

**Sudan University of Science &
Technology
College of graduate studies**

**Evaluation of Visceral Leishmaniasis in Gadarif State
Using Ultrasonography**

تقويم داء اللشمانيا الحشوي في ولاية القضارف عن طريق التصوير بالموجات فوق الصوتية

*A Thesis Submitted for the Requirement of PhD Degree in Medical
Ultrasound*

By:

Ahmed Mohamed Barakat Ahmed

Supervisor:

Dr. Elsafi Ahmed Abdulla Balla

Co-supervisor:

Dr. Mohamed Elfadil Mohamed Gar-elnabi

2017



Approval Page

(To be completed after the college council approval)

Name of Candidate: AHMED MOHAMED BARAKAT AHMED

Thesis title: Evaluation of visceral leishmaniasis in Gadarif state using ultrasonography

Degree Examined for: ph.D

Approved by:

1. External Examiner

Name: Hussein A. Hassan

Signature: [Signature] Date: 20/2/2017

2. Internal Examiner

Name: Caroline Edward Ayad

Signature: [Signature] Date: 20/2/2017

3. Supervisor

Name: Dr. MOHAMED ELHADIL MOHAMED GAREL NABI

Signature: [Signature] Date: 20/2/2017

بسم الله الرحمن الرحيم

قال تعالى:

(وَيَلْمُوكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا)

صدق الله العظيم

سورة الأسراء/ آية رقم (85)

Abstract

This study was intended to assess and evaluate the visceral leishmaniasis in Gadarif state using abdominal ultrasound scan in order to assess the feature changes in abdominal viscera due to this parasitic infection, A retrospective case–control study was conducted among 215 patients (male = 140 and female = 75), coming from kala-azar endemic areas (areas from where VL is regularly reported Gadarif state) with fever of more than 15 days and not responding to antimalarial and antibiotics during the period June 2012 to February 2017 Gadarif state hospital - Sudan. The data of this study was collected by using ultrasound machines. The result of this study showed that the commonest ultrasound findings in VL participants were hepatomegally and splenomegaly. While this study reveals that the most affected gender were male (65.1%), the majority from south Gadarif state region accounted for (62.8%) where usually patient experiences distended bladder and vomiting in (39.5%) and (34.9%) respectively, lower corner of the liver appear to be rounded in (55.3%) which indicated the persistent of hepatomegaly, as well (46.5%) showed hyperecogenic and (83.7%) homogenous liver texture. Same texture noted for spleen and both kidneys. Other complications such as focal liver lesions (4.7%) and dilated portal vein (2.3%) were detected. In conclusion ultrasound scanning presents an effective manifestation of VL, because it can illustrated the impact and consequences of this disease in various abdominal organs such as liver and spleen in respect to duration of disease, which in turn allowing the possibility to

treat these complications and prevents the deterioration of a patient's health status.

المستخلص

كان الغرض من هذه الدراسة تقدير وتقييم داء اللشمينيا الحشوي في ولاية القصارف باستخدام الفحص بالموجات فوق الصوتية للبطن من أجل تقييم التغييرات في أحشاء البطن بسبب هذا المرض ، اجريت هذه الدراسة التحليلية على 215 مريضا (الذكور، 140 و الإناث، 75) في المناطق الموبوءة باللشمينيا لمناطق التي عادة ما ينتشر فيها المرض مثل ولاية القصارف مع حمى لأكثر من 15 يوما وعدم الاستجابة لعلاج الملاريا اوالمضادات الحيوية وذلك في الفترة من يونيو 2012 وحتى فراير 2017 في تمت الدراسة مستشفيات ولاية القصارف بالسودان . تم جمع البيانات عن طريق اجهزة الموجات فوق الصوتية . أظهرت نتائج الدراسة ان الأكثر شيوعا في نتائج الموجات فوق الصوتية عند مرضي اللشمينيا هو تضخم الكبد و الطحال ، في حين أن هذه الدراسة تكشف أن الجنس الأكثر اصابة بين المرضي هم من الذكور (65.1%) ، وكانت الغالبية من جنوب منطقة ولاية القصارف (62.8%) حيث يعاني معظم المرضي من انتفاخ المثانة والقيء في (39.5%) و (34.9%) على التوالي ، هناك استدارة في الزاوية اليمنى السفلية من الكبد في (55.3%) هذا يشير إلى تضخم الكبد وزيادة صدويتها في (64.5%) مع تجانس نسيج الكبد في (83.7%) ، نفس نسق النسيج لوحظ في الطحال والكليتين. مضاعفات أخرى مثل آفات الكبد المحورية (4.7%) و تمدد الوريد البابي في (2.3%). في الختام الفحص بالموجات فوق الصوتية له دور فعال في تتبع اثار اللشمينيا ، وذلك لفعاليتها في الكشف عن النتائج المترتبة على هذا المرض

في مختلف أعضاء البطن مثل الكبد و الطحال وذلك حسب مدة الاصابة بالمرض، مما يتيح إمكانية علاج هذه المضاعفات، و بذلك يقلل من تدهور الحالة الصحية للمريض.

Dedication

I solely dedicate this work to:

My parents

My wife and children

Acknowledgments

Praise is to the almighty Allah who gifted me the knowledge and the ability to write this thesis.

I would like to express my sincere thanks to Dr. Mohamed Elfadil Mohamed Gar-elnabi for his continuous and valuable guidance, supervision, faithful encouragement and patience and appreciable efforts during preparation of this thesis and the whole course, what so ever I thank him it is just a glimpse of what he uses to give without waiting for reward. Also I would like to thank Dr. Elsafi Ahmed abdullah for his support and caring during this study.

Finally all and greatest thanks are to everyone who has participated in completion and improvement of this study.

List of contents

Contents	Page number
Abstract (English)	ii
Abstract (Arabic)	iii
Dedication	iv
Acknowledgements	v
List of contents	vi
List of abbreviations	vii
Chapter one: Introduction	1
1-1 The problem of the study	3
1-2 Objectives	4
1-3 Significant of the study	4
1-4 Overview of the study	5
Chapter Two: literature review	7
2.1. Normal Liver Size and Echogenicity	7
2.2. Spleen sonographic appearance	9
2.3. Pathology under study	12
2.3.1. Patho-clinical features of visceral leishmaniasis	14
2.3.2. Symptoms and signs of visceral leishmaniasis	17
2.3.3. Differential diagnosis of VL	20
2.3.4. Treatment of leishmaniasis	21
2.4. Image quality in ultrasound	22
2.5. Ultrasound Examination Technique	25
2.6. Previous studies	29
Chapter three: material and methods	37
3-1 Materials	39
3-2 Methods	40
3-2-1 Design of the study	40
3.2.2. Population of the study	40
3.2.3. Sample size and type	40
3.2.4. Place and duration of the study	40
3.2.5. Methods of data collection	40

3.2.6 Technique	40
3.2.7 Inclusion criteria	41
3.2.8 Exclusion criteria	41
3.2.9 statistical analysis	41
3.2.10 Ethical issues	4
Chapter four: Results	42
Chapter five: Discussion, conclusion and recommendation	56
5-1 Discussion	56
5-2 conclusion	59
5-3 Recommendations	60
References	61
Appendix A: master data sheet	64
Appendix B: Ultrasound images	66

List of abbreviation

Neural tube defects, amniotic band syndrome (ABS), limb-body-wall complex (LBWC), Choroids Plexus Cysts (CPC)

Abbreviation	Meaning
ABS	Amniotic Band Syndrome
AC	Abdominal circumference
VL	Visceral leishmaniasis
HIV	human immunodeficiency virus
FOV	field of view
RLD	right lateral decubitus
DCL	Diffuse Coetaneous Leishmaniasis
IVC	Inferior veni cava
GB and CBD	Gallbladder and common bile duct
PCR	Polymerase Chain reaction

CL

Cutaneous Leishmaniasis

MCL

Mucocutaneous Leishmaniasis

Chapter one

Introduction

The viscera are one of a vital part of the body and any problem of it may leave a profound effect on the patient, so a systematic approach to the sonographic examination of the viscera is needed. In the last two decades ultrasound facilities have revolutionized the scope of diagnosing the viscera. The development of new ultrasound techniques and real-time scanners has played an important role in detection of various diseases. Visceral leishmaniasis (VL), is a chronic deadly disease of the viscera (liver, spleen, etc) due to infection by the microscopic parasite named *Leishmaniadonovani*, also called kala-azar, black sickness, ponos, (Assam, dumdum and black fevers). Kala-azar first came to the attention of Western doctors in 1824 in Bangladesh, The term "kala-azar" comes from India, it is derived from kala which means black and the -azar for disease,

today, it is used interchangeably with the scientific name visceral leishmaniasis for the most acute form of the disease caused by *Leishmania donovani* or medically known as visceral leishmaniasis and in popular English as black fever. (Alvar J et al. 2008).

The name "*Leishmaniadonovani*" honors two men: the British pathologist William Boog Leishman who in 1903 wrote about the protozoa that causes kala-azar and the researcher Charles Donovan who made the same discovery independently the same year. As they published their discovery almost simultaneously, the species was named for both of them — *Leishmaniadonovani*. (Jawhar NM. 2011).

L. donovani, the agent of (VL), is transmitted to humans via tiny and silent adult female sandfly bites which found in tropical or temperate regions throughout the world, in an area of Asia (India), Africa (Sudan) and Europe (Mediterranean region) - old world - it spread through *Phlebotomus* genus, and the species Such as *L. donovani* and *L. infantum* while in north and south America (Brazil & Mexico) - new world - it spread through the *Lutzomyia* genus and the species is *L. chagasi*, or about 88 countries during the world, all together there are an estimated half million(500,000) and several hundred cases yearly . Also it has been reported in military personnel returning from the Persian Gulf, 350 million people living in that parts worldwide are the most vulnerable, others at risk include people traveling through these areas such as development workers and soldiers. (Gorg C. 2007).

The parasitic life cycle of leishmania includes the sand fly and an appropriate host. Humans are one of those hosts. The Leishmania organisms multiply in the cells causing hyperplasia of it and the symptoms are usually resembling those of incipient pulmonary tuberculosis (fever, enlargement of the spleen, and the liver (splenomegaly & hepatomegaly), weight loss, secondary infections such as pneumonia etc...) and the disease is often confused with malaria. VL has become the second most fatal parasitic disease in world If is not treated, it is 100% causing death without treatment, claiming 600,000 victims annually. Only malaria causes a higher number of deaths. Most of the victims are from rural poor. Ninety percent of all cases occur in five countries: India, which has the greatest burden of the disease, Bangladesh, Brazil, Nepal and Sudan. The incubation period is difficult to evaluate precisely. It can develop over several months or years and generally two to six months, but can range from ten days to many years. Long incubation period up to ten years have occasionally reported, related to clinical outcome of symptomatic infection flowing immune system alteration. The onset of the disease may be sudden or gradual. (Kala-azar Govt. [2015](#)).

Three major types of leishmaniasis occur in humans – visceral, cutaneous and mucocutaneous as following:

Visceral leishmaniasis (systemic), affects the entire body. This form occurs 2 - 8 months after a person is bitten by the Infector insect, the parasite invades the liver, spleen, bone marrow and lymph nodes. Other organs (intestines, lung) and

tissues (skin) may also become involved as they contain the elements of mononuclear phagocytes' system. In immune suppressed individuals and in advanced cases all body organs are involved. (Visceral Leishmaniasis, Govt. [2015](#)).

Cutaneous leishmaniasis, affects the face, arms, and legs as skin ulcers which take time to heal and mucus membranes. It is caused by *Leishmania aethiopica* in Ethiopia and Kenya, *L. pifanoi* in Venezuela and species of the *L. viannia* and *L. mexicana* subclass in south and central America. (Alvar J, et al.1997).

Mucocutaneous leishmaniasis, the parasite invades the mucous membranes and causes ulcers in the nose, mouth and parts of the sinuses which can result in lesions and deformity of the face. It is caused by *Leishmania viannia* in south and central America. (Pintado V, et al. 2001).

Recently VL has increased amongst HIV/AIDS patients which could result in a health disaster, so urgent action should be taken. Co-infections of HIV and VL accelerated the onset of AIDS and shortening the life expectancy of HIV-infected people, so the spread of co-infection may become a new threatening. Patients with VL are also susceptible to secondary bacterial infections. Untreated the disease has an extremely high mortality. The only means of protection to eradicate VL is to develop adequate social, medical health care and quality assurance as well as good medication, while the growth of rural poverty and the destruction of the health system is creating conditions for an expansion of the

epidemic to other regions. Leishmaniasis diagnosed in the hema- laboratory by direct visualization of the Leishmani donovani bodies. (Alwar J, et al. 2012).

1.1. Problem of study

Visceral leishmaniasis is a major public health problem in Sudan. It threatens all the states especially tropical ones because Sudanese don't visit clinics unless they were late in their problem especially when it deals with the dangerous disease as in this study, it can be diagnosed by ultrasound, but the diagnosis still depend on the experience of the sonographer and the efficiency of the ultrasound machine used and in many occasion other imaging modalities such as MRI were need it for confirmation. Also subclinical forms of the disease remain undiagnosed and become a clinical challenge to the treating physician but diagnosis of VL by laboratory investigation need a sample by using a needle aspiration as this is harmful and costive procedure, fortunately ultrasound can put a quick solution of the harm and cost for the patient as well integration of the impact of the disease on visceral organs can reveals the presence of VL.

1.2 Objectives of study

1.2.1 General Objective

The general objective of this study was to explore the value of medical ultrasound in evaluating and characterizing visceral leishmaniasis as early as possible in order to speed the process of the treatment.

1.2.2. Specific objectives:

- To measure right and left lobes of the liver.
- To obtain the association of the gallbladder size and wall thickness with presence of visceral leishmaniasis.
- To measure the spleen size and weight.
- To determine kidneys position, size and shape.
- To get diameters and wall thickness of the main portal vein, common bile, aorta and inferior vena cava.
- To find the shape, texture and echogenicity of the visceral organs.
- To correlate the lab investigations with visceral organs dimension and characteristics.
- To assess the gender incidence.

1.3 Significance of study

This study will provide a low cost method in diagnosis of kala-azar as will save the patient from harm that come from needle aspiration, ultrasound is easy and quick method of evaluation so no time spends will be lost. As well this study will show the impact of visceral leishmaniasis on visceral organs and hence an approach to easily diagnose Visceral leishmaniasis objectively.

