31]

In polarography both the dropping mercury electrode[48] and the hanging mercury drop electrode[49]

In cosmetics, Mercury, as thiomersal, is widely used in the manufacture of mascara. Minnesota became the first state in the United States to ban intentionally added mercury in cosmetics, giving it a tougher standard than the federal government.[50]

Gold and silver mining: Historically, mercury was used extensively in hydraulic gold mining in order to help the gold to sink through the flowing water-gravel mixture. Thin gold particles may form mercury-gold amalgam and therefore increase the gold recovery rates.[31] Large-scale use of mercury stopped in the 1960s. However, mercury is still used in small scale, often clandestine, gold prospecting. It is estimated that 45,000 metric tons of mercury used in California for placer mining have not been recovered.[51] Mercury was also used in silver mining.(52]

2.2.4 Health Effects

Mercury has no known function in normal human physiology. Mercury and its compounds have been used in medicine, although they are much less common today than they once were, now that the toxic effects of mercury and its compounds are more widely understood. Mercury (I) chloride has traditionally been used as a diuretic, topical disinfectant, and laxative. The mercury containing

organohalide Mercurochrome is still widely used but has been banned in the United States and some other countries. [27]

2.2.4.1 Absorption, Transport, and Excretion

Routes of exposure include

I - inhalation, primarily aselemental mercury vapor but occasionally as dimethyl mercury.

II - ingestion, as $HgCl_2$, and also consumption of high-mercury foods such as certain fish species.

III - Cutaneous, methyl mercury is rapidly absorbed through skin, even through latex gloves.

IX – injection, liquid mercury and mercury-containing tattoo pigments are relatively inert due to low water solubility.Water-soluble forms of mercury can cause rapid tissue destruction.

X - Dental amalgams likely cause a slight increase in blood and urine mercury levels with uncertain but probably have insignificant health consequences.

Inhaled mercury vapor is retained in the lungs to about 80%, whereas liquid metallic mercury passes the gastrointestinal tract almost unabsorbed. [53]

Mercury enters the food chain primarily by volcanic activity and manmade sources such as coal combustion and smelting. Most of the dietary intake comes from consumption of meat and fish products, with an estimated dietary intake of approximately 3 g/day. [54]

There is relatively little bioaccumulation of mercury. Half-lives vary according to the form of mercury and the fluid sampled, from 5 days in blood for phenyl mercury to 90 days in urine for chronic exposure to inorganic mercury.

Normally, the highest accumulation of mercury is in kidney, liver, spleen, and brain. Mercury can accumulate in pituitary and thyroid glands, the pancreas, and the reproductive organs. [55]

The kidney is the major storage organ after elemental or inorganic mercury exposure. However, large amounts are transported to the brain after the inhalation of elemental mercury of methyl mercury (MeHg). Methyl mercury is efficiently absorbed from the gastrointestinal tract, and distribution to tissues, including the brain, appears complete in 48 hours. Movement of MeHg across the blood-brain barrier appears to be dependent on coupling with the amino acid cystine. [55]

2.2.5 Toxicity and safety:

Elemental and inorganic forms of Hg are predominantly absorbed through the respiratory tract, while organic Hg is mainly absorbed and bioaccumulated through the gastrointestinal tract because of its highly lipophilic nature [8]. Hg is removed from the human body through urine or feces, and the half-life of total Hg in the blood was found to be 57 days on average in a Japanese study. [10]

The toxicity of high-level Hg exposure is well known from Minamata disease in Japan outbreaks of which occurred in 1956 and 1965 due to the consumption of Hg-contaminated sea food. [11] and in Iraq.[12]

In addition to neurological toxicity, high levels of Hg exposure affect various human organs, including the cardiovascular, endocrine, reproductive, and immune systems. [13] The mechanisms of its toxicity have been suggested to involve degeneration, oxidative stress, and changes in the energy metabolism of the cell, but are not fully understood [14]

Mercury and most of its compounds are extremely toxic and must be handled with care; in cases of spills involving mercury (such as from certain thermometers or fluorescent light bulbs), specific cleaning procedures are used to avoid exposure and contain the spill. [56]

Preindustrial deposition rates of mercury from the atmosphere may be about (4ng/L of ice deposit). Although that can be considered a natural level of exposure,

regional or global sources have significant effects. Volcanic eruptions can increase the atmospheric source by 4–6 times(57)

Mercury toxicity is a result of protein binding, which results in a change of structure and function. The most significant result of this interaction is the inhibition of many enzymes. Binding to intestinal proteins after ingestion of inorganic mercury results in acute gastrointestinal disturbances. Ingestion of moderate amounts may result in severe bloody diarrhea because of ulceration and necrosis of the gastrointestinal tract. In severe cases, this may lead to shock and death.(27)

Mercury in all forms poisons cellular function by altering the tertiary and quaternary structure of proteins and by binding with sulfhydryl and selenohydryl groups. Consequently, mercury can potentially impair function of any organ, or any subcellular structure. The chief target organ of mercury vapor is the brain, but peripheral nerve function, renal function, immune function, endocrine and muscle function, and several types of dermatitis have been described [58].

With massive acute exposure to mercury vapor, erosive bronchitis and bronchiolitis potentially leading to respiratory failure may be accompanied by CNS symptoms such as tremor or erethism(59)

Chronic exposure to clinically significant doses of mercury vapor usually produces neurological dysfunction. At low-level exposures, nonspecific symptoms like weakness, fatigue, anorexia, weight loss, and gastrointestinal disturbance have been described [60]. Higher exposure levels are associated with mercurial tremor: fine muscle fasciculations punctuated every few minutes by coarse shaking.

Erethism may also be observed: severe behavior and personality changes, emotional excitability, loss of memory, insomnia, depression, fatigue, and in

severe cases delirium and hallucination [61]. Gingivitis and copious salivation have been described [62].

These symptoms may regress with cessation of exposure, but in many cases do not. Persistent neurological symptoms are common.(63)

2.3 Previous studies

An epidemiological study with risk analysis of liver diseases in the general population living in methyl mercury polluted area. J Epidemiol Community Health. By Futatsuka M,et al. in Minamata area, at 1992, they did not have a higher prevalence of liver disease than other areas. [64]

Association of serum lead and mercury level with cardiometabolic risk factors and liver enzymes in a nationally representative sample of adolescents, by Poursafa P, et al. in the CASPIAN, at 2014.a study of 320 they found that there is no significant associations were found between blood Hg levels and aspartate amino transferase (AST) or alanine transaminase (ALT) levels. [65]

Chapter three

Materials & Methodology

3.Material and methods:

3.1 Study design:

Descriptive cross sectionalanalytical study,carried out in Abu Hamad city at the period from February to July 2018 to evaluate the effect of mercury exposture on liver function tests.

3.2 Study area :

This study was done in Abu Hamad city – River Nile State – Northern Sudan.

3.3 Study population :

Gold mining workers in different age and duration of exposed to mercury

3.4 Inclusion criteria:

People exposed to mercury, and others doesn't exposed to mercury(control group)

3.5 Exclusion criteria:

Persons who have liver problems or alcoholism.

3.6 Sample size:

There were50 of gold mining workers selected randomly as test group, and 30 healthy non exposedvolunteers were selected randomly as control group.

3.7 Sampling :

Three ml of venous blood samples were collected by standard procedure in vaccuated plain blood container, serum was separated and tested.

3.8 Data collection tools :

Interview questionnaire was applied and filled, which contain name, age, duration of mercury exposer, history of liver disease and investigations (LFTs).

3.9 Methods

3.9.1 Estimation of serum (total) protein:

Protein in the samples reacts with copper (11) ion in alkaline medium forming a colored complex.