1.4 Overview of the study

This study falls into five chapters; with chapter one is an introduction which includes an idea about visceral leishmaniasis, problem of the study objective and significance of the study. Chapter two is literature review which

presents theoretical background and scholar studies concerning diagnosis of visceral leishmaniasis, while chapter three include material used to collect data and the method employed to collect the data. Chapter five presents the results of the study using tables, figures and finally chapter five gives discussion of the result including conclusion and offer suggestion as recommendation.

Chapter Two

Literature Review

2.1. Normal Liver Size and Echogenicity

The upper border of the liver lies approximately at the level of the fifth intercostals space at the mid-clavicular line. The lower border extends to or slightly below the costal margin. An accurate assessment of liver size is difficult with real-time ultrasound equipment because of the limited field of view. Go sink and Leominster proposed measuring the liver length in the mid-hepatic line. In 75% of patients with a liver length of greater than 15.5 cm, hepatomegaly is present. Measured the liver in a longitudinal and antero-posterior diameter in both the mid-clavicular line and the midline and correlated these findings with gender, age, height, weight, and body surface area. They found that organ size increases with height and body surface area and decreases with age. The mean longitudinal diameter of the liver in the mid-clavicular line in this study was 10.5 cm, with standard deviation (SD) of 1.5 cm, and the mean mid-clavicular antero-posterior diameter was 8.1 cm (SD 1.9 cm). In most patients, measurement of the liver length suffices to measure liver size. Reidel's lobe is a tongue like extension of the inferior tip of the right lobe of the liver, frequently found in as the nice women. The normal liver is homogeneous, contains fine level echoes, and is

either minimally hyperechoic or isoechoic compared to the normal renal cortex. The liver is hypoechoic compared to the spleen. This relationship is evident when the lateral segment of the left lobe is elongated and wraps around the spleen. (Gosink BB, Leymaster CE. 1981). (Lafortune M, Madore F, Patriquin H, Breton G1991).

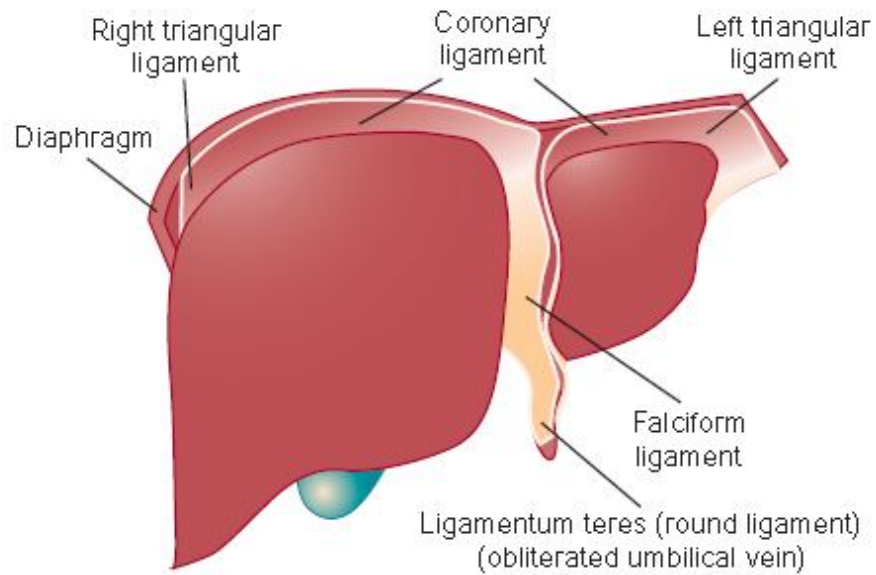


Figure (2.1) Hepatic ligaments, Diagram of anterior surface of the liver.

(Roumak.2011).

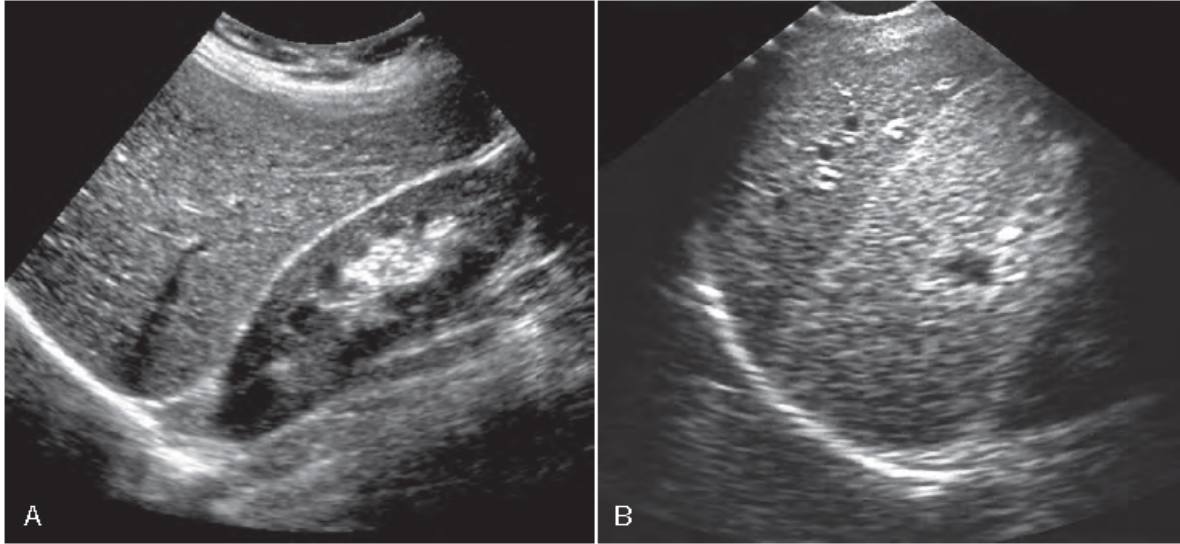


Figure (2.2) Normal liver echogenicity. A, The liver is more echogenicity than the renal cortex. B, The liver is less echogenicity than the spleen, as seen in many thin women, whose left lobe of the liver wraps around the spleen. (Catalano O, et al. 2006).

2.2 Spleen sonographic appearance

The shape of the normal spleen is variable. The spleen consists of two components joined at the hilum: a super medial component and an infer lateral component. More superiorly on transverse scanning, the spleen has a typical fat, “inverted comma” shape, with a thin component extending inferiorly and another component extending medially, either superior to or adjacent to the upper pole of the kidney. This second component (super medial) can be seen to indent the gastric fundus on plain films of the abdomen or in barium studies. As the scan plane moves inferiorly, only the inferior component of the spleen is seen. This component (infer lateral) can be outlined by a thin rim of fat above the splenic

flexure, as seen on a plain abdominal film. It may extend inferiorly to the costal margin and present clinically as a palpable spleen. However, either the superomedial or the inferolateral component can enlarge independently, without enlargement of the other component. It is important to recognize the normal structures that are related to the spleen. The diaphragm cradles the spleen posteriorly, superiorly, and laterally. The left liver lobe may extend into the left upper quadrant superior and lateral to the spleen. The fundus of the stomach and lesser sac are medial and anterior to the splenic hilum. The gastric fundus may contain gas or fluid, which should not be confused with a fluid collection. The tail of the pancreas lies posterior to the stomach and lesser sac. It approaches the hilum of the spleen, closely related to the splenic artery and vein. Consequently, the spleen can be used as a “window” to evaluate the pancreatic tail area. The left kidney generally lies inferior and medial to the spleen. A useful landmark in identifying the spleen and splenic hilum is the splenic vein, which generally can be demonstrated without difficulty. The normal splenic parenchyma is homogeneous. The liver is generally considered to be more echogenic than the spleen, but in fact the echogenicity of the parenchyma is higher in the spleen than in the liver. Using a dual-image setting, the operator may compare the echogenicity of these two organs. The impression that the liver has greater echogenicity results from its large number of reflective vessels. As in measuring other body structures, it is helpful to have measurements that establish the upper limits of normal. The size of a normal spleen depends on gender, age, and body height. The range of the “normal sized” adult spleen, combined with its complex

three dimensional shape, makes it difficult to establish a normal range of Sonographic measurements. Ideally, the clinician would assess splenic volume or weight. Techniques have been developed to measure serial sections of the spleen by planimetry and then compute the volume of the spleen by adding the values for each section. However, these techniques are cumbersome and not popular. The most frequently used method is “eyeballing” the size. Unfortunately, this method of assessment requires considerably more experience than is necessary for other imaging techniques and is relatively inaccurate. Various authors have used different methods to measure splenic size. The length of the spleen measured on a coronal or coronal oblique view that includes the hilum is the most common technique. (Gorg C, Eur J Radiol 2007). (Niederau C, et al. 1983)

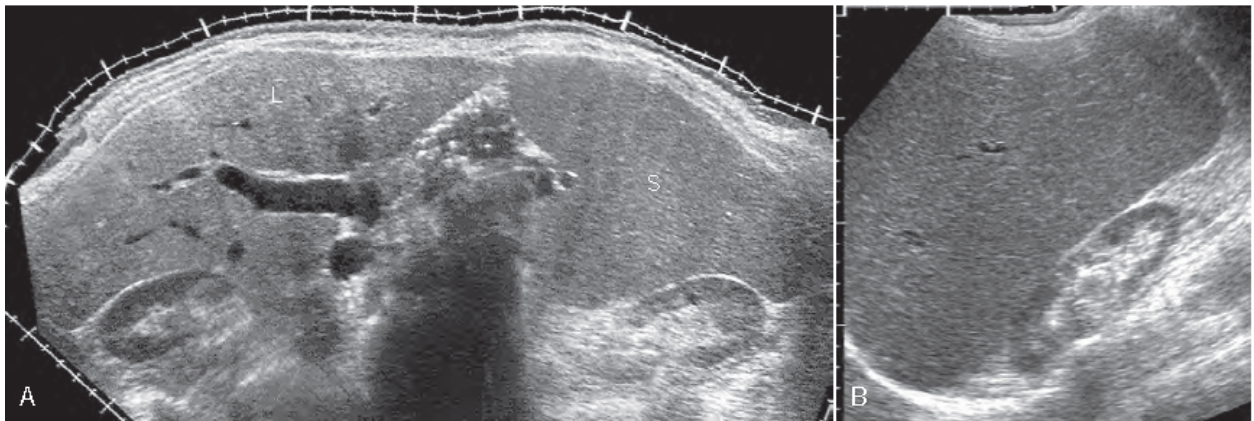


Figure (2.3) Splenomegaly. A, Transverse, and B, coronal extended–field of view (FOV) (Siescape), images demonstrate marked splenic (S) enlargement, L, liver. (Niederau C, et al. 1983).

This view can be obtained during deep inspiration or quiet breathing. Importantly, this method correlates well with the splenic volume, particularly when performed with the patient in the right lateral decubitus (RLD) position. Multiple studies have tried to establish monograms of spleen size. In a study of 703 normal adults, the length of the spleen was less than 11 cm, the width (breadth) less than 7 cm, and the thickness less than 5 cm in 95% of patients, an upper limit of normal splenic length of 12 cm for girls and 13 cm for boys (≥ 15 years). (Rosenberg et al. 1991)

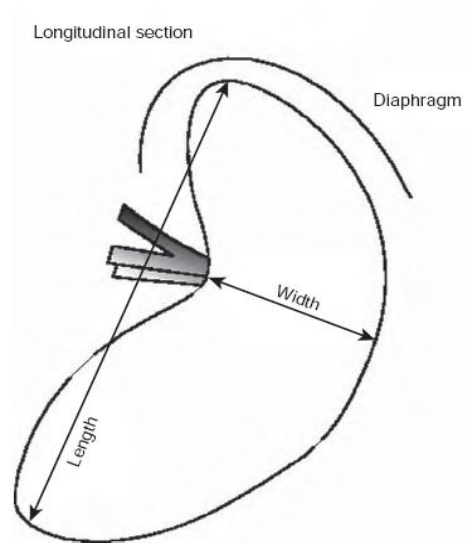
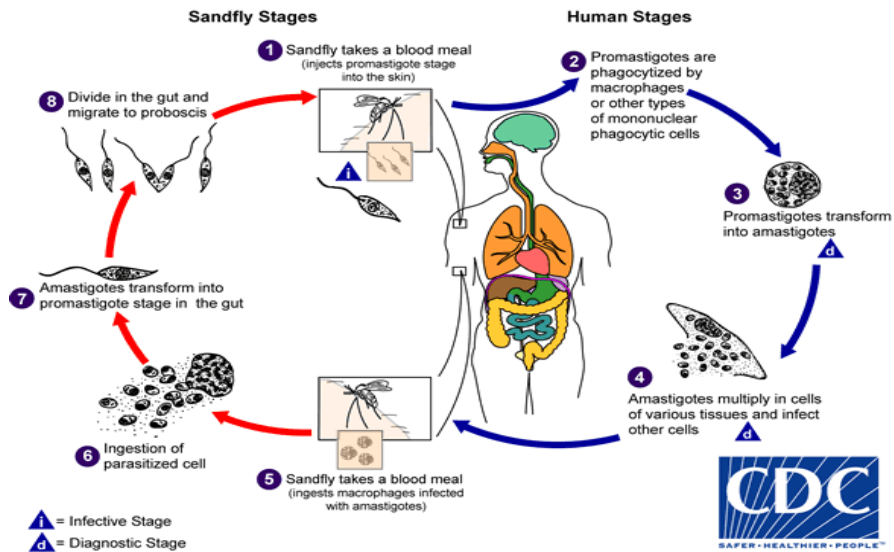


Figure (2.4) splenic measurement, Diagram shows sonographic approach to measuring splenic length and width. Splenic size is best measured by obtaining a coronal view that includes the hilum. (Lamb et al. 2002).

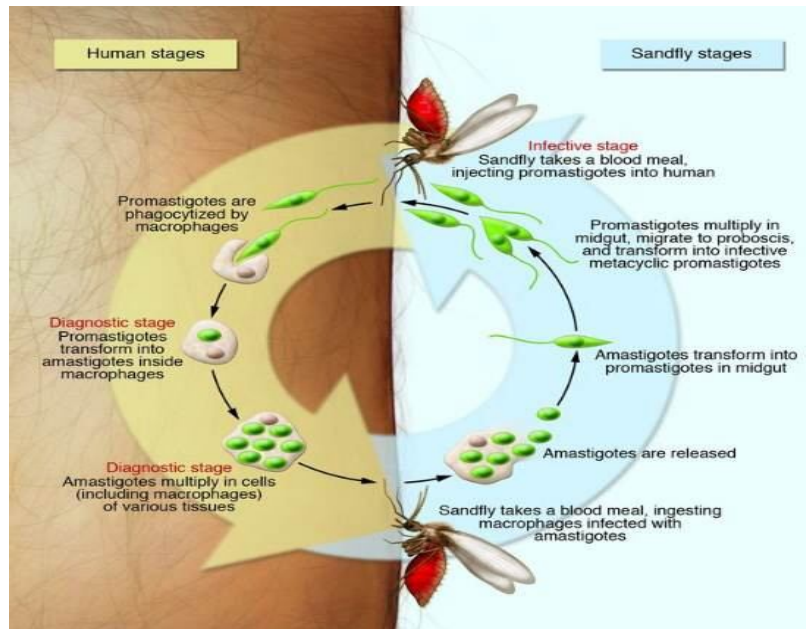
2.3. Pathology under study

Life cycle of leishmaniasis in human: Infected female Sand fly takes a blood meal from a healthy person and injects the metacyclic promastigotes into the host blood stream. Macrophages will be attracted to the infection site to up take the pathogen, which enters a sac-like organelle known as parasitophorous vacuole. Multiple functions of macrophage are recognized in Leishmania life cycle. They serve as host cells for Leishmaniasis replication and as a source of cytokines which modulate the T cell-mediated response that may kill the parasite. Inside the macrophage the promastigotes stage change shapes, lose the flagellum and -become round amastigote as an adapting mechanisms to allow the parasite survival within the macrophages. The mechanisms through which the parasite resists killing within the toxic environment of the phagolysosome remain incompletely defined. The location of Leishmania-infected macrophage differs according to the parasite species, effectiveness of the immune system, and the host temperature. For example, *L. douovani* can live at the high body temperature within deep organs such as spleen, liver, lymph glands, bone marrow and other tissue of the reticulo-endothelial system, but *L. major* remains in the external tissue macrophages. Amastigotes start divide by asexual binary fission and accumulate in the macrophage until it ruptured to release the amastigotes. Then amastigotes will be picked up by new circulating or other local macrophages, this cycle continues and results in one of the clinical symptoms of Leishmaniasis with different species having different tropism for macrophages in particular organ in the host. Finally, the infection can be transmitted among

people via sandflies feeding on macrophages containing amastigotes. (Edward 1989). (Jaryaram 2001)



(A)



(B)

Figure (2.5 A & B) life cycle of leishmaniasis in human and sandfly. (Jaryaram 2001)

2.3.1. Patho-clinical features of visceral leishmaniasis

Leishmania donovani parasitizes reticulo-endothelial cells and is found in greatest numbers in those organs which are particularly rich in this tissue. Its presence leads to great proliferation of macrophage-type cells. As a result, the liver and especially the spleen enlarge, and the red bone marrow extends beyond its normal limits. Reticulo-endothelial tissues in the lymphatic glands, in the lungs, in the intestinal wall, and in the skin, are sometimes heavily parasitized; the degree to which these are affected varies with the strain of parasite and from one case to another. There are almost no tissues from which *Leishmania* have not at some time been recovered on post-mortem of cases of kalaazar, and for this reason almost every secretion or discharge from the body at some time has been reported to yield parasites. Histologically the outstanding feature of parasitized tissue is the enormous proliferation of cells of the macrophage type, their presence overshadows the normal structure of the organ and many of the macrophages in the tissue will be seen to contain *Leishmania*. The spleen becomes much engorged and expands until it may largely fill the abdomen; there is little fibrous tissue formation. In the liver, the Kupffer cells which usually are heavily parasitized, proliferate freely. That fibrous tissue

formation is not a feature of the histological picture in kalaazar is shown by the return to normal size of an enormously enlarged spleen and liver, and the restoration to normal histological structure after effective treatment. The so-called leishrnanial fibrosis of the liver is believed to result due to fibrosis from other causes including malnutrition and not directly from the infection. In the peripheral blood there is an absolute leucopenia to below 4000 per cu. mm, due to marked diminution in the number of the granulocytes with an associated mononucleosis, The neutropenia in the later stages of the disease may become a total agranulocytosis, development commonly is followed by the fatal complication, cancrum oris. There is a slowly progressive anaemia, the red cells falling in number between 2 and 3 million per cu.mm, the red cells tend to be hyperchromic and macrocytic, but nucleated cells are unusual. Red cell fragility is increased, the sedimentation rate is always high; and the platelet count is reduced. The serum indirect bilirubin concentration is usually raised. The total plasma protein is low and there is an inversion of the albumin/globulin ratio, the serum albumin being much reduced as a result of liver dysfunction and the gamma globulin increased partly from increased antibody production. This reversal in the balance of serum protein provides the basis for the formol-gel and other serum tests employed in the diagnosis of kala-azar. (Bekaert ED, et al. 1992) and (Kallel R, et al. 1993).

The blood and plasma changes may be contributed to in old-standing cases by hyper splenism, in it is early stages kala-azar is not easy to diagnose on clinical grounds when its presence is unsuspected. There is indefinite ill-

health and lassitude, but there are no constant physical signs. (Bekaert ED, et al. 1989).

Recent medical findings, there are irregular fever, which is intermittent, remittent, or continuous, recurs irregularly. The patient is rarely prostrated, and does not usually suffer from the subjective symptoms of fever. A low blood pressure, and a high pulse rate; the changes in the blood picture soon become evident and these, particularly the leucopenia, should suggest the diagnosis. The outstanding physical signs are progressive enlargement of the spleen and, to a lesser extent, of the liver. In the early stages the spleen usually enlarges downwards about one inch during each month of the disease; ultimately it may largely fill most of the abdomen and extend into the pelvis. The liver enlarges to a lesser degree, but commonly reaches more than half way to the umbilicus. From time to time patients may be encountered in whom the spleen enlarges but the liver does not, or there may be gross enlargement of the liver unassociated with enlargement of the spleen. The enlarged spleen and the enlarged liver are neither painful nor tender. Sometimes there is jaundice, especially late in the disease, it is held to be of b & d prognostic significance. In cases of kala-azar from China and the Sudan general enlargement of the lymphatic glands has been reported, but this is not a feature of the disease elsewhere. Though the appetite usually is good and indeed may be voracious, there is steady bodily wasting, in time the patient becomes emaciated, with a protuberant abdomen due to the gross swelling of the spleen and of the liver. The skin is dry and rough, in dark-skinned races the natural pigmentation of the skin over the malar bones,

temples and around the mouth is deepened, it is from this that the disease derives its name (Black Sickness). The hair becomes dry and brittle and tends to fall out, even children may become almost bald, but the hair grows again after specific treatment of the infection. Edema of the extremities is not uncommon, particularly in the undernourished and debilitated. (Collin S, et al. 2004).

The lungs are invariably involved to some degree, an irritant cough is usual, though physical signs of a pulmonary lesion adequate to account for it are absent. Bronchopneumonia and similar complications, due to superadded infections, are common; these are probably attributable to a diminished resistance to infection associated with the leucopenia. Diarrhea and even dysentery are common, probably arising from superadded infections. Comparatively mild and self-terminating cases of kalaazar may occur, the mortality from the untreated disease is very high, death usually follows within 2 years of the onset.(Pintado V, et al. 2001).

2.3.2. Symptoms and signs of visceral leishmaniasis

Ultrasound is a useful imaging modality in the tropical diseases, showing the manifestations of VL in the liver, spleen, kidneys, Aorta, portal vein, IVC, GB and CBD.

Clinical symptoms present by the multiplication of the parasites within the human host macrophages whose immune system failed to destroy the parasite. According to the location of macrophage which harbors the parasite, four major clinical forms of Leishmaniasis are distinguished: Cutaneous Leishmaniasis (CL),

Diffuse Cutaneous Leishmaniasis (DCL), Mucocutaneous Leishmaniasis (MCL) and Visceral Leishmaniasis (VL). Features of this form of leishmaniasis include fever (95%), hepatomegaly (greatly enlarged liver) (60%), splenomegaly (greatly enlarged spleen) (95%), lymphadenopathy (75%), diarrhea (40%), weight loss (80%), anemia (75%), skin darkening and death (95%) if patient remain untreated according to WHO report. Scanning imaging has been used primarily to localize (signs and complications for evaluation of VL. Abdominal U/S has increased the rate of positive result when invasive procedures are done. Always clinical examinations confirmed other modality like U/S, CT..etc.(Geneva: WHO. 2010).

Splenomegally is defined by Abdel Gafar as spleen size >12 cm as measured by US along its longer dimension. Paulin, et al classify splenomegally into moderate (11-20 cm), and huge splenomegally if >20 cm. Splenomegally should not be confused with hypersplenism. The former is statement about the size of spleen, while the latter about the spleen function, these may coexist or they may not. Splenomegaly and hepatomegaly are the more common signs VL and increased echogenicity of the kidneys with normal size on ultrasound in patients with VL. Hepatomegally is an enlarge liver usually span longer than 12 cm at the right mid clavicle line. However, liver size on physical examination is only an approximation and should be accurately measured by an abdominal ultrasound, CT or MRI. (Yetter EM, Acosta KB, Olson MC, Blundell K. 2003).

Symptoms of Visceral Leishmaniasis (kala azar)



Figure (2.6) Hepato-splenomegally. (Jaryaram 2001)

LEISHMANIASIS

- Vector borne disease
- A wide clinical spectrum
 - VISCERAL
 - PKDL
 - DIFFUSE CUTANEOUS
 - CUTANEOUS
 - MUCOCUTANEOUS

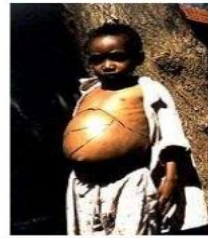


Figure (2.7) Major types of leishmaniasis. (Jaryaram 2001).

2.3.3. Differential diagnosis of VL

VL should be differentiating from mimicking diseases such as malaria, tropical splenomegally, portal hypertension, chronic bilharziasis, tuberculosis, myeloproliferative and lympho-proliferative conditions by clinical and specific laboratory investigations.

Diagnosis of VL: Diagnoses depend on visualizing the organisms within macrophage by collecting samples from the patient lesion by aspirate or biopsy. These collected materials can be stained by Giemsa, cultured in NNN or analyzed by PCR (Polymerase Chain reaction), which sensitively detect the parasite. On the other hand serological diagnosis such as ELISA is reliable especially in *Leishmania donovani* infection but they cannot differentiate between

asymptomatic, past or active infection. Pancytopenia is commonly seen, hypergammaglobulinemia, on which aldehyde test is based on, also is commonly seen. (Das P, et al. 2010).

Laboratory methods of diagnosis VL: (1) Demonstration of the parasite in materials obtained from patients, by (a) microscopy (b) culture (c) animal inoculation. (2) Demonstration of antibodies by using (a) specific leishmanial antigens or (b) non-specific antigens. (3) Non-specific serum tests. (4) Absence of hypersensitivity to leishmanial antigen. And (5) Contributory findings in clinical laboratory tests. - For microscopic demonstration of the parasite, the materials collected from the following: (a) Peripheral blood: The amastigotes are present in the peripheral blood, inside circulating monocytes less often in neutrophils, but the numbers are so scanty that a direct smear may not show them. Chances of detecting them are somewhat improved by examination of a thick blood film or of the leucocytic edge in a blood smear. However, it is best to examine buffy coat smears, though even these are not often found positive, in buffy coat preparations positive in about 70% of cases, (direct agglutination test – D A T), (b) Bone marrow: The most common diagnostic specimen collected is the bone marrow aspirate. Generally, the sternal marrow is aspirated by puncturing the sternum at level of the 2nd or 3rd intercostal space, using a sternal puncture needle. Bone marrow aspirate is positive in up to 86% cases, (c) Splenic aspirate: are richer in parasites and so more valuable for diagnosis. But the procedure can sometimes cause dangerous bleeding and so should be done carefully and only when a marrow examination is inconclusive. Parasites can be

demonstrated in splenic puncture in about 95% cases, (d) Liver aspirates positive in about 70% of cases. 2- Cultures are made on Novy-MacNeal-Nicolle (NMN) medium. 3- Animal inoculation is not used for routine diagnosis. When necessary the hamster is the animal employed. The materials are inoculated intraperitoneally or intradermally into the skin of the nose and feet. The inoculated animals are kept at 23-26°C. In positive cases, the parasite can be demonstrated: in smears taken from ulcers or nodules developing the sites of cutaneous inoculation, or from the spleen. Animal inoculation is a very sensitive method, but takes several weeks to become positive. 4- Other serological tests: A number of tests have been described for demonstrating specific antibody using leishmanial antigens prepared from cultures. These include complement fixation, counterimmunoelectrophoresis, immunofluorescence and ELISA tests. In Kala-azar, the immunofluorescent antibody (IFA) titer usually rises to 64 or above and declines slowly after treatment, eventually becoming negative. (Muniaraj M, et al. 2012).

2.3.4. Treatment of leishmaniasis

Visceral or cutaneous leishmaniasis can usually be cured in immunocompetent individuals. Pentavalent antimonials can be used where the parasites are sensitive to these drugs, but resistance is a major problem in some areas. Other drugs such as allopurinol, amphotericin B or liposomal amphotericin B, and miltefosine may also be used. Most of the drugs used to treat leishmaniasis must be given parenterally. Visceral leishmaniasis in AIDS patients

is often resistant to treatment, and many patients relapse. Side effects of drugs include pancreatitis, cardiac and renal toxicity.(Rosenthal E, et al. 2000).

Prevention and control: Control of leishmania infections relies primarily on the following: 1. early diagnosis and treatment of infected person. 2. Avoid sandfly bites by using insect repellents, parathyroid impregnated bed nets and curtains. 3. Staying away of endemic areas and stopping outdoor activities especially at the insect active time. 4. Vector control by using light traps, sticky papers and insecticides. 5. Destruction of reservoirs such as: infected dogs and rodents, and 6. Setting human residents, away from animal reservoir habitants, where sand fly usually breeds. (Alwar J, et al. 2012).

2.4. Image quality in ultrasound

The key determinants of the quality of an ultrasound image are its spatial, contrast, and temporal resolution,as well as freedom from certain artifacts.