3.9.2 Estimation of serum Albumin:

Albumin in the presence of bromocresol green at slightly acid pH produces a colour change of the indicator from yellow –green to green blue. The intensity of the color formed is proportional to the albumin concentration in the sample.

3.9.3 Aspartate amino transferees (AST/GOT):-

Aspartate aminotranseferase:9(AST OR GOT)catalyzes the transferees of the of (Astor Got)catalyzes the transferees of the amino group from aspartate to 2 oxoglutarate forming oxaloacetate and glutamate .the catalytic concentration is determined from the rate of decrease of NADH ,measured at 340 nm by means of the malate dehdrogenenase (MDH)coupled reaction .

Aspartate +2 - 0x0glutarate$\xrightarrow{AST}$ Oxaloacetate +Glutamate

Oxaloacetate +NADH+H^+ $\xrightarrow{\text{MDH}}$ MALATE+NAD^+

3.9.4 Alanine amino transferase (ALT or GPT):

Catalyzes the transfer of amino group from alanine to 2 oxoglutarate, forming pyruvate and glutamate, the catalytic concentration is determine from the rate decrease of NADH measured at 340 nm, by means of the lactate dehydrogenase (LDH)coupled reaction

Alanine +2- Oxoglutrate$\xrightarrow{ALT}$Pyruvate +glutamate

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$Lactate +NAD^+

3.9.5 Alkaline phosphatase:-

Alkaline phosphate (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrohpenylphosphate to 2-amino2 methyl-1-propanol (AMP) liberating 4 nitrophenol .the catalytic concentration is determined from the rate of 4 nitrophenol formation .measured at 405 nm .

4 - Nitrophenylphosphate +AMP$\xrightarrow{ALP}$AMP- phosphate +4- Nitro phenol.

3.9.6Mercury :

A small amount of the coal or fly ash sample (0.05-1.00 gms, depending on the mercury content) is weighed into a sample boat (typically nickel). The boat is heated in an oxygen rich furnace, to release all the decomposition products, including mercury. These products are then carried in a stream of oxygen to a catalytic section of the furnace. Any halogens or oxides of nitrogen and sulfur in the sample are trapped on the catalyst. The remaining vapor is then carried to an amalgamation cell that selectively traps mercury. After the system is flushed with oxygen to remove any remaining gases or decomposition products, the amalgamation cell is rapidly heated, releasing mercury vapor. Flowing oxygen carries the mercury vapor through an absorbance cell positioned in the light path of a single wavelength atomic absorption spectrophotometer. Absorbance is measured at the 253.7 nm wavelength as a function of the mercury concentration in the sample. Adetection limit of 0.005 ng (nanogram) of mercury is achievable with a 25 cm path length cell, while a 2 cm cell allows a maximum concentration of 20 µg (microgram) of mercury.

3.10 Data analysis technique:

The collected data was analyzed with spsssoft ware computer program, to obtained correlation; coefficient. Also paired t-test was used for calculating degree of variation, p.value<0.05 was considered to be significant.

3.11 Ethical clearance

Informed consent was attached to each questionnaire to be obtained from the patient. There was full commitment precaution sample taken and privacy and confidentiality.

Chapter four

Results

4. Result:

This descriptive cross sectional analytical study that aimed to evaluate LFTs among gold mining workers in Abu Hamad city in northern Sudan, at the period from February to July 2018.

Table (4.1) shows Comparison of serum T. protein, s. albumin, ALP, AST, ALT, GGTand Mercury between test group and control.

Table (4.2) shows Comparison of serum T. protein, s. albumin, ALP, AST, ALT and GGT between test group in normal mercury level and high mercury level.

Table (4.3) shows compression between serum T. protein, s. albumin, ALP, AST, ALT, GGT, Mercury and duration of mercury exposure.

Table (4.4) shows compression between serum T .protein, s. albumin, ALP, AST, ALT, GGT, Mercury and age.

Figure (4.1): Distribution of study group according to age.

Figure (4.2): Distribution of study group according to duration of exposure.

Figure (4.3): relationship between mercury and albumin concentration.

Figure (4.4): relationship between mercury and GGT concentration.

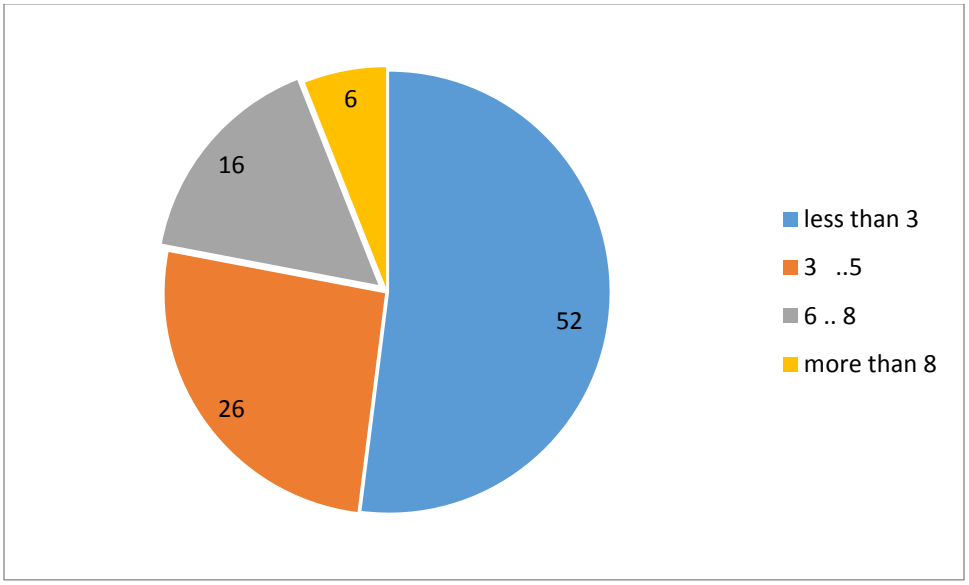

Figure (4.1): Distribution of study group according to age groups:

Table (4.1)shows Comparison of serum T.protein, s. albumin, ALP, AST, ALT, GGTand Mercury between test group and control:

Parameters	Frequency of control	Frequency of test	Mean of test	Mean of control	Sig.(2-tailed)
T.protein	30	50	7.8	7.6	0.258
Albumin	30	50	3.9	4.7	0.000*
ALP	30	50	82.8	63.1	0.000*
AST	30	50	20.9	19.9	0.469
ALT	30	50	21.2	21.5	0.833
GGT	30	50	38.0	18.9	0.000*
Mercury	30	50	63.6	3.0	0.002*

Independent t- test

*****Significant level is <0.05

.

Table (4.2)shows Comparison of serum T.protein, S. albumin, ALP, AST, ALT and GGT between test group in normal mercury level and high mercury level:

	Normalmercury level		High mercury level		
Parameter	**Frequency**	**Mean**	**Frequency**	**Mean**	**Sig.(2-tailed)**
T.protein	12	7.8	38	7.8	0.603
Albumin	12	3.8	38	3.9	0.679
ALP	12	82.1	38	83.1	0.881
AST	12	23.1	38	20.9	0.160
ALT	12	20.2	38	21.5	0.607
GGT	12	19.8	38	43.8	0.000*

Independent t- test

*****Significant level is <0.05

Table (4.3) shows compression between serum T.protein, S.albumin, ALP, AST, ALT, GGT, Mercuryand duration of mercury exposure:

Parameter	Duration of exposure	Frequency	Mean	P.value
Protein	Less than 3 years	26	7.7	0.093
	3 - 5 years	13	7.9	
	6 - 8years	8	8.1	
	More than 8 years	3	7.6	
Albumin	Less than 3 years	26	3.8	0.197
	3 - 5 years	13	3.9	
	6 - 8years	8	4.0	
	More than 8 years	3	3.6	
ALP	Less than 3 years	26	82.1	0.690
	3 - 5 years	13	81.7	
	6 - 8years	8	89.7	
	More than 8 years	3	75.6	
AST	Less than 3 years	26	19.5	0.121
	3 - 5 years	13	22.1	
	6 - 8years	8	24.7	
	More than 8 years	3	17.6	
ALT	Less than 3 years	26	21.4	0.497
	3 - 5 years	13	19.5	
	6 - 8years	8	24.1	
	More than 8 years	3	19.0	
GGT	Less than 3 years	26	36.0	0.054
	3 - 5 years	13	32.9	
	6 - 8years	8	54.5	
	More than 8 years	3	34.0	
Mercury	Less than 3 years	26	83.9	0.549
	3 - 5 years	13	43.3	
	6 - 8years	8	45.5	
	More than 8 years	3	23.8	

One way ANOVA

*****Significant level is <0.05

Table (4.4) shows compression between serum T.protein, s. albumin, ALP, AST, ALT, GGT,Mercury and age:

Parameter	Age	Frequency	Mean	P.value
Protein	Less than 20 years	4	7.7	0.813
	20 - 30 years	30	7.8	
	31 - 40 years	9	7.8	
	More than 40 years	7	7.9	
Albumin	Less than 20 years	4	3.8	0.665
	20 - 30 years	30	3.9	
	31 - 40 years	9	3.9	
	More than 40 years	7	3.9	
ALP	Less than 20 years	4	83.7	0.718
	20 - 30 years	30	81.1	
	31 - 40 years	9	89.5	
	More than 40 years	7	81.0	
AST	Less than 20 years	4	18.2	0.441
	20 - 30 years	30	20.2	
	31 - 40 years	9	23.4	
	More than 40 years	7	21.8	
ALT	Less than 20 years	4	22.2	0.719
	20 - 30 years	30	21.5	
	31 - 40 years	9	21.8	
	More than 40 years	7	18.3	
GGT	Less than 20 years	4	29.2	0.057
	20 - 30 years	30	35.1	
	31 - 40 years	9	53.1	
	More than 40 years	7	36.3	
Mercury	Less than 20 years	4	42.7	0.679
	20 - 30 years	30	70.1	
	31 - 40 years	9	81.9	
	More than 40 years	7	24.1	

One way ANOVA

*****Significant level is <0.05

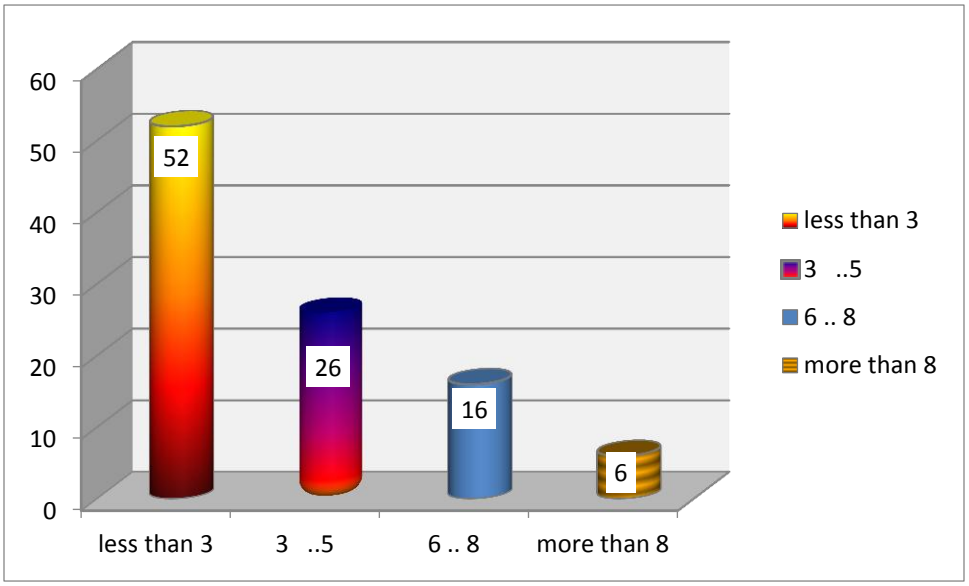

Figure (4.2): Distribution of study group according to duration of exposure

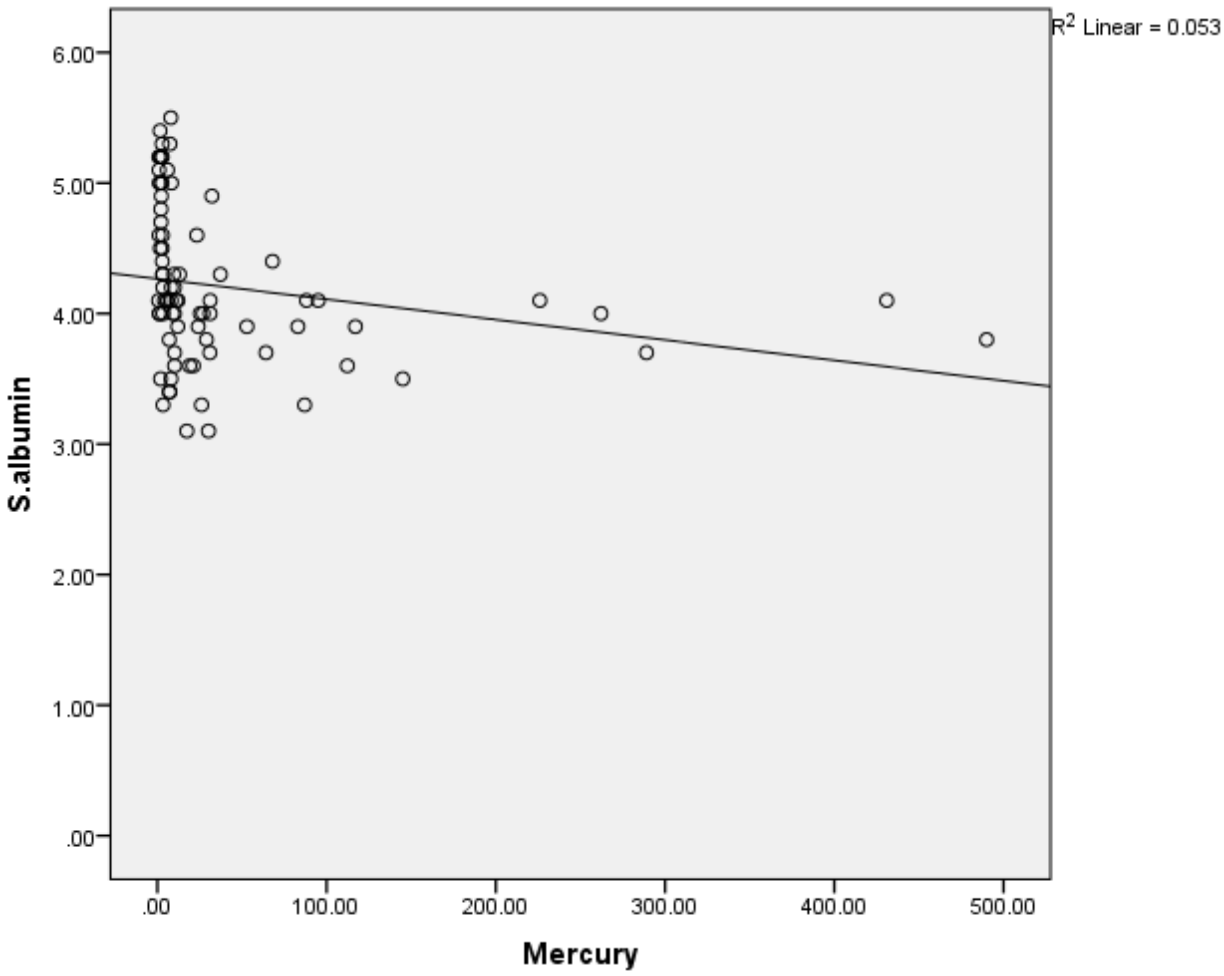

Figure (4.3): relationship between mercury and albumin concentration

Personal correlation -0.23

P.value 0.04

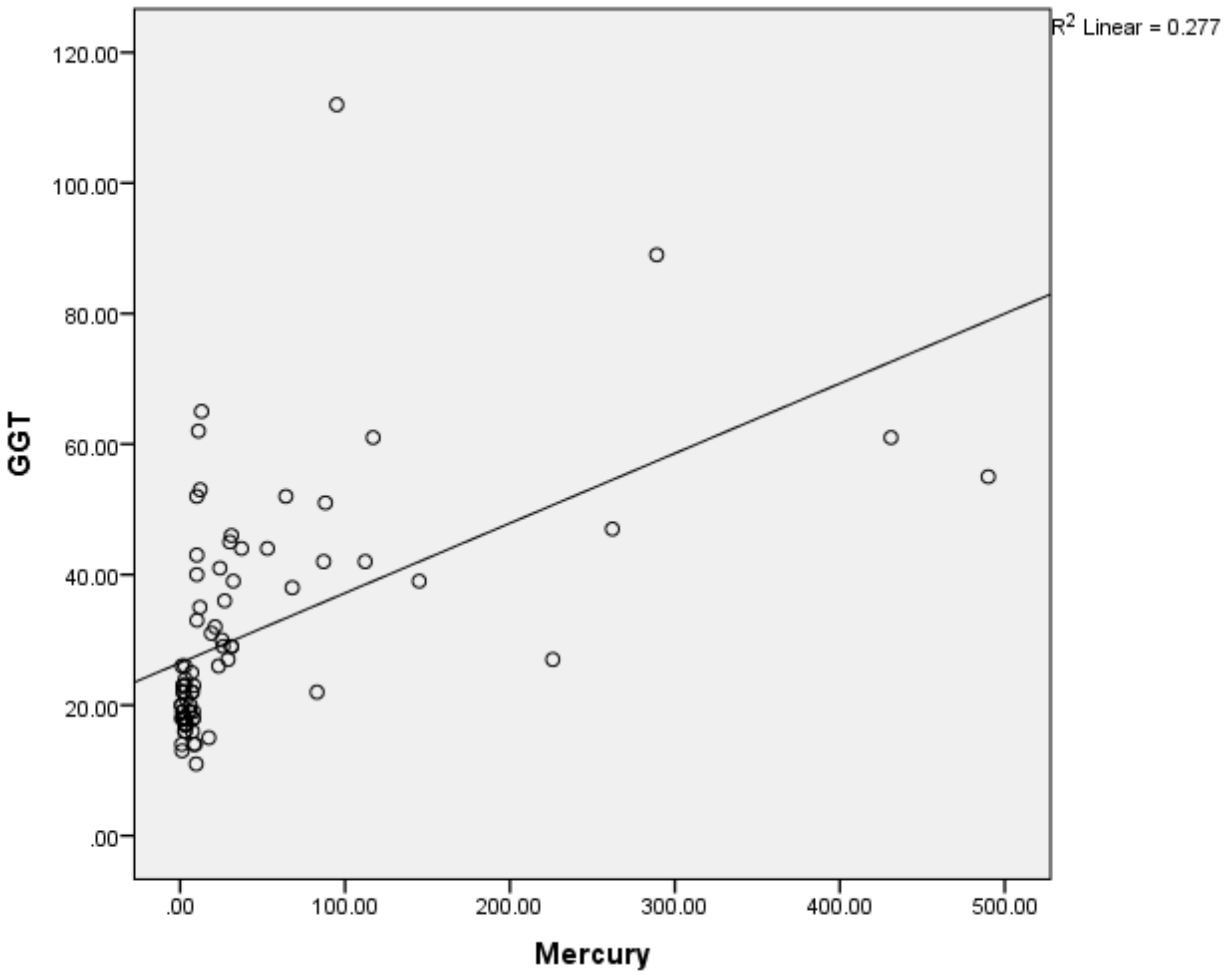

Figure (4.4): relationship between mercury and GGT concentration.

Personal correlation 0.526

P.value 0.000

Chapter five

Discssion

Conclusion

Recommendations

5.1 Discussion:

The present study was carried out to investigate liver function tests and serum mercury among gold mining in Abu Hamad city, River Nile state in Sudan during at the period from February to July 2018.

80 blood samples were collected, a 50 of these samples were collected from gold mining as test group, and 30 samples were collected from health individual as control group.

Statistical analysis of results reviled that, there was significant increase in serum GGT, mean serum GGT in test group was (38.0 IU/L) when compared with control group was (18.9 IU/L) with P.value of 0.000, and significant increase in serum level of ALP in test group was (82.8 IU/L) when compared with control group of (63.1IU/L) with P.value of 0.000, and there was significant decrease in synthetic function of liver because the mean of serum albumin in test group was (3.9 g/dL) when compared with control group was (4.7 g/dL) with P.value of 0.000, there was no significant differences in other liver parameters between test and control group. The mean level of serum mercury in test group was (63.6) and in control group was (3.0) with p.value of 0.002.

Present study reviled that there was significant effect of mercury level on serum GGT because the mean of GGT in group of normal mercury level was (19.8IU/L) when compared with pathologic level of mercury was (43.8IU/L) with p.value of 0.000, that mean the mercury had significant effect on serum GGT level. Our study result agrees with study conducted by Futatsuka M,et al. in Minamata area, at 1992 and the study conducted by Poursafa P, et al. in the CASPIAN, at 2014.

There was negative correlation between serum albumin and serum mercury level because r= -0.23, and p.value was 0.04, and positive correlation with S.GGT r= 0.526, with p.value of 0.000. Present study results reviled that there was no

significant effect of age and duration of mercury exposure on liver parameters, because p.value were > 0.05.

5.2 Conclusion:

On the basis of our result we conclude that:

- Mercury exposure associate with an elevate serum concentration of GGT
- Mercury exposure associate with an elevate serum concentration of ALP.
- Mercury exposure associate with decrease serum concentration of Albumin.
- Mercury exposure not associate with serum concentration of AST,ALT,and total protein level.

5.3 Recommendations

- Educate gold miners to the risks of unsafe use of mercury.
- Gold prospectors are alerted to safe disposal of mercury-contaminated waste.
- Replacing traditional extraction method with modern motorized methods.
- Conduct periodic examinations of those exposed to mercury.

Chapter Six

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Appendix

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6-2 Appendixes:

ShendiUniversity
Faculty of Graduate Studies and Scientific Research

Questionnaire about LFTs among gold mining workers in Abu Hamed city 2018

Name:……..

1. Age groups:

a. < 20 ( ) b. 20 - 30 ( ) c. 31 - 40 ( )
d. >40 ( ).

2. When you start gold mining work?

a. < 3 years ( ) b. 3 –5 years ( ) c. 6 - 8years ( )
d. > 8 years( ).

3.Are you alcoholism?

a. Yes ( ) b. No ( )

4. Are you suffer from liver problems?

a. Yes ( ) b. No ( )

<u>Lab investigations:</u>

<u>1-LFTs:</u>

a. Total protein……g/dl NR(6.4 – 8.5)
b. Albumin……g/dl NR (3.5 – 5.0)
c. ALT……..…u/l NR (less than 41)
d. AST…….u/l NR (less than 40)
e. GGT……u/l NR (less than 55)
f. ALP…….u/l NR (less than 115)

<u>2-Mercury level</u>:……ng/ml NR (less than 10)

بسم الله الرحمن الرحيم

اقـــــرار بالموافقة

الاسم: .....
العمر.....العنوان:.....