Spatial Resolution, the ability to differentiate two closely situated objects as distinct structures is determined by the spatial resolution of the ultrasound device. Spatial resolution must be considered in three planes, with different determinants of resolution for each. Simplest is the resolution along the axis of the ultrasound beam, or axial resolution. With pulsed wave ultrasound, the transducer introduces a series of brief bursts of sound into the body. Each ultrasound pulse typically consists of two or three cycles of sound. The pulse length is the product of the wavelength and the number of cycles in the pulse.

Axial resolution, the maximum resolution along the beam axis, is determined by the pulse length. Because ultrasound frequency and wavelength are inversely

related, the pulse length decreases as the imaging frequency increases. Because the pulse length determines the maximum resolution along the axis of the ultrasound beam, higher transducer frequencies provide higher image resolution. For example, a transducer operating at 5 MHz produces sound with a wavelength of 0.308 mm. If each pulse consists of three cycles of sound, the pulse length is slightly less than 1 mm, and this becomes the maximum resolution along the beam axis. If the transducer frequency is increased to 15 MHz, the pulse length is less than 0.4 mm, permitting resolution of smaller details. In addition to axial resolution, resolution in the planes perpendicular to the beam axis must also be considered. (Niederau C, et al. 1983). (Gorg C. 2007).

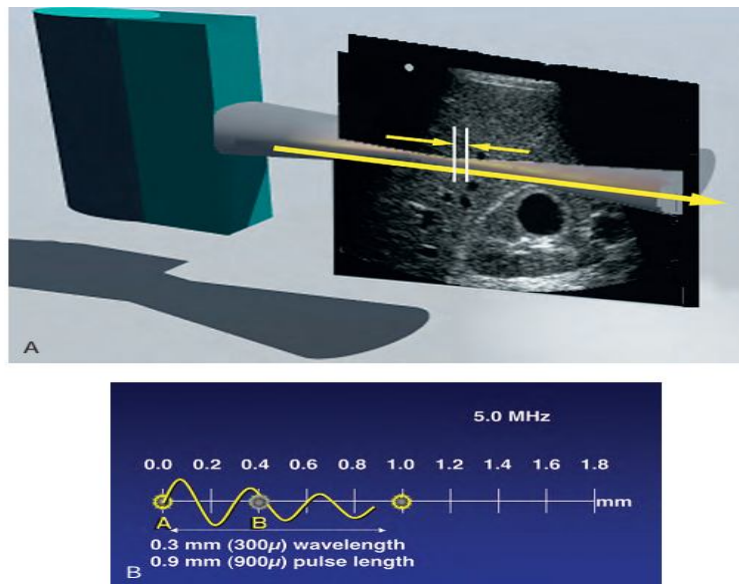


Figure (2.8) shows axial resolution. (Gorg C. 2007).

Lateral resolution, refers to resolution in the plane perpendicular to the beam and parallel to the transducer and is determined by the width of the ultrasound beam. Azimuth resolution, or elevation resolution, refers to the slice thickness in the plane perpendicular to the beam and to the transducer. Ultrasound is a tomography method of imaging that produces thin slices of information from the body, and the width and thickness of the ultrasound beam are important determinants of image quality. Excessive beam width and thickness limit the ability to delineate small features and may obscure shadowing and enhancement from small structures, such as breast micro calcifications and small thyroid cysts. The width and thickness of the ultrasound beam determine lateral resolution and elevation resolution, respectively. Lateral and elevation resolutions are significantly poorer than the axial resolution of the beam. Lateral resolution is controlled by focusing the beam, usually by electronic phasing, to alter the beam width at a selected depth of interest. Elevation resolution is determined by the construction of the transducer and generally cannot be controlled by the user. (From Lamb PM, et al. 2002).

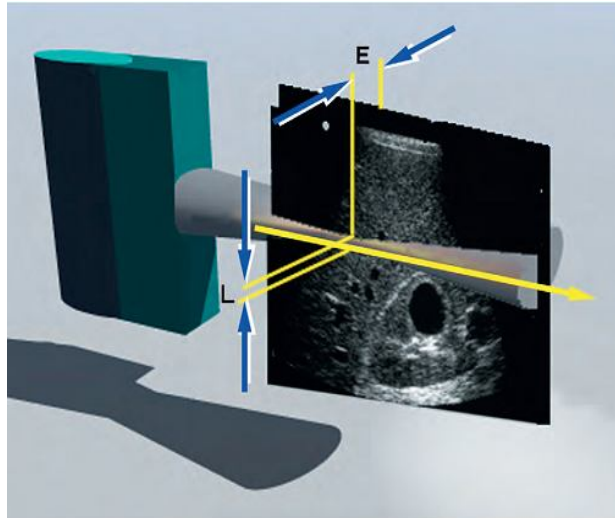


Figure (2.8) shows lateral resolution. (Gorg C. 2007).

2.5. Ultrasound Examination Technique

Patient Preparation, It is recommended that a patient undergo a period of fasting prior to upper abdominal imaging to maximize the distension of the gall bladder and to reduce food residue and gas in the upper GI tract which may reduce image quality or precluded liver imaging. This is essential for full imaging of the liver and related biliary tree but may not be required in an acute situation such as trauma where imaging of the gall bladder is not immediately essential. A patient may take small amounts of still water by mouth prior to scan, particularly for taking any medications. There is some evidence that smoking can reduce image quality when scanning upper abdominal structures and it is good practice to encourage a patient not to smoke for 6-8 hours prior to US scan. Smoking increases gas intake into upper GI tract and may reduce image quality. Also, some chemicals in tobacco are known to cause contraction of the smooth muscle

of the GI tract and this can cause contraction of the gall bladder, even when fasting has occurred, and the gall bladder cannot be scanned. (Christoph, 2004)

Sonographic Technique, TAUS usually begins with the patient in the supine position. The examiner is on the patient's right side and the ultrasound machine is on the same side toward the head of the bed. A 3.5 MHz curvilinear transducer is the most common one used in adults. The curvilinear transducer requires a larger, flatter surface for optimal contact. When a smaller "footprint" (size of the contact surface) is necessary, such as viewing through an intercostal space, a phased array transducer can be used. Ideally, prior to TAUS, the patient should fast for 6 h. This decreases bowel gas and allows gallbladder distension. Standard scanning planes for TAUS are: longitudinal (sagittal, coronal) and transverse. Most TAUS scanning is done with light contact with coupling accomplished with gel. When holding the transducer, it is helpful to stabilize your hand by placing the base of the hypothenar eminence against the body. This allows for fine probe movement during the examination. The initial transducer placement depends on the type of study or organ of interest. The same is true for the initial transducer orientation. Transducer movement during TAUS includes all the techniques previously described. (Ellen, 2014).

The patient should be examined from the sub- to the intercostals in the decubitus position as well in the modified, slightly oblique, positions with the right arm above the head and the right leg stretched during all respiration cycles to identify the best approach and to avoid artifacts caused by the thorax.

Examination in the standing position is also helpful owing to the liver moving caudally with gravity. Scanning from the sub- or intercostals probe positions (depending on the individual anatomy) avoids interposed lung, which can occur in the right poster lateral (superficial) parts of the liver when using the intercostals approach. There are other examination techniques that can also be used, but these will not be mentioned here in detail. The anatomy and examination technique are explained in the videos available online. (Jan 2013).

One measurement of liver size is done in the mid-clavicular line from highest peak of the diaphragm down to the caudal liver end. This has a maximum dimension 18 cm. Another possibility to measure the liver size is in the mid-clavicular line to measure ventrodorsal dimension (depth) and cranio-caudal dimension (length). The maximum length is 15 cm and depth 13 cm, maximum for both dimensions together is 28 cm. In many diseases, the caudate lobe is larger than the rest. In the liver cross section, measurement of this lobe relative to the rest, the quotient should be normally less than 0.55. (Tuma 2013).

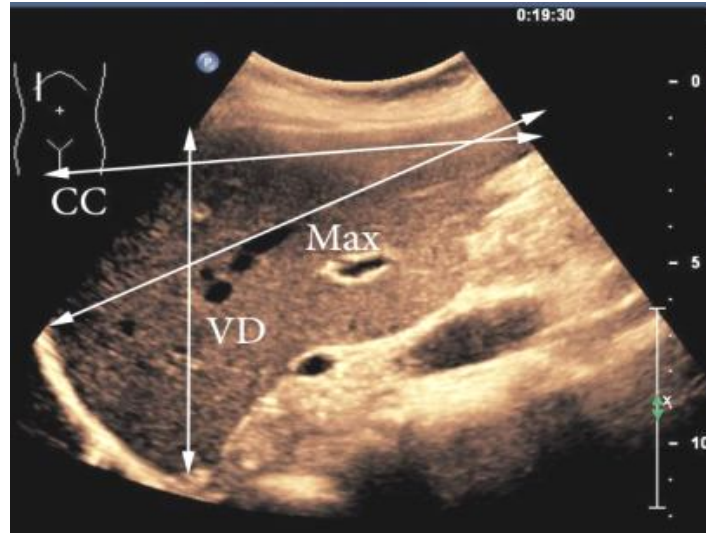


Figure (2.9) Measurement of liver size: Length CC- cranio-caudal depth, VD- ventrodorsal and the maximum distance of diaphragmatic dome to the lower edge of the liver in the MCL Max. (Tuma 2013).

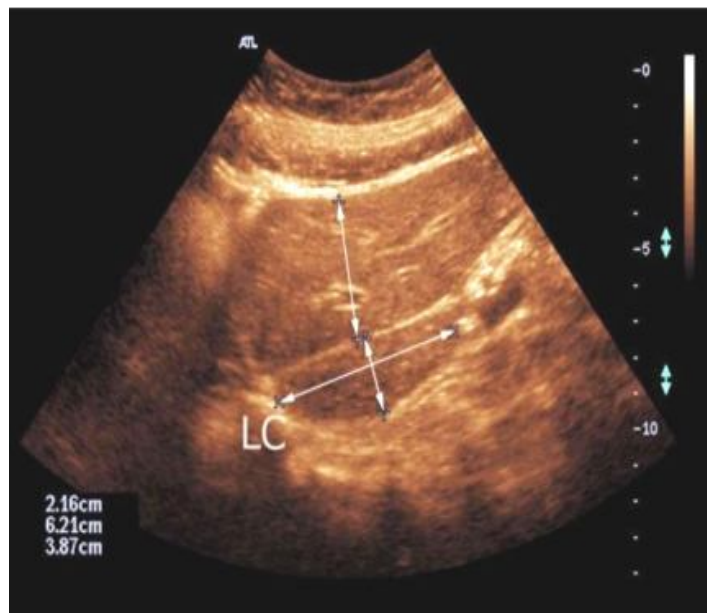


Figure (2.10) Measurement of the size of the caudate lobe and the overlying segments. (Tuma 2013).

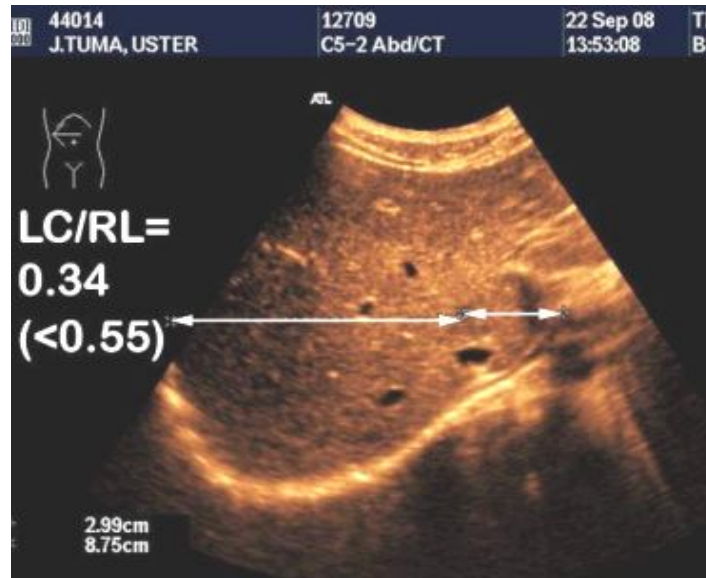


Figure (2.11) Measurement of the size of the caudate lobe and the right lobes. The ratio of caudate lobe CL / right lobes, RL should be <0.55 (here 0.34, normal) (Tuma 2013).

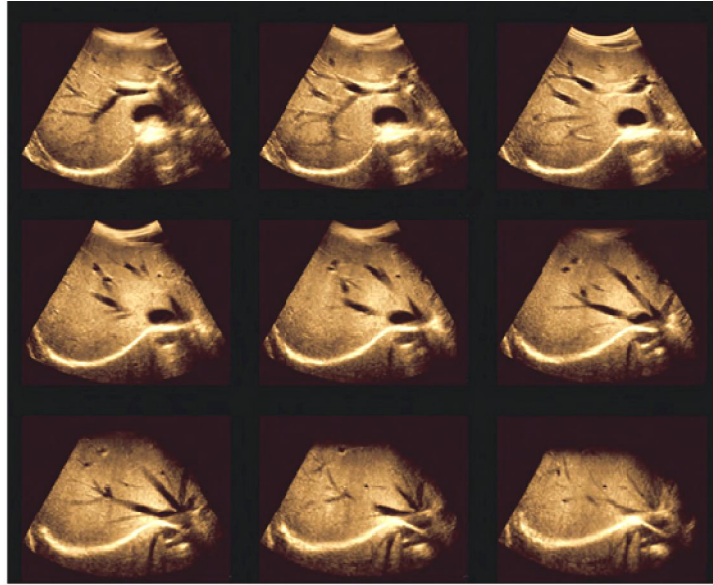


Figure (2.12) Ultrasound images show normal liver. (Tuma 2013).

2.6. Previous studies

Chakrabarti et al. (2013), who studied cases of visceral leishmaniasis (VL) over a 2-year period among immunocompetent patients who presented to a rural medical college in West Bengal, India to determine a clinical and hematological profile among these patients. We studied a total of 36 cases of VL; the male to female ratio of the cases was 1.6:1 and the mean age was 20.1 ± 11.1 years. A detailed history, physical examination, hemogram, bone marrow or splenic aspiration and chest x-ray were conducted on all cases. A CT-scan of the thorax and fiberoptic bronchoscope were performed in selected cases. Fever and

splenomegaly were present in all cases. Weakness, abdominal pain, bleeding, and hepatomegaly were seen in 63.9, 27.8, 8.3 and 58.3% of cases, respectively. Pancytopenia, bicytopenia, leukopenia and thrombocytopenia were seen in 58.3, 41.7, 61.1 and 83.3% of cases, respectively. Five patients (13.9%) had cough, 2 (5.6%) had hemoptysis, 6 (16.7%) had an abnormal chest x-ray and 3 (8.3%) had localized reticulo-nodular opacities on a CT-scan of the thorax. Broncho alveolar lavage showed gram-positive cocci in 2 cases (5.6%). One patient died of acute respiratory distress syndrome. Cytopenia was common among the series of VL patients. Pulmonary complications, usually secondary infection, were less frequent (found in 13.9% cases) but was fatal in one patient.