أوافق بمحض إرادتي المشاركة في البحث العلمي المتعلق بدراسة قياس وظائف الكبد عند المنقبين عن الذهب بمدينة أبوحمد.

إعداد الطالب :منصور علي محمد قسم السيد
بعد ان شرح لي بانة لا يترتب علي أي أذى جسدي او نفسي و أعلم أن المشاركة في هذا البحث لن تؤثر علي بأي حال من الاحوال كما يحق لي الانسحاب بدون إبداء أسباب الانسحاب من هذا البحث.
البحث بإشراف: د. عبدالوهاب عابدين سعيد

التوقيع..... التاريخ.....

Direct mercury analyzer :

Milestone's DMA-80 Direct Mercury Analyzer is redefining mercury analysis. Many laboratories still employ Cold Vapor Atomic Absorption (CVAA) or ICP-MS as their mercury analysis techniques of choice, however, these techniques require sample preparation which can prove tedious, costly, and time-consuming. The DMA-80 on the other hand, requires no sample preparation and delivers results, comparable to those obtained on CVAA and ICP-MS, in as little as 5 minutes.

Full automated chemistry analyzer A15 Biosystem :

A15 is a compact and easy to use automatic analyzer, designed especially for small laboratories as their main analyzer offering the best performance and maximum efficiency. The A15 is easily adaptable to any work routine due to the flexibility in the installation of samples and reagents. A15's performance (low water consumption, minimal maintenance, high quality constituents and significant savings in the use of consumables) optimizes the operating cost of the laboratory. With the automatic analyzer A15, BioSystems provides a complete system using our dedicated reagents for Clinical Chemistry and Turbidimetry designed to achieve the best possible performance.

COD 11592 50 mL	COD 11593 200 mL	COD 11598 500 mL
STORE AT 2-8ºC		
Reagents for measurement of ALP concentration Only for *in vitro* use in the clinical laboratory		

ALKALINE PHOSPHATASE (ALP) - AMP

BioSystems REAGENTS & INSTRUMENTS

CE

ALKALINE PHOSPHATASE (ALP) - AMP
2-AMINO-2-METHYL-1-PROPANOL BUFFER (IFCC)

PRINCIPLE OF THE METHOD

Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm[1].

$$\text{4 – Nitrophenylphosphate + AMP} \xrightarrow{\text{ALP}} \text{AMP – phosphate + 4 - Nitrophenol}$$

CONTENTS

	COD 11592	COD 11593	COD 11598
A. Reagent	1 x 40 mL	1 x 160 mL	4 x 100 mL
B. Reagent	1 x 10 mL	1 x 40 mL	2 x 50 mL

COMPOSITION

A. Reagent: 2-Amino-2-methyl-1-propanol 0.4 mol/L, zinc sulfate 1.2 mmol/L, N-hydroxyethylethylenediaminetriacetic acid 2.5 mmol/L, magnesium acetate 2.5 mmol/L, pH 10.4.

B. Reagent: 4-Nitrophenylphosphate 60 mmol/L.

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank over 1.200 at 405 nm (1 cm cuvette).

REAGENT PREPARATION

Working Reagent:

- Cod. 11592 and 11593: Transfer the contents of one Reagent B vial into a Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 months at 2-8ºC.
- Cod. 11598: Transfer 25 mL of one Reagent B vial into a Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 months at 2-8ºC.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 25, 30 or 37ºC and able to read at 405 nm.

– Cuvettes with 1 cm light path.

SAMPLES

Serum and plasma collected by standard procedures.

Alkaline phosphatase in serum or plasma is stable for 7 days at 2-8ºC. Heparin may be used as anticoagulant.

PROCEDURE

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Note 1)

Working Reagent	1.0 mL
Sample	20 µL

3. Mix and insert the cuvette into the photometer.
4. Record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The ALP catalytic concentration in the sample is calculated using the following general formula:

$$\Delta A/\text{min} \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs} = U/L$$

The molar absorbance (ε) of 4-nitrophenol at 405 nm is 18450, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.02, the sample volume (Vs) is 0.02, and 1 U/L are 0.0166 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

ΔA/min	x 2764 = U/L x 46.08 = µkat/L

REFERENCE VALUES

Reaction temperature	men	women
25ºC, up to	75 U/L = 1.25 µKat/L	68 U/L = 1.13 µKat/L
30ºC, up to[2]	87 U/L = 1.45 µKat/L	80 U/L = 1.33 µKat/L
37ºC, up to[2]	115 U/L = 1.92 µKat/L	105 U/L = 1.75 µKat/L

Values at 25ºC are obtained from those at 30ºC by using a conversion factor. Concentrations in growing children are higher and highly variable.These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 1.0 U/L = 0.017 µkat/L

– Linearity limit: 1200 U/L = 20 µkat/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean Concentration	CV	n
61 U/L = 1.02 µkat/L	1.0 %	20
244 U/L = 4.07 µkat/L	0.7 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
61 U/L = 1.02 µkat/L	3.4 %	25
244 U/L = 4.07 µkat/L	1.1 %	25

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Lipemia (triglycerides < 10 g/L) and bilirubin (< 20 mg/dL) do not interfere. Hemoglobin (> 2.5 g/L) interfere. Other drugs and substances may interfere[3].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Alkaline phosphatase catalyzes the hydrolysis of organic phosphate monoesters at alkaline pH. The enzyme is present in practically all tissues of the body, especially at or in the cell membranes, and it occurs at particularly high concentrations in placenta, intestinal epithelium, kidney tubules, osteoblasts and liver.

The form present in the sera of normal adults originates mainly in the liver and bone.

Elevated serum ALP is found in patients with bone disease associated with increased osteoblastic activity (Paget's disease, primary and secondary hyperparathyroidism, bone tumors, rickets, osteomalacia, bone fractures) and also in patients with hepatobiliary disease (obstructive jaundice, hepatitis, hepatotoxicity caused by drugs, liver cancer). Physiological changes, such as bone growth and pregnancy, may cause increases in ALP levels[4,5].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37 °C. Part 9. Reference procedure for the measurement of catalytic concentration of alkaline phosphatase. *Clin Chem Lab Med* 2011; 49:1439-1446.
2. Rosalki SB, Foo AY, Burlina A, at al. Multicenter evaluation of iso-ALP test kit for measurement of bone alkaline phosphatase activity in serum and plasma. *Clin Chem* 1993; 39:648-652.
3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.
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COD 11547 2 x 250 mL	COD 11573 1 x 250 mL
STORE AT 2-8ºC	
Reagents for measurement of albumin concentration Only for *in vitro* use in the clinical laboratory	

CE

BioSystems
REAGENTS & INSTRUMENTS

ALBUMIN
BROMOCRESOL GREEN

PRINCIPLE OF THE METHOD

Albumin in the sample reacts with bromocresol green in acid medium forming a coloured complex that can be measured by spectrophotometry[1].

CONTENTS

	COD 11547	COD 11573
A. Reagent	2 x 250 mL	1 x 250 mL
S. Standard	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent: Acetate buffer 100 mmol/L, bromocresol green 0.27 mmol/L, detergent, pH 4.1.

S. Albumin Standard: Bovine albumin. Concentration is given on the label. Concentration value is traceable to the Standard Reference Material 927 (National Institute of Standards and Technology, USA).

STORAGE

Reagent (A): Store at 2-8ºC.

Albumin Standard (S): Store at 2-8ºC, once opened.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.200 at 630 nm (1 cm cuvette).

– Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer able to read at 630 nm (610 - 670 nm).