Abdalla et al. (2014), stated that visceral leishmaniasis (VL) has been a major health burden on the Sudanese patients and to the health authorities in Sudan. Delay in diagnosis of VL leads to serious complications and eventually death. This study aimed to study the ultrasonographic findings of VL in Sudanese patients. 50 Sudanese patients who were clinically diagnosed as VL, attended to Ultrasound Department and were scanned for abdomen. Subject's ages, duration of the disease, liver, spleen, lymph nodes, portal vein and inferior vena cava (IVC) diameter, presence of ascites and plural effusion, echogenicity of kidneys and pancreas were evaluated. VL affected the age group 15 – 30 years in 20 patients (40%) constituting the high incidence and it was less common in age group <15 years (10%). The disease was common in males (76%) than females (24%) with a ratio of (3.6: 1). The Eastern states constituted the high incidence of

the diseases among the other states 27 (54.0%), and Khartoum State constituted the lower incidence 1(2%). Splenomegally, hepatomegally, lymphadenopathy were the most common ultrasound findings. Portal vein, IVC dilatations were less common and were significantly affected with duration of diseases at p value <0.0001. VL associated with asites and plural effusion was found in the sample and has statistically significant relation at p value< 0.02. Ultrasound detected changes in kidneys and pancreases echogenicity with prolonged disease duration. Ultrasonography has the capability of diagnosis of VL and by using ultrasound complications could be diagnosed earlier and treated before damaging the spleen, liver, lymph nodes or death to occur.

Nabi et al. (2015), who stated that Leishmaniasis is a disease caused by an intracellular protozoa parasite transmitted by the bite of a female sandfly (Phlebotomus species). This vector borne disease has wide clinical spectrum with visceral leishmaniasis being the most severe form. We report a case of visceral leishmaniasis from a Kashmir valley of Jammu and Kashmir (India) where the vector for leishmaniasis does not exist. Only one case of leishmaniasis has been reported from valley. This is the second case, which was reported because of rarity and delay in diagnosis even though it was imported. A 44 Year old male patient presented with intermittent, low grade fever associated with chills and rigors, night sweating for 3 years, took antibiotic and antipyretic, partially responded, was never evaluated properly. Patient had history of travel to outside state. In state like Jammu and Kashmir where tuberculosis is more

prevalent visceral leishmaniasis is very unlikely to be diagnosed. On evaluation patient had pancytopenia and hepato-splenomegaly with bone marrow revealing inclusion bodies (LD bodies both intracellular and extra cellular) thus confirming the diagnosis of visceral leishmaniasis.

Daher et al. (2008), stated that visceral leishmaniasis is an endemic disease caused by various species of *Leishmania*. We made a retrospective study of 57 consecutive patients with visceral leishmaniasis in Brazil. Patients with visceral leishmaniasis were identified using the registries of the São José Infectious Diseases Hospital. The sample was divided into two groups: patients with serum creatinine (Scr) $<1.3\text{mg/dL}$ and $\text{Scr} \geq 1.3\text{mg/dL}$. We compared these two groups for differences in clinical manifestations and laboratory features. Patients' mean age was 28 ± 18 years old; 74% were male. The main clinical symptoms and signs presented in the initial evaluation were: fever (97%), splenomegaly (96.4%), weight loss (95.5%), pallor (93.6%), cough (89.7%), hepatomegaly (87.2%), asthenia (83.3%), anorexia (82.9%) and vomiting (73.9%). Acute renal failure was found in 15 patients (26.3%) and eight of these patients had ARF before amphotericin B administration. The mean age was higher in the group with $\text{Scr} \geq 1.3\text{mg/dL}$. Death occurred in three cases; all deaths occurred with $\text{Scr} \geq 1.3\text{mg/dL}$. There were no significant differences in the frequencies of the clinical symptoms and signs between the two groups. The laboratory data and demographic characteristics were significantly worse in the $\text{Scr} \geq 1.3\text{mg/dL}$

group. Renal dysfunction is an important feature of this disease; it is associated with important morbidity and can increase mortality.

Bu" kte et al., (2003) studied a case for Visceral leishmaniasis is a severe disease caused by the intracellular protozoa *Leishmania donovani*. Diagnosis is based on examination of bone marrow or serology. The role of imaging techniques as diagnostic tools remains to be established in visceral leishmaniasis. We report multiple nodular lesions in the liver and spleen on ultrasonography and computed tomography in a patient with visceral leishmaniasis. To our knowledge, this is the first reported case of multiple nodular hepatosplenic lesions in visceral leishmaniasis.

Lal et al. (2015), aimed of visceral leishmaniasis (VL), a protozoan disease, is 100 % fatal if left untreated. Anemia is common in VL which plays a role in expression of clinically overt VL disease. Laboratory clues are scarce for strengthening clinical suspicion for severity in VL. Hypertriglyceridemia has emerged as a new concept for the diagnosis and prognosis in VL. The present study is aimed at correlating the magnitude of hypertriglyceridemia with the severity in VL. **Materials and methods** A retrospective case–control study was conducted between January 2012 to December 2013 among 124 patients coming for treatment from VL endemic areas, who had fever of more than 15 days and did not respond to antimalarials and antibiotics. The parasitologically confirmed VL cases (n = 87) were categorized as mild/moderate (n = 60) and severe (n = 27) groups according to WHO classification for anemia and parasite

burden. Serum triglycerides were assayed in VL groups along with controls (n = 37). Results Serum triglyceride level was significantly higher in VL than controls [mean values were 173.50 ± 47.67 versus 127.1 ± 53.79 mg/dl, respectively ($p < 0.0001$)]. Triglyceride level was significantly higher in severe than in mild/moderate group of VL [211.3 ± 50.2 mg/dl versus 134 ± 45.09 mg/dl, respectively ($p < 0.0001$)]. Hypertriglyceridemia (>161.7 mg/dl) was noted in all severe VL patients, compared to 31.66 % of mild or moderate group ($p < 0.0001$). There was no significant difference between mild/moderate VL and controls. Conclusions It is hypothesized that hypertriglyceridemia could be of additional diagnostic benefit to assess the probability and severity of VL in endemic areas.

Prasad et al. (2009), who stated that we conducted this study to observe evidence of portal hypertension in children with visceral leishmaniasis (VL). Eighty-eight consecutive cases (50 male) of VL were subjected to Ultrasonography. Those with evidence of portal hypertension also underwent upper gastrointestinal endoscopy and liver biopsy. Eight patients had portal hypertension as evidenced by dilated caliber of portal and splenic veins. Two patients had periportal, splenic and peripancreatic collaterals and one patient had cavernous transformation of portal vein. Out of eight patients, four patients had esophageal and gastric varices. Liver biopsy was done in four patients and revealed hepatic sinusoidal dilations without any evidence of fibrosis. Portal hypertension may be an independent manifestation of VL and remain undiagnosed unless a physician maintains a high index of suspicion.

Mao et al. (2014) stated that the spleen is one of the most commonly involved organs of visceral leishmaniasis (VL). However, there were few reports about imaging findings of splenic leishmaniasis, especially regarding MRI findings. This case report describes a 45 years old male patient from Zhejiang province of southeastern China, who was admitted for persistent fever of unknown origin, with splenomegaly and multiple hypodense/low echo nodules on CT/Ultrasonography (USG) studies. MRI showed multiple nodules with concentric rings in the spleen on T2-weighted imaging(T2WI), with no obvious diffusion restriction on diffusion weighted imaging (DWI), and gradual ring-like enhancement after intravenous administration of contrast medium. So MRI suggested necrotic granulomatous lesion. By reviewing the clinical history and following positive serological leishmania antibody test, the patient was finally confirmed a recent infection with VL. The patient received antimony gluconate therapy intravenously. At 4 months follow-up, the contrast-enhanced abdominal MRI showed that the size of the spleen was returned to normal and the splenic lesions were completely resolved except for reduced infarction compared with the previous MRI. This is the first case which was performed MRI examination completely. Meanwhile, it is the second case which MRI findings were reported. As for the characteristics of MRI in this case, there are several features, which are helpful for giving the diagnosis and differential diagnosis of VL.

Mahmoud (2014), studied the visceral leishmaniasis (VL), also known as kala azar, is a parasitic disease that caused by infection with *Leishmania* parasites,

which are spread by the bite of phlebotomine sand flies. An ultrasound examination is strongly advocated for the promote diagnosis and treatment of VL for long term follow up and evaluation of unresponsive cases. The aim of this study was to assess the consequences of VL in the liver, spleen and pancreas of affected participants by using ultrasound. Materials and Methods: A prospective cohort study was conducted in the period of January 2012 to March 2014 in the ultrasound department of Tropical Disease Hospital (TDH) in Khartoum-Sudan, among a group of 100 participants (84% males, 16% females and their ages ranges from 15 to 45 years) positive to VL and had been diagnosed by laboratory tests; either serological or Napier's Aldehyde test. The Aloka portable ultrasound machine equipped with 3.5 MHz convex probe was used for abdominal ultrasound scanning. Standard Statistical Package for the Social Sciences (SPSS) was used to analyze the results. Results: The commonest ultrasound findings in VL participants were hepatomegaly (100%), splenomegaly (100%) and ascites (50%). Other complications such as lymphadenopathy (35%), focal splenic lesions (34%), dilated portal vein (7%) and shrinkage liver (4%) were detected. Conclusion, Ultrasound scanning presents an effective role in VL, because of its ability to detect the consequences of this disease in various abdominal organs such as liver, spleen and pancreas earlier, which in turn allowing the possibility to treat these complications and prevents the deterioration of a patient's health status.

YAZICI et al.(2014), stated that visceral leishmaniasis (VL) is one of the parasitic infections causing different pathogeneses of various systems including intra

abdominal solid organs. *L. donovani* and *L. infantum*, particularly in Turkey, have been diagnosed in systemic infections. In the present case study, a 43-year-old woman with left upper abdominal pain, persistent fever and splenic pathology according to the radiological findings was investigated. Laboratory findings showed elevated liver function tests and anemia while radiological studies revealed splenomegaly, and nodular infiltration and laceration of the spleen. Because of enlarged intra abdominal lymph nodes observed during surgery, a lymph node biopsy and a liver biopsy were also performed along with the splenectomy. Results from the pathological examination of the spleen were nonspecific and the liver biopsy confirmed a diagnosis of granulomatous hepatitis. Further examination of the hepatic granulomatosis including parasitic evaluation and serological evaluation with the rK39 dipstick test revealed VL. In conclusion, in cases of visceral organ pathology accompanied by persistent fever, and hematological disorders, parasitic infections, particularly VL should be considered in the differential diagnosis.

Chapter Three

Materials and Methods

3.1. Materials:

Table (3.1) (A) an ultrasound machine of facilities as shown in the following table:

U/S machine name	Honda	Eucup 7	General Electric
Model	HS 2000	Eucup 7	LOGIQ 5
Movement	Portable	Mobile	Mobile
Type of probe	Curvilinear	Curvilinear	Curvilinear
Energy of probe	5 MHz	3.5 MHz	3.5 MHz



Figure (3.1) Shows Honda 2000 Machine which used in this study



Figure (3.2) Shows General electric LOGIQ 5 which used in this study



Figure (3.3) Shows ALOKA SSD 500 machine which used in this study

- (B) All these machines have computer for data analysis, printer (Sony) with

thermal papers.

3.2. Methods

This study was conducted at Omdurman Tropical Diseases Teaching Hospital in Sudan during the period from 2016-2017. 50 Sudanese patients who were positively diagnosed as visceral leishmaniasis were included while non-Sudanese patients and normal subjects were excluded. Data including (a) personal information (age, gender, locality and duration of illness) (b) Ultrasonic findings of abdominal examination (size of spleen, liver, caliber of portal vein and vena cava and echogenicity of kidneys) were all been evaluated. Data were collected on data sheet and were statistically analyzed using SPSS programmed version 16. The ultrasound examinations were done after explaining the procedure to the patients. The patients came fasting for 8 hours, positioned in the couch comfortably in supine position. Coupled gel was applied to the abdomen, the patients were allowed to breathe quietly and deeply. The transducer was chosen and the gain was corrected. The scanning was taken in all directions (longitudinal and transverse views). As for spleen the long axis was measured (normal size <13 cm, mild splenomegaly 13 – 15 cm, moderate splenomegaly 15.1 – 19.9 cm, marked splenomegaly >20 cm). Liver was measured in mid-clavicular line (normal size <13 cm, mild hepatomegaly 13.1 – 15 cm, moderate hepatomegaly 15.1 – 18 cm, and marked hepatomegaly >18 cm). Regarding IVC diameter (normal < 2.4 cm, dilated if >2.5 cm) and portal vein diameter (normal 13

mm, dilated >13 mm). Concerning the kidneys size were measured in long axis (normal 8 - 12 cm, increased >12 cm).

3.2.1. Design of the study

This study is analytic study used abdominal ultrasound scan to evaluate the disease of VL.

3.2.2. Population of the study

The population of this study was patient living in the tropical disease areas particularly those coming from gadarif state

3.2.3. Sample size and type

Sample included in this study was more 215 patient having VL.

3.2.4. Place and duration of the study

This study was carried out in gadrif state hospitals and Omdurman Tropical Diseases Teaching Hospital in Sudan in period from 2013 - 2017.

3.2.5. Methods of data collection

Using a special data collection sheet (questionnaire), sample of 215 patient were studied by trans abdominal ultrasound scanning and data was collected using a data collecting sheet which designed to evaluate liver anatomy, size, texture, shape, surface characteristics (outlines) and right liver lobe corner.

3.2.6 Technique (Imaging protocols) - Trans abdominal U/S scanning

(a) Position of the patient: The patient should lying supine comfortably, but may need to be rotated obliquely, he should be relaxed, and breathing quietly,

lubricates the lower abdomen because hair anywhere on the abdomen will trap air bubbles so apply coupling agent generously.

(b) Choice of transducer: Uses a curve linear probe of 3.5 MHZ frequency.