SAMPLES

Serum or plasma (EDTA, citrate or heparine) collected by standard procedures.

Albumin in serum is stable for 3 days at 2-8ºC.

PROCEDURE

1. Pipette into labelled test tubes: (Notes 1, 2)

	Blank	Standard	Sample
Albumin Standard (S)	—	10 µL	—
Sample	—	—	10 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

2. Mix thoroughly and let stand the tubes for 1 minute at room temperature.
3. Read the absorbance (A) of the Standard and the Sample at 630 nm against the Blank. The colour is stable for 30 minutes.

CALCULATIONS

The albumin concentration in the sample is calculated using the following general formula:

$$\frac{A\ Sample}{A\ Standard} \times C\ Standard = C\ Sample$$

REFERENCE VALUES

Serum[2]:

Newborn, 2 to 4 days	28-44 g/L
4 days to 14 years	38-54 g/L
Adult	35-50 g/L
> 60 years	34-48 g/L

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 1.1 g/L.

– Linearity limit: 70 g/L.

– Repeatibility (within run):

Mean Concentration	CV	n
26.2 g/L	1.4 %	20
42.1 g/L	1.0 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
26.2 g/L	1.9 %	25
42.1 g/L	1.9 %	25

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 3). Details of the comparison experiments are available on request.

– Interferences: Bilirubin (>10 mg/dL), lipemia (triglycerides >7.5 g/L) and hemoglobin (>2.5 g/L) may affect the results. Other drugs and substances may interfere[3].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Albumin is the most abundant protein in human plasma. It has three main functions: it contributes towards maintaining the colloid oncotic pressure of plasma, it acts as non-specific transport vehicle for many nonpolar compounds and it is a source of endogenous amino acids.

Hyperalbuminemia is of little diagnostic significance except in dehydration[2].

Hypoalbuminemia is found as a result of several factors: reduced synthesis caused by liver diseases; reduced absorption of amino acids due to malabsorption syndromes or malnutrition; increased catabolism as a result of inflammation or tissue damage; altered distribution between intravascular and extravascular space due to increased capillary permeability, overhydration or ascites; abnormal losses caused by renal disease (nephrotic syndrome, diabetes mellitus, chronic glomerulonephritis, systemic lupus erythematosus), gastrointestinal tract disease (ulcerative colitis, Crohn's disease) or skin damage (exfoliative dermatitis, extensive burns); congenital absence of albumin or analbuminemia[2,4].

Albumin plasma concentrations, although important for management and follow-up, have very little value in diagnosis[2].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. This reagent may be used in several automated analysers. Instructions for many of them are available on request.
2. Albumin reaction with bromocresol green is immediate. It is not recommended to delay readings, since other proteins react slowly.
3. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

1. Doumas BT, Watson WA and Biggs HG. Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta* 1971: 31: 87-96.
2. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.

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EN ISO 13485 and EN ISO 9001 standards

COD 11832 1 x 50 mL	COD 11533 1 x 200 mL	COD 11568 1 x 500 mL	COD 11562 1 x 1 L
STORE AT 2-8ºC			
Reagents for measurement of ALT/GPT concentration Only for *in vitro* use in the clinical laboratory			

ALANINE AMINOTRANSFERASE (ALT/GPT)

BioSystems
REAGENTS & INSTRUMENTS

CE

ALANINE AMINOTRANSFERASE (ALT/GPT)
IFCC

PRINCIPLE OF THE METHOD

Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the lactate dehydrogenase (LDH) coupled reaction[1,2,3].

$$\text{Alanine} + 2-\text{Oxoglutarate} \xrightarrow{\text{ALT}} \text{Pyruvate} + \text{Glutamate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{Lactate} + \text{NAD}^+$$

CONTENTS

	COD 11832	COD 11533	COD 11568	COD 11562
A. Reagent	1 x 40 mL	1 x 160 mL	1 x 400 mL	1 x 800 mL
B. Reagent	1 x 10 mL	1 x 40 mL	1 x 100 mL	1 x 200 mL

COMPOSITION

A. Reagent: Tris 150 mmol/L, L-alanine 750 mmol/L, lactate dehydrogenase > 1350 U/L, pH 7.3.

B. Reagent: NADH 1.9 mmol/L, 2-oxoglutarate 75 mmol/L, Sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L.

WARNING: H302: Harmful if swallowed. EUH031: Contact with acids liberates toxic gas. P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, absorbance of the blank lower than 1.400 at 340 nm (1 cm cuvette).

AUXILIARY REAGENTS

C. Reagent (cod 11666): Pyridoxal phosphate 10 mmol/L. 5 mL.

REAGENT PREPARATION

Working Reagent: Pour the contents of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B (Note 1). Stable for 1 month at 2-8ºC.

Working Reagent with Pyridoxal Phosphate (Note 2): Mix as follows: 10 mL of Working Reagent + 0.1 mL of Reagent C (cod 11666). Stable for 6 days at 2-8ºC.

ADDITIONAL EQUIPMENT

- Analyzer, spectrophotometer or photometer with cell holder thermostatable at 30 or 37ºC and able to read at 340 nm.
- Cuvettes with 1 cm light path.

SAMPLES

Serum and plasma collected by standard procedures.

Alanine aminotransferase in serum and plasma is stable for 7 days at 2-8ºC. Use heparin or EDTA as anticoagulant[7].

PROCEDURE

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Note 3)

Reaction temperature	37ºC	30ºC
Working Reagent	1.0 mL	1.0 mL
Sample	50 µL	100 µL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. After 1 minute (Note 1), record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The ALT/GPT concentration in the sample is calculated using the following general formula:

$$\Delta A/\text{min} \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs} = U/L$$

The molar absorbance (ε) of NADH at 340 nm is 6300, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.05 at 37ºC and 1.1 at 30ºC, the sample volume (Vs) is 0.05 at 37ºC and 0.1 at 30ºC, and 1 U/L are 0.0166 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

	37ºC	30ºC
ΔA/min	x 3333 = U/L x 55.55 = µkat/L	x 1746 = U/L x 29.1 = µkat/L

REFERENCE VALUES

Reaction temperature	37ºC	30ºC
Without pyr-P, up to[3]	41 U/L = 0.68 µkat/L	29 U/L = 0.48 µkat/L
With pyr-P, up to[1]	65 U/L = 1.08 µkat/L	35 U/L = 0.58 µkat/L

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 1.6 U/L = 0.027 µkat/L
- Linearity limit: 800 U/L = 13.3 µkat/L. For higher values dilute sample 1/10 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean Concentration	CV	n
43 U/L = 0.72 µkat/L	1.8 %	20
192 U/L = 3.2 µkat/L	2.8 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
43 U/L = 0.72 µkat/L	5.3 %	25
192 U/L = 3.2 µkat/L	2.7 %	25

- Sensitivity: 0.3 ΔmA·L/U·min = 0.00502 ΔmA·L/µkat·min
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.
- Interferences: Hemolysis (hemoglobin 10 g/L) and bilirubin (20 mg/dL) do not interfere. Lipemia (triglycerides 2 g/L) may affect the results. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

The aminoatransferases catalyze the formation of glutamic acid from 2-oxoglutarate by transfer of amino groups. ALT is normally present in various tissues but its higher concentrations are found in liver and kidney.

The serum concentration of ALT is elevated in hepatitis and other forms of hepatic disease associated with necrosis: infectious mononucleosis, cholestasis, cirrhosis, metastasic carcinoma of the liver, delirium tremens, and after administration of various drugs, such as opiates, salycilates or ampicillin[5,6].