(c) Scanning technique: Start with a longitudinal scans from the xiphoid process and we must be angle the probe sharply to the right for setting the gain, move the probe to the right until the right lateral border of the liver appears clearly in the center of the screen. Then move the probe to the left until the left lateral border of the liver appears clearly in the center of the screen, return to the right until the right lateral border of the liver appears clearly in the center of the screen, then adjusted the gain of the image and freeze it, measured the liver by putting the probe sagittal, sub costal and in mid-clavicular line. Any area appears in abnormal must be viewed in several projections then rock slide and saving the image. After that do a transverse scanning to measure the liver lobes transversely. During scan researcher also evaluate the liver shape, texture and Echogenecity if they are normal or abnormal, saving the image and print out, then to complete the abdomen examination by the scanning other abdominal organs mainly involved in the study.

3.2.7. Inclusion criteria

The study was included all patients with VL at age between (20-90years), female or male.

3.2.8. Exclusion criteria

Patient with hepatitis and other liver disease or abdominal disease was excluded.

3.2.9. Statistical analysis

All data were presented as mean±SD values. Data were analyzed by an independent t- test and by correlation analysis with the use of the SPSS (Inc., Chicago, Illinois version 16). A value of P<0.05 was considered significant.

3.2.10. Ethical issues

- There was official written permission to Gadaref state hospitals and centers, Aumdrman tropical hospital to take the data.
- No patient data were published, also the data was kept in personal computer with personal password.

Chapter Four

Results

This study intended to evaluate the VL using abdominal US in Gadarif state region in which responding to the area of higher rate of VL, the result showed that:

Table (4.1) demonstrate the frequency distribution of gender for 215 patient with VL

Gender	Frequency	Percent
Male	140	65.1
Female	75	34.9
Total	215	100.0

Table (4.2) demonstrate the frequency distribution of common region affected by VL for 215, patient where the main region where the study was conducted is Gadarif state

Region	Frequency	Percent
--------	-----------	---------

Gadarif city	10	4.7
East Gadarif	20	9.3
West Gadarif	45	20.9
North Gadarif	5	2.3
South gadarif	135	62.8
Total	215	100.0

Table (4.3) demonstrate the frequency distribution of patient signs and symptoms

Signs	Frequency	Percent
distended bladder	85	39.5
Vomiting	75	34.9
nasal bleeding	40	18.6
welling	15	7.0
Total	215	100.0

Table (4.4): frequency distribution of patient symptoms

Symptoms	Frequency	Percent
loss of appetite	140	65.1
pain	35	16.3
fever	40	18.6
Total	215	100.0

Table (4.5) showed frequency distribution of presence of family history for VL

History Family	Frequency	Percent
no	135	62.8
yes	80	37.2
Total	215	100.0

Table (4.6) showed frequency distribution of shape of lower corner of the liver for VL patient

Lower corner of liver	Frequency	Percent
Triangle	96	44.7
Round	119	55.3
Total	215	100.0

Table (4.7) showed frequency distribution of liver Echogenicity for VL patients

Echogenicity	Frequency	Percent
isoechogenic	60	27.9
hyperechogenic	100	46.5
hypoechoic	55	25.6
Total	215	100.0

Table (4.8) showed frequency distribution of liver echo texture for VL patients

Texture	Frequency	Percent
homogenous	180	83.7
heterogeneous	10	4.7
mixed	25	11.6
Total	215	100.0

Table (4.9) showed frequency distribution of presence and absence of liver mass for VL patients

Mass	Frequency	Percent
no	205	95.3
yes	10	4.7
Total	215	100.0

Table (4.10) showed frequency distribution of presence and absence of liver cyst or VL patients.

Cyst	Frequency	Percent
no	210	97.7
yes	5	2.3
Total	215	100.0

Table (4.11) showed frequency distribution of liver fatty changes for VL patients

Fatty stat	Frequency	Percent
no	140	65.1
all fatty	70	32.6
focal fatty	5	2.3
Total	215	100.0

Table (4.12) showed frequency distribution liver shape during US scan for VL patients

Liver Shape	Frequency	Percent
regular	185	86.0
irregular	30	14.0
Total	215	100.0

Table (4.13) showed frequency distribution of CBD shape for VL patients

CBD shape	Frequency	Percent
normal	210	97.7
abnormal	5	2.3
Total	215	100.0

Table (4.14) showed frequency distribution of Echogenicity of right kidney for VL patients

Echogenicity of right kidney	Frequency	Percent
isoechogenic	80	37.2
hyperechogenic	115	53.5
hypoechoic	20	9.3
Total	215	100.0

Table (4.15) showed frequency distribution of Echogenicity of left kidney for VL patients

Echogenicity of left kidney	Frequency	Percent
isoechogenic	75	34.9
hyperechogenic	130	60.5
hypoechoic	10	4.7
Total	215	100.0

Table (4.16) showed frequency distribution of Echo texture of right kidney for VL patients

echo texture of right kidney	Frequency	Percent
homogenous	145	67.4
heterogeneous	50	23.3
mixed	20	9.3
Total	215	100.0

Table (4.17) showed frequency distribution of Echo texture of left kidney for VL patients

Left kidney Texture	Frequency	Percent
homogenous	135	62.8
heterogeneous	50	23.3
mixed	30	14.0
Total	215	100.0

Table (4.18) showed frequency distribution of mass presence in right kidney for VL patients

Mass in right kidney	Frequency	Percent
no	210	97.7
yes	5	2.3
Total	215	100.0

Table (4.19) showed frequency distribution of cyst presence in right kidney for VL patients

Right kidney Cyst	Frequency	Percent
no	185	86.0
yes	30	14.0
Total	215	100.0

Table (4.20) showed frequency distribution of cyst presence in left kidney for VL patients

Cyst in left kidney	Frequency	Percent
no	190	88.4
yes	25	11.6
Total	215	100.0

Table (4.21) showed frequency distribution of kidney shape

kidney Shape	Frequency	Percent
Irregular	45	20.9

Regular	170	79.1
Total	215	100.0

Table (4.22) showed frequency distribution of echotexture of spleen

Texture of Spleen	Frequency	Percent
Homogenous	115	53.5
heterogeneous	25	11.6
Mixed	75	34.9
Total	215	100.0

Table (4.23) showed frequency distribution of Echogenicity of the spleen

Echogenicity of spleen	Frequency	Percent
isoechogenic	85	39.5
hyperechogenic	110	51.2
hypoechoic	20	9.3
Total	215	100.0

Table (4.24) showed frequency distribution of portal vein shape

Shape of the Portal vein	Frequency	Percent
regular	200	93.0
irregular	15	7.0
Total	215	100.0

Table (4.25) showed the group statistic of study variables with its mean and SD for 215 patient

Lower corner of liver		N	Mean	Std. Deviation
Disease duration	triangle	96	1.4144	1.11347
	round	119	3.1185	6.16720
TWB	triangle	96	4374.84	1701.745
	round	119	3662.86	2110.142
Hp	triangle	96	7.6302	1.90717
	round	119	6.7580	2.29686
Size of liver Rt lobe	triangle	96	122.34	30.071
	round	119	140.88	33.496
Size of liver Lt lobe	triangle	96	62.86	20.779
	round	119	77.35	11.506
GB Wall thickness	triangle	96	2.0729	0.84908
	round	119	2.2353	1.04724
GB Lumen width	triangle	96	13.95	3.062
	round	119	12.53	3.244
Aorta Caliber	triangle	96	11.48	2.783
	round	119	14.86	2.267
Aorta thickness	triangle	96	1.81	0.529
	round	119	2.19	0.655
CBD Caliber	triangle	96	3.36	0.896
	round	119	3.55	0.851
CBD Thick mass	triangle	96	.0145	0.00521
	round	119	.0165	0.01030
Length of Rt kidney	triangle	96	87.27	18.253
	round	119	106.24	12.149
Width of Rt kidney	triangle	96	39.90	11.415
	round	119	51.22	9.028
Length of Lt kidney	triangle	96	87.74	25.720
	round	119	101.66	22.163
Width of Lt kidney	triangle	96	42.88	10.301
	round	119	47.85	7.050
Spleen length	triangle	96	127.13	17.287
	round	119	172.96	15.409
Spleen width	triangle	96	53.34	17.343

	round	119	75.87	9.050
Caliber Portal vein	triangle	96	9.24	2.046
	round	119	10.40	2.958
Thickness Portal vein	triangle	96	2.79	3.662
	round	119	2.41	2.437
Caliber IVC	triangle	96	10.56	2.431
	round	119	12.82	3.681
Thickness IVC	triangle	96	2.07	0.943
	round	119	2.32	0.637

Table (4.26) an independent sample t-test performed for Ultrasonography measurement carried for the abdominal viscera.

Variables	t	Sig. (2-tailed)
	t-test for Equality of Means	
	t	p-value
Disease duration	2.671	0.008
TWB	2.677	0.008
Hp	2.982	0.003
Size Rt lobe	4.221	0.000
Size Lt lobe	6.477	0.000
GB Wall thickness	1.228	0.221
GB Lumen W	3.268	0.001
Aorta Caliber	9.808	0.000
Aorta thickness	4.611	0.000
CBD Caliber	1.519	0.130
CBD Thick mass	1.725	0.086
Length of the Rt kidney	9.108	0.000
Width of the Rt kidney	8.122	0.000
Length of the Lt kidney	4.259	0.000
Width of the Lt kidney	4.190	0.000
Length of the spleen	20.530	0.000
Width of the spleen	12.257	0.000
Caliber Portal vein	3.274	0.001
Thickness Portal vein	.910	0.364
Caliber of IVC	5.174	0.000
Thickness of IVC	2.279	0.024

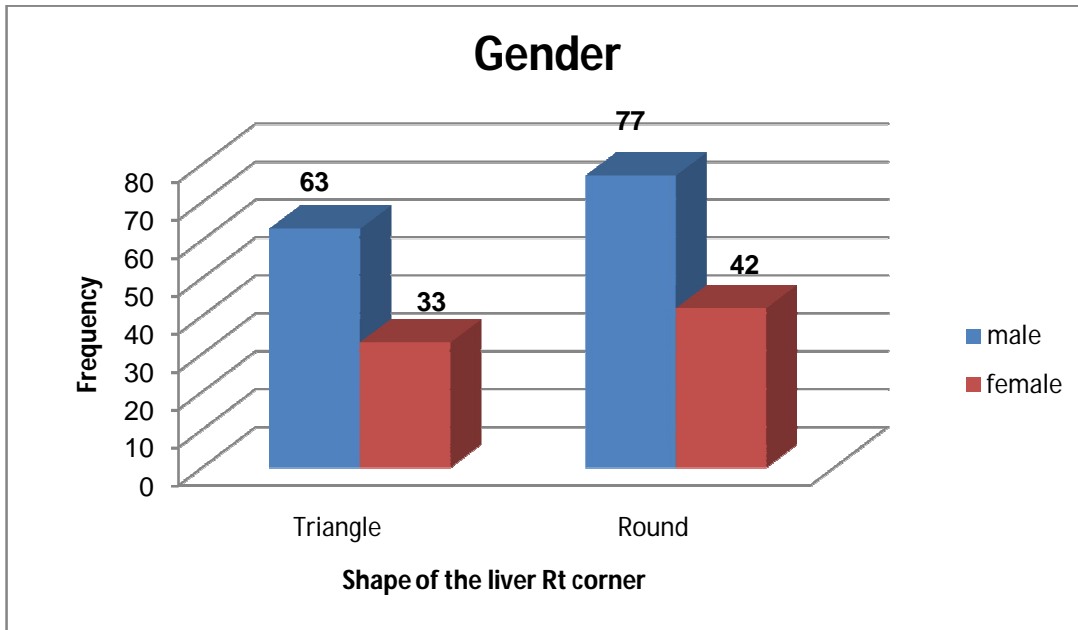


Figure 4-1 bar graph shows the distribution of gender in respect to shape of the liver Rt corner

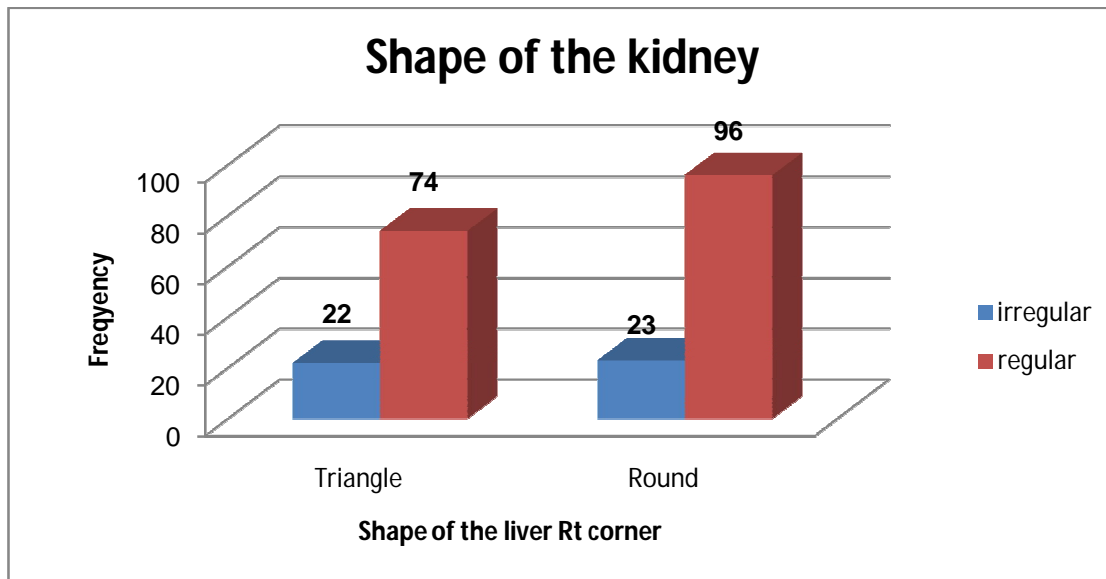


Figure 4-2 bar graph shows the distribution of shape of kidney in respect to shape of the liver Rt corner

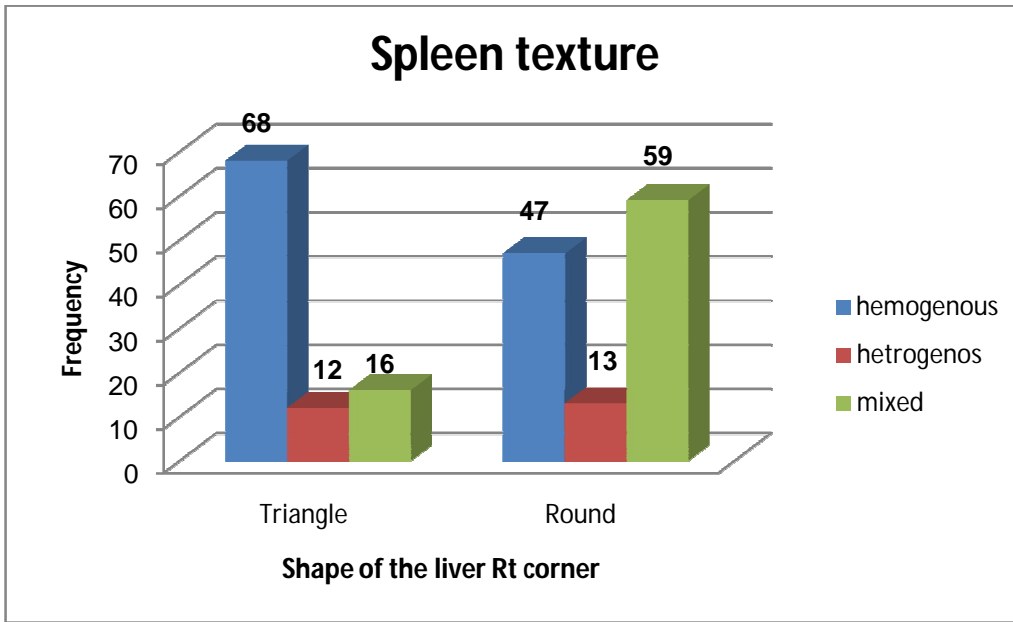


Figure 4-3 bar graph shows the distribution of spleen texture in respect to shape of the liver Rt corner

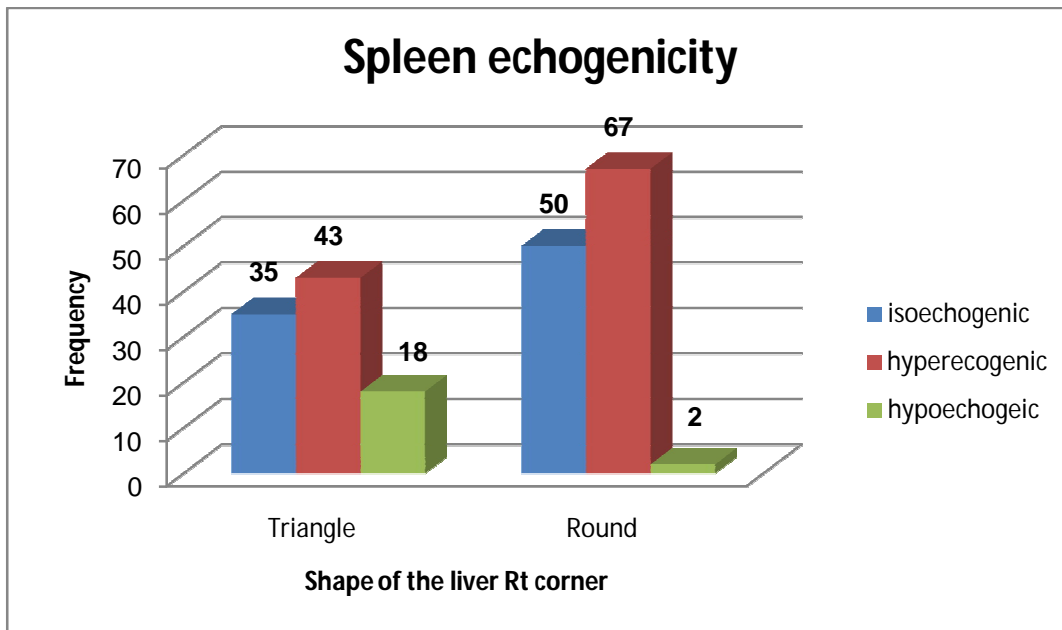
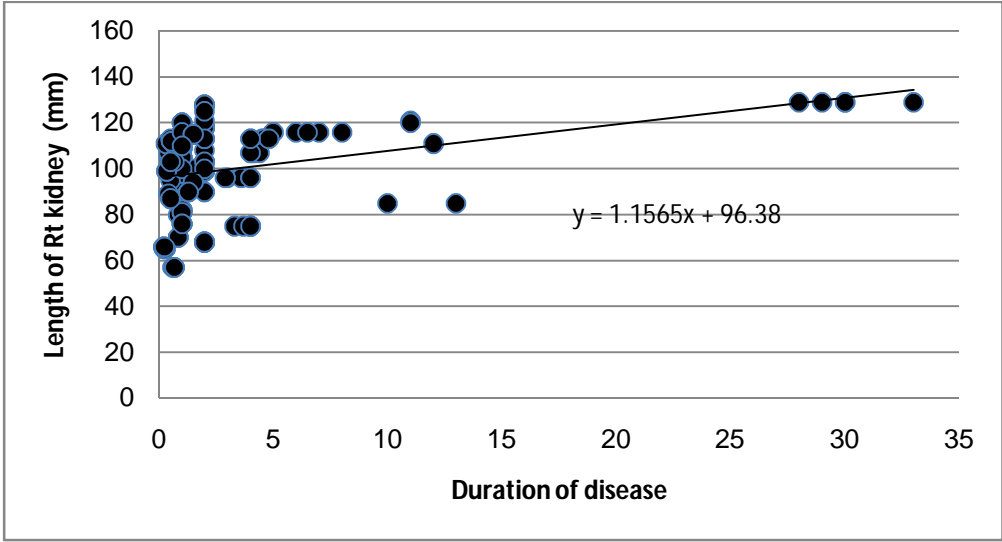
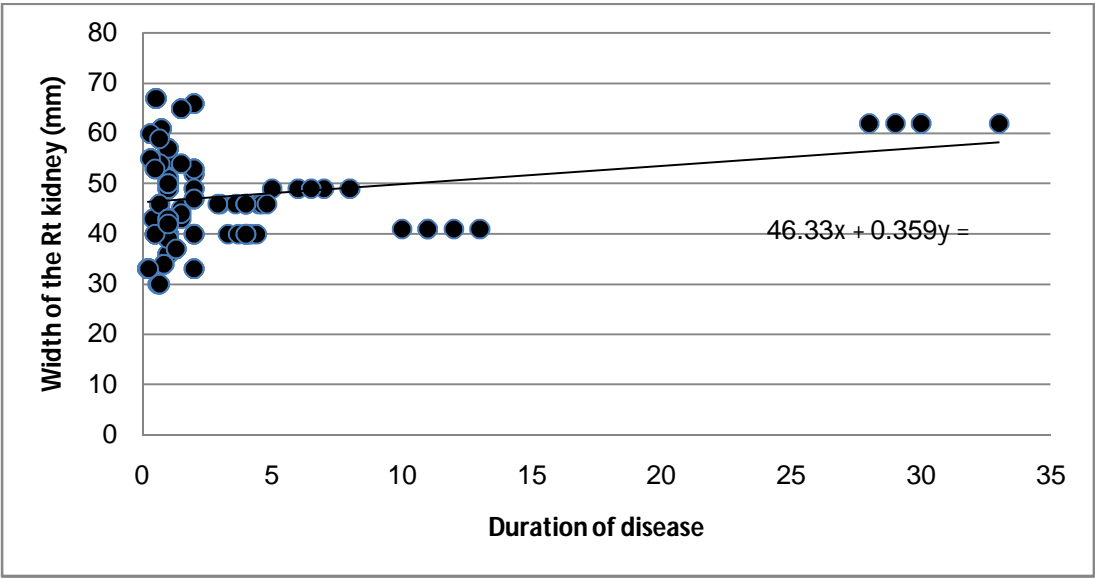


Figure 4-4 bar graph shows the distribution of echogenicity in respect to shape of the liver Rt corner

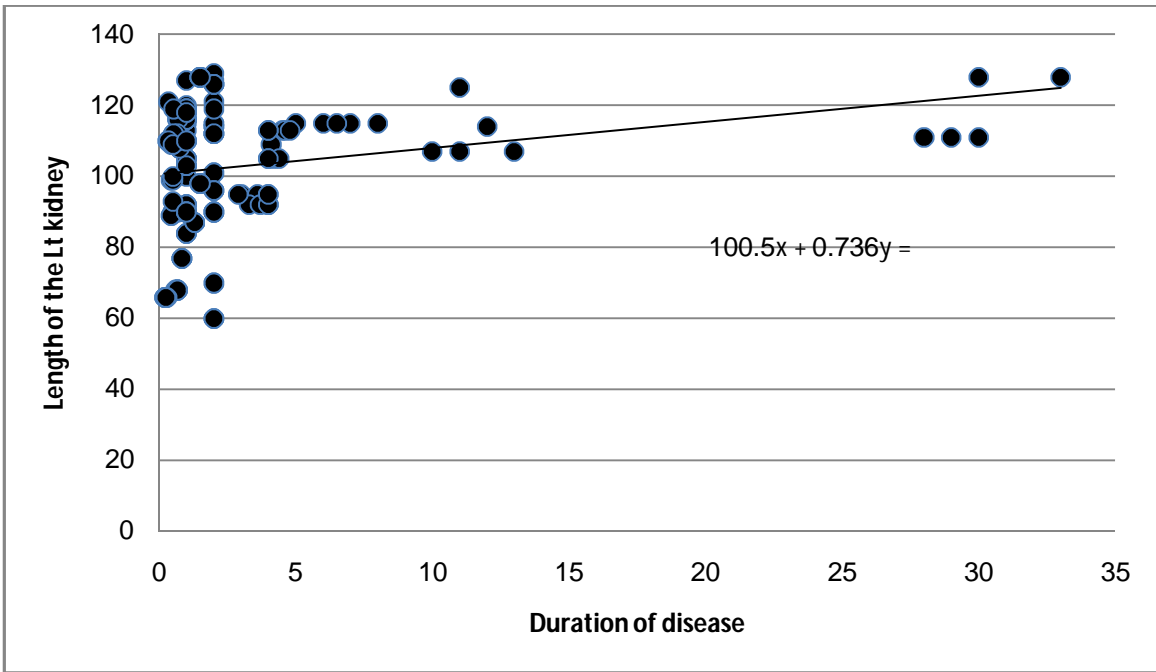


(A)

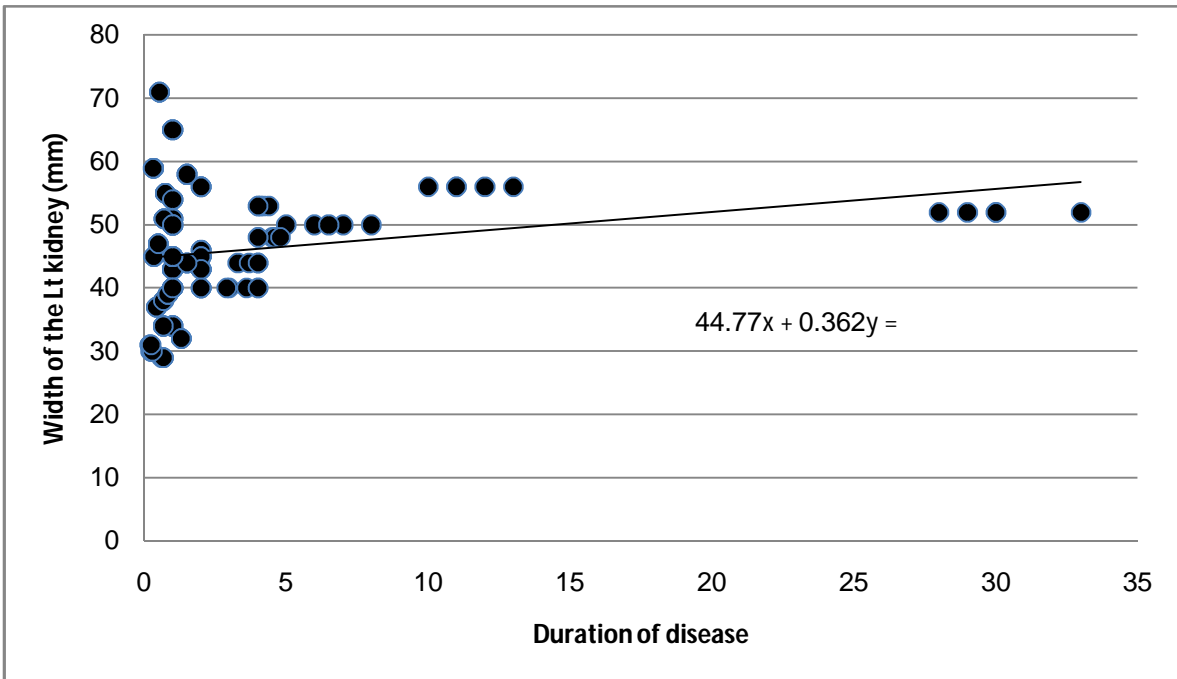


(B)

Figure 4-5 scatter plot shows a liner direct relationship of disease duration with, (A) Rt kidney length and (B) Rt kidney width.