Serum ALT concentration can also be elevated in skeletal or cardiac muscle disease[5,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. The initial absorbance of the reaction mixture may be out of range in some photometers with a low maximum absorbance reading. For these photometers it is recommended to prepare the Working Reagent by mixing in the proportion: 5 mL Reagent A + 1 mL Reagent B.
2. The IFCC recommended method specifies the addition of pyridoxal phosphate. The delay time before measurements should then be increased to 2 minutes.
3. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. IFCC primary reference procedures for the measurement of catalytic activity concentrations of enzymes at 37ºC. Part 4. Reference procedure for the measurement of catalytic concentration of alanine aminotransferase. *Clin Chem Lab Med* 2002; 40: 718-724.
2. IFCC reference procedures for measurement of catalytic concentrations of enzymes: corrigendum, notes and useful advice. *Clin Chem Lab Med* 2010; 48: 615-621.
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4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
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6. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
7. Word Health Organization (WHO). Use of anticoagulants in diagnostic laboratory investigations. Document WHO/DIL/LAB/99.1, Rev.2; 2002.

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03/2018

COD 11800 1 x 50 mL	COD 11500 2 x 250 mL	COD 11572 1 x 250 mL	COD 11553 1 x 1 L
STORE AT 2-30ºC			
Reagents for measurement of protein concentration Only for *in vitro* use in the clinical laboratory			

PROTEIN (TOTAL)

CE

BioSystems
REAGENTS & INSTRUMENTS

PROTEIN (TOTAL)
BIURET

PRINCIPLE OF THE METHOD

Protein in the sample reacts with copper (II) ion in alkaline medium forming a coloured complex that can be measured by spectrophotometry[1].

CONTENTS

	COD 11800	COD 11500	COD 11572	COD 11553
A. Reagent	1 x 50 mL	2 x 250 mL	1 x 250 mL	1 x 1 L
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent. Copper (II) acetate 6 mmol/L, potassium iodide 12 mmol/L, sodium hydroxide 1.15 mol/L, detergent.

DANGER: H314: Causes severe skin burns and eye damage. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

S. Protein Standard. Bovine albumin. Concentration is given on the label. Concentration value is traceable to the Standard Reference Material 927 (National Institute of Standards and Technology, USA).

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Reagent (A): Store at 2-30ºC.

Protein Standard (S): Store at 2-8ºC, once opened.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.150 at 545 nm.

– Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer able to read at 545 ± 10 nm

SAMPLES

Serum or heparinized plasma collected by standard procedures. Stable for 8 days at 2-8ºC.

Anticoagulants other than heparin should not be used.

PROCEDURE

1. Pipette into labelled test tubes: (Note 1)

	Blank	Standard	Sample
Distilled water	20 µL	—	—
Protein Standard (S)	—	20 µL	—
Sample	—	—	20 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

2. Mix thoroughly and let stand the tubes for 10 minutes at room temperature.
3. Read the absorbance (A) of the Standard and the Sample at 545 nm against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

The protein concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = C_{Sample}$$

REFERENCE VALUES

Serum, adults[2]:

Ambulatory	64-83 g/L
Recumbent	60-78 g/L

Concentrations are lower in child. Plasma total protein concentration is 2 to 4 g/L higher due to the presence of fibrinogen as well as some other trace proteins[2].

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 4.6 g/L.

– Linearity limit: 150 g/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatability (within run):

Mean Concentration	CV	n
44 g/L	1,1 %	20
57 g/L	0,9 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
44 g/L	1,8 %	25
57 g/L	1,9 %	25

– Sensitivity: 5 mA·L/g

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.

– Interferences: Hemoglobin (2.5 g/L) and lipemia interfere. Bilirubin (20 mg/dL) does not affect the results. Other drugs and substances may interfere[3].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Most of the plasma proteins are synthesized by the liver. The major exception to this is the immunoglobulins which are produced by plasma cells found in the spleen, lymph nodes and bone marrow.

The two general causes of alterations of serum total protein are a change in the volume of plasma water and a change in the concentration of one or more of the serum proteins.

Hyperproteinemia can be caused by dehydration (inadequate water intake, severe vomiting, diarrhea, Addison's disease, diabetic acidosis) or as a result of an increase in the concentration of specific proteins (immunoglobulins in chronic infections, multiple myeloma)[2,4].

Hypoproteinemia may be caused by hemodilution (salt retention syndromes, massive intravenous infusions), by an impaired synthesis (severe malnutrition, chronic liver disease, intestinal malabsorptive disease), or by an excessive protein loss due to a chronic kidney disease or severe burns[2,4].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. This reagent may be used in several automated analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

1. Gornall AG, Bardawill CS, David MM. Determination of serum proteins by means of the Biuret reaction. *J Biol Chem* 1949; 177: 751-766.
2. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
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M11500i-22

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07/2016

COD 11584 1 x 50 mL	COD 11520 1 x 200 mL
STORE AT 2-8ºC	
Reagents for measurement of γ-GT concentration Only for *in vitro* use in the clinical laboratory	

GAMMA-GLUTAMYLTRANSFERASE (γ-GT)

BioSystems
REAGENTS & INSTRUMENTS

CE

GAMMA-GLUTAMYLTRANSFERASE (γ-GT)
IFCC

PRINCIPLE OF THE METHOD

Gamma-glutamyltransferase (γ-GT) catalyzes the transfer of the γ-glutamyl group from γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine, liberating 3-carboxy-4-nitroaniline. The catalytic concentration is determined from the rate of 3-carboxy-4-nitroaniline formation[1,2,3].

$$\gamma\text{ – Glutamyl – 3 – carboxy – 4 – nitroanilide + Glycylglycine} \xrightarrow{\gamma\text{-GT}} \gamma\text{ – Glutamyl – glycylglycine + 3 – carboxy – 4 - nitroaniline}$$

CONTENTS

	COD 11584	COD 11520
A. Reagent	1 x 40 mL	1 x 160 mL
B. Reagent	1 x 10 mL	1 x 40 mL

COMPOSITION

A. Reagent: Glycylglycine 206.25 mmol/L, sodium hydroxide 130 mmol/L, pH 7.9.

WARNING: H315: Causes skin irritation. H319: Causes serious eye irritation. P280: Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P332+P313: If skin irritation occurs: Get medical advice/attention.

B. Reagent: γ-Glutamyl-3-carboxy-4-nitroanilide 32.5 mmol/L.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank over 1.000 at 410 nm or over 1.450 at 405 nm (1 cm cuvette).

REAGENT PREPARATION

Working Reagent: Pour the contents of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B. Stable for 2 months at 2-8ºC.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 25, 30 or 37ºC and able to read at 405 nm or 410 nm (Note 1)

– Cuvettes with 1 cm light path.

SAMPLES

Serum and plasma collected by standard procedures.

Gamma-glutamyltransferase in serum and plasma is stable for 5 days at 2-8ºC. Use heparin or EDTA as anticoagulant.

PROCEDURE

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Note 2)

Working Reagent	1.0 mL
Sample	100 µL

3. Mix and insert the cuvette into the photometer.
4. Record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The γ-GT concentration in the sample is calculated using the following general formula:

$$\Delta A/\text{min} \times \frac{Vt \times 10^6}{\varepsilon \times l \times Vs} = U/L$$

The molar absorbance (ε) of 3-carboxy-4-nitroaniline at 410 nm is 7908 and at 405 nm is 9900, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.1, the sample volume (Vs) is 0.1, and 1 U/L are 16.67 nkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

	405 nm	410 nm
ΔA/min	x 1111 = U/L x 18.52 = µkat/L	x 1391 = U/L x 23.19 = µkat/L

REFERENCE VALUES

Reaction temperature	Men		Women	
	U/L	µkat/L	U/L	µkat/L
25ºC	< 22	< 0.37	< 15	< 0.25
30ºC	< 35	< 0.59	< 24	< 0.40
37ºC[1]	< 55	< 0.92	< 38	< 0.64

Values at 25ºC and 30ºC are obtained from those at 37ºC by using a conversion factor. These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 1.6 U/L = 0.03 µkat/L.

– Linearity limit: 600 U/L = 10.0 µkat/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean Concentration	CV	n
31 U/L = 0.52 µkat/L	1.6 %	20
99 U/L = 1.65 µkat/L	0.5 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
31 U/L = 0.52 µkat/L	4.8 %	25
99 U/L = 1.65 µkat/L	1.4 %	25

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Hemoglobin (> 5 g/L), bilirubin (> 10 g/L) and lipemia (triglycerides > 4 g/L) may affect the results. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Gamma-glutamyl transferase is found in highest concentration in liver, the renal tubules and intestines although it is also present in other tissues such as the pancreas, prostate, salivary glands, seminal vesicles, brain and heart.

Gamma-glutamyl activity is elevated in any and all forms of liver disease, showing highest values in cases of intra or posthepatic biliary obstruction. High elevations are also observed in patients with metastatic neoplasm of the liver. In pancreatitis and some pancreatic malignancies, enzyme activity may be moderately elevated[5,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. The IFCC recommended method specify a wavelenght of 410 nm. However, measurements can also be carried out at 405 nm. In this case, the reagent initial absorbance is almost duplicated and the factor used for calculations is different (see calculations).
2. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

BIBLIOGRAPHY

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2. IFCC reference procedures for measurement of catalytic concentrations of enzymes: corrigendum, notes and useful advice. *Clin Chem Lab Med* 2010; 48: 615-621.
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4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
5. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
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COD 11830 1 x 50 mL	COD 11531 1 x 200 mL	COD 11567 1 x 500 mL	COD 11561 1 x 1 L
STORE AT 2-8ºC			
Reagents for measurement of AST/GOT concentration Only for *in vitro* use in the clinical laboratory			

ASPARTATE AMINOTRANSFERASE (AST/GOT)

BioSystems REAGENTS & INSTRUMENTS

CE

ASPARTATE AMINOTRANSFERASE (AST/GOT)

IFCC

PRINCIPLE OF THE METHOD

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction[1,2,3].

$$\text{Aspartate} + 2\text{-Oxoglutarate} \xrightarrow{\text{AST}} \text{Oxalacetate} + \text{Glutamate}$$

$$\text{Oxalacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+$$

CONTENTS

	COD 11830	COD 11531	COD 11567	COD 11561
A. Reagent	1 x 40 mL	1 x 160 mL	1 x 400 mL	1 x 800 mL
B. Reagent	1 x 10 mL	1 x 40 mL	1 x 100 mL	1 x 200 mL

COMPOSITION

A. Reagent: Tris 121 mmol/L, L-aspartate 362 mmol/L, malate dehydrogenase > 460 U/L, lactate dehydrogenase > 660 U/L, pH 7.8.

WARNING: H315: Causes skin irritation. H319: Causes serious eye irritation. P280: Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P332+P313: If skin irritation occurs: Get medical advice/attention.

B. Reagent: NADH 1.9 mmol/L, 2-oxoglutarate 75 mmol/L, Sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L

WARNING: H302: Harmful if swallowed. EUH031: Contact with acids liberates toxic gas. P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth.

For further warnings and precautions, see the product safety data sheet (SDS).

STORAGE

Store at 2-8ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagents: Presence of particulate material, turbidity, absorbance of the blank lower than 1.400 at 340 nm (1 cm cuvette).

AUXILIARY REAGENTS

C. Reagent (cod 11666): Pyridoxal phosphate 10 mmol/L. 5 mL.

REAGENT PREPARATION

Working Reagent: Pour the contents of the Reagent B into the Reagent A bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A + 1 mL Reagent B (Note 1). Stable for 1 month at 2-8ºC.

Working Reagent with Pyridoxal Phosphate (Note 2): Mix as follows: 10 mL of Working Reagent + 0.1 mL of Reagent C (cod 11666). Stable for 6 days at 2-8ºC.

ADDITIONAL EQUIPMENT

– Analyzer, spectrophotometer or photometer with cell holder thermostatable at 30 or 37ºC and able to read at 340 nm.

– Cuvettes with 1 cm light path.

SAMPLES

Serum and plasma collected by standard procedures.

Aspartate aminotransferase in serum and plasma is stable for 7 days at 2-8ºC. Use heparin as anticoagulant[7].

PROCEDURE

1. Bring the Working Reagent and the instrument to reaction temperature.
2. Pipette into a cuvette: (Note 3)

Reaction temperature	37ºC	30ºC
Working Reagent	1.0 mL	1.0 mL
Sample	50 µL	100 µL

3. Mix and insert the cuvette into the photometer. Start the stopwatch.
4. After 1 minute (Note 1), record initial absorbance and at 1 minute intervals thereafter for 3 minutes.
5. Calculate the difference between consecutive absorbances, and the average absorbance difference per minute (ΔA/min).

CALCULATIONS

The AST/GOT concentration in the sample is calculated using the following general formula:

$$\Delta A/\text{min} \times \frac{Vt \times 10^6}{\varepsilon \times l \times VS} = \text{U/L}$$

The molar absorbance (ε) of NADH at 340 nm is 6300, the lightpath (l) is 1 cm, the total reaction volume (Vt) is 1.05 at 37ºC and 1.1 at 30ºC, the sample volume (Vs) is 0.05 at 37ºC and 0.1 at 30ºC, and 1 U/L are 0.0166 µkat/L. The following formulas are deduced for the calculation of the catalytic concentration:

	37ºC	30ºC
ΔA/min	x 3333 = U/L x 55.55 = µkat/L	x 1746 = U/L x 29.1 = µkat/L

REFERENCE VALUES

Reaction temperature	37ºC	30ºC
Without pyr-P, up to[4]	40 U/L = 0.67 µkat/L	25 U/L = 0.42 µkat/L
With pyr-P, up to[2]	50 U/L = 0.83 µkat/L	30 U/L = 0.50 µkat/L

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit: 1.67 U/L = 0.028 µkat/L

– Linearity limit: 800 U/L = 13.3 µkat/L. For higher values dilute sample 1/ 10 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean Concentration	CV	n
38 U/L = 0.63 µkat/L	1.4 %	20
119 U/L = 1.98 µkat/L	1.5 %	20

– Reproducibility (run to run):

Mean Concentration	CV	n
38 U/L = 0.63 µkat/L	5.9 %	25
119 U/L = 1.98 µkat/L	3.8 %	25

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

– Interferences: Lipemia (triglycerides 2 g/L) interfere. Bilirubin (20 mg/dL) and hemolysis (hemoglobin 10 g/L) do not interfere. Other drugs and substances may interfere[5].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

The aminotransferases catalyze the formation of glutamic acid from 2-oxoglutarate by transfer of amino groups. AST is found in highest concentration in the liver and heart muscle but it is also abundant in skeletal muscle, kidney and pancreas.

The serum concentration of AST is elevated in hepatitis and other forms of hepatic disease associated with necrosis: infectious mononucleosis, cholestasis, cirrhosis, metastasic carcinoma of the liver, delirium tremens, and after administration of various drugs[4,6].

Serum AST concentration is also elevated after myocardial infarction, in sketetal muscle disease (as progressive muscular distrophy), in acute pancreatitis or hemolytic disease and other[4,6].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. The initial absorbance of the reaction mixture may be out of range in some photometers with a low maximum absorbance reading. For these photometers it is recommended to prepare the Working Reagent by mixing in the proportion: 5 mL Reagent A + 1 mL Reagent B.
2. The IFCC recommended method specifies the addition of pyridoxal phosphate. The delay time before measurements should then be increased to 2 minutes.
3. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.

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