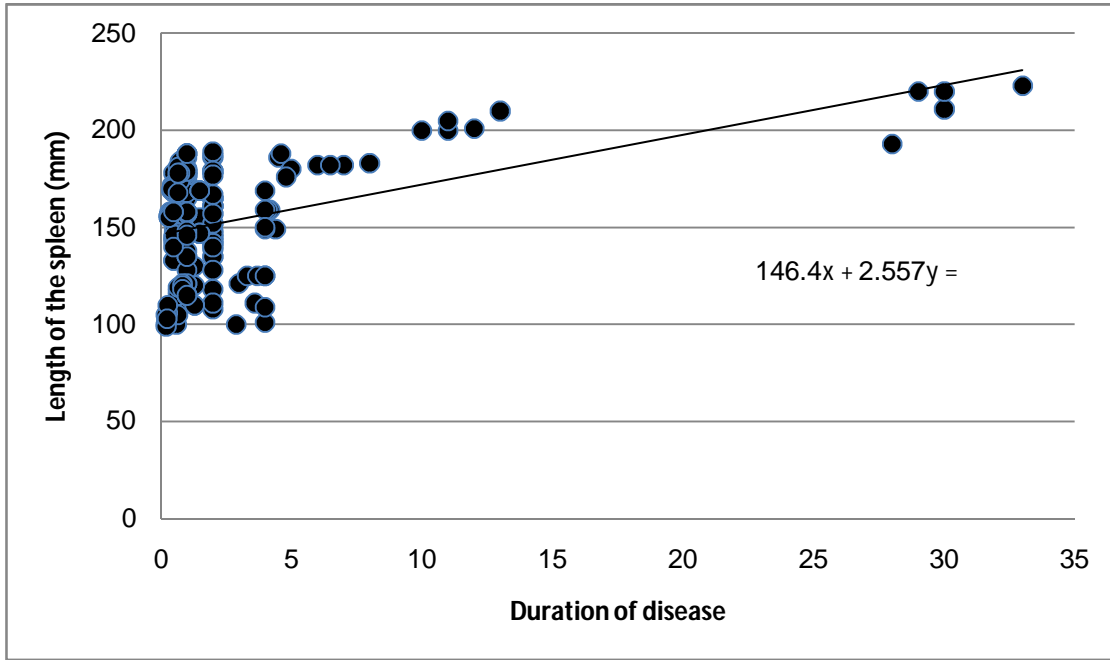


(A)

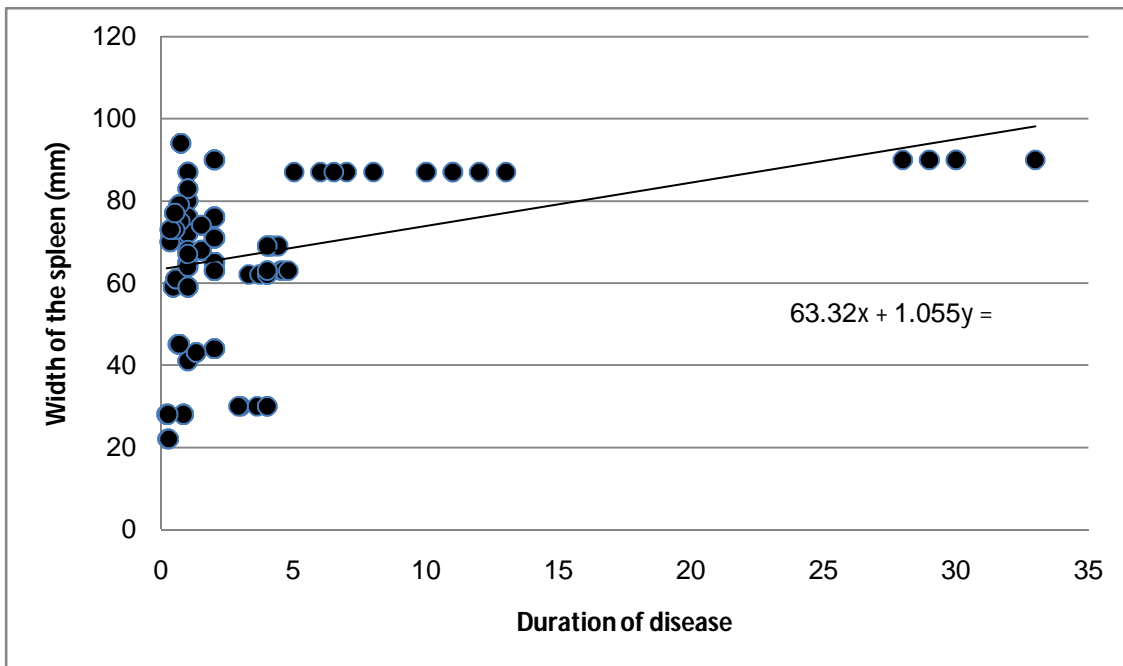


(B)

Figure 4-6 scatter plot shows a liner direct relationship of disease duration with, (A) Lt kidney length and (B) Lt kidney width.

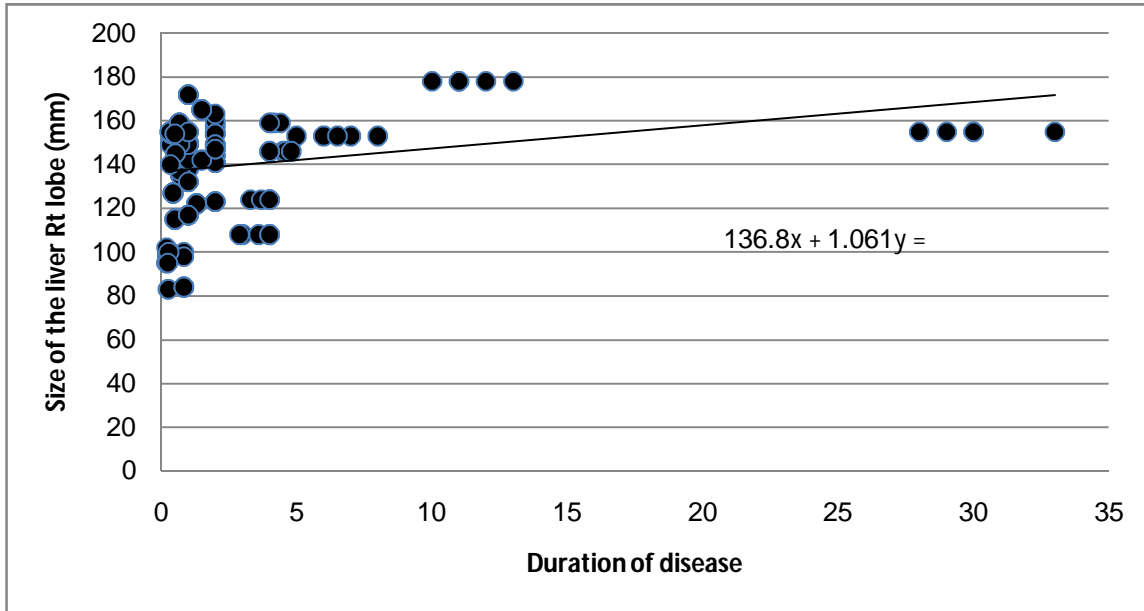


(A)

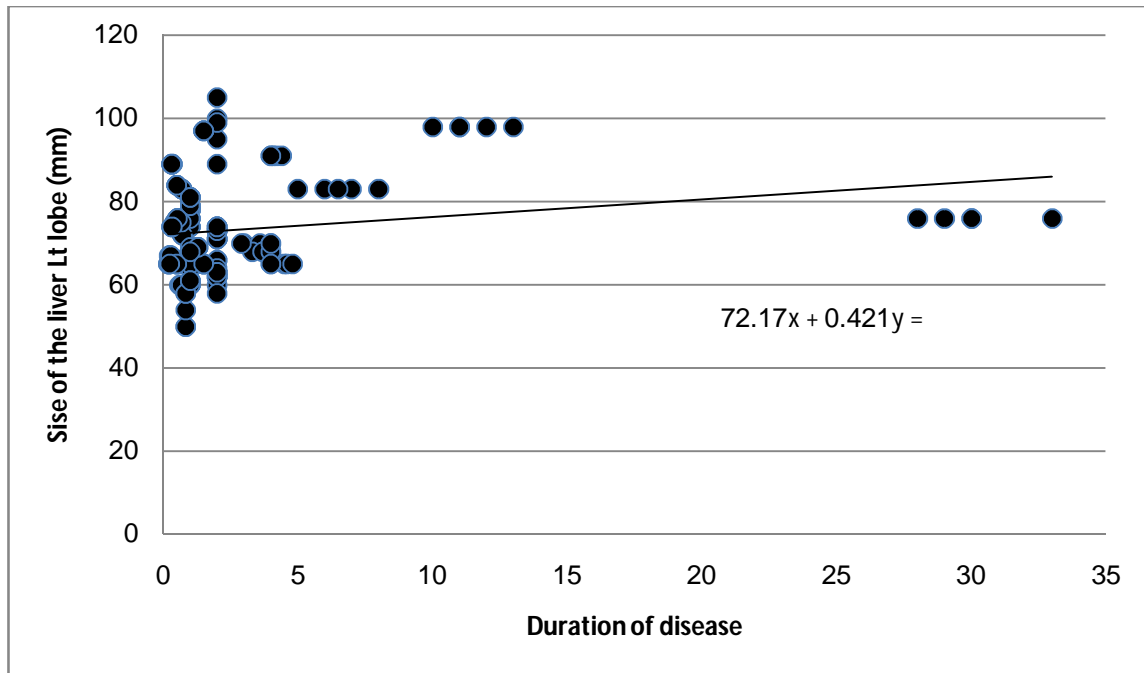


(B)

Figure 4-7 scatter plot shows a liner direct relationship of disease duration with, (A) spleen length and (B) spleen width.



(A)



(B)

Figure 4-8 scatter plot shows a linear direct relationship of disease duration with, (A) size of the liver Rt lobe and (B) size of the liver Lt lobe.

Chapter five

Discussion, conclusion and Recommendation

5.1. Discussion

This study was intended to characterize the visceral leishmaniasis disease in Gadarif state population using ultrasound scan. Ultrasound was done to measure visceral organs dimension correlated to laboratory test for TWB, HP, which included, size of the liver Rt and Lt lobe, gall bladder wall thickness, GB lumen width, aortic caliber, aortic thickness, CBD Caliber, CBD Thick mass, right kidney length, right kidney width, left kidney length, left kidney width, SPL, SPW, caliber of the portal vein, PV wall thickness, Caliber and wall thickness of IVC, which having mean \pm SD of 16.42 \pm 9.809, 2.3400 \pm 4.67663, 3980.77 \pm 1966.386, 7.1474 \pm 2.17084, 132.60 \pm 33.248 mm, 70.88 \pm 17.798mm, 2.1628 \pm .96505mm, 13.16 \pm 3.235mm, 13.35 \pm 3.018mm, 2.02 \pm .630, 3.47 \pm .874, .0156 \pm .00846mm, 97.77 \pm 17.849mm, 46.16 \pm 11.603mm, 95.44 \pm 24.750mm, 45.63 \pm 8.981mm, 143.63 \pm 30.134mm, 17.457 \pm 65.81mm, 9.88 \pm 2.649, 2.58 \pm 3.044, 11.81 \pm 3.372, and 2.21 \pm .796 mm respectively. where the result revealed that: Significant two tailed t-test was performed for all abdominal measurement done by using ultrasound where confidence level equal to 95% and $p < 0.05$ is consider as significant associations for Size Rt lobe, size of left liver lobe, GB Wall thickness, GB lumen wall, Aorta Caliber, Aorta thickness, CBD Caliber, CBD Thick mass, Rt

Length kidney, Rt kidney width, left kidney length and width, SPL Length and width, Caliber Portal vein, Thickness Portal vein, Caliber IVC, and Thickness IVC, where a significant difference noted for all these measure for patient with visceral Leishmaniasis except for GB wall thickness ($p=0.221$), CBD caliber ($p=0.130$), and for portal vein thickness, these result indicate that this type of disease and infection strongly affect the measurement of abdominal organs and blood vessels including the portal system and biliary tree also. This study revealed that male were more frequently affected by this type of disease where more than 140 patient come with VL represented about 65.1% of the data collected and female accounted for 34.9% (75 patient) from total study population. The frequency distribution of common region affected by VL for 215 patient where the main region where the study was conducted is Gadarif state; revealed that most area affected by this type of disease. The disease is found everywhere in the world except Australia and Antarctica. However, Over 90 percent of visceral cases occur in India, Bangladesh, South Sudan, Sudan, Brazil, Ethiopia. As previously stated Sudan is one of the common area affected by this disease, inside this country Gadarif state where more frequently affected by KAL-ZAR (second name of VL), this study intended to measure the distribution among the state, south Gadarif where mostly affected, followed by west and east of the state (62.8%, 20.9% and 9.3%) respectively as in table (4.4).

According to the [World Health Organization \(WHO\)](#), poverty is a determining factor for the disease. Leishmaniasis often occurs in areas where the following conditions are common poverty, malnutrition, famine, illiteracy, large migrations caused by urbanization, emergency situations or environmental changes. In this study signs and symptoms were assessed which are distended bladder, vomiting, nasal bleeding, and swelling as (85, 75, 40, 15) frequency and (39.5%, 34.9%, 18.6%, and 7.0%) respectively as in table (4.5), Kim 2015 who stated that symptoms often don't appear for months after the bite. Most cases are apparent two to six months after infection. Symptoms include weight loss, weakness, cough, fever that lasts for weeks or months, enlarged spleen, enlarged liver, and decreased production of red blood cells (RBCs), bleeding, other infections, night sweats, thinning hair, scaly skin and dark, ashen skin. Patient symptoms as in this research most patient come by loss of appetite in 140 patient (65.1%) from total study population. Table (4.6) This study revealed that 37.2% (80 patient) having positive family history and the rest with no history of this disease accounted for 62.8% (135 patient) this indicate that the VL tend to have close relation with family history. Table (4.7).

Lower liver corner can indicate the presence and absence of liver enlargement (hepatomegaly) this study showed that more than 119 patient having rounded liver. VL affects many organs in abdomen including liver and other organ. US having a major role in assessing this type of disease and even management criteria. Liver echogenicity was grouped in three different classes as iso, hypo

and hyperchoenic appearance in US in which having frequency of (60-27.9%, 100-46.5% and 25.6% respectively as in table (4.7) this indicate that the majority of the patient come with hyper echogenecity and fatty status with homogenous echo texture in about 180- 83.7% patient as in table (4.8). Some times this type of disease associated with presence of mass or cystic changes in 4.7% and 2.3% respectively, which indicate that no clear association with this pathologic changes and VL. as in table (4.9), (32.6%) of having fatty liver with irregular liver texture in 14% of patient. Focal liver appearance found in 2.3% of data. CBD was evaluated and abnormal shape noted to be in 2.3% with rest normal data, kidney echogenecity assessed in order to detect the changes associated VL, most of the patient come with hyper echoic, homogenous echogenecity as in 53.5%, 60.5% and 67.4%, 62.8% for right and left kidney respectively. While no mass or cystic changes affect the kidney except for to have cyst in 11.6% while irregular shape noted in more than 20% of both kidneys. Ultrasound of the spleen showed homogenous echo texture in 53.5% of the patient and 51.2% was hyper echoic spleen texture. Independent sample t-test was performed according the corner of the liver whatever it can be triangular or rounded one for all study variably table (4.25) showing the difference in the these parameters in which the all variables showed significant variation among the liver corners at p value <0.05 , (CL=95%) except for GB wall thickness, CBD caliber and thick mass, and portal vein thickness showed no significant difference in which indicate that the effect in these organ might be not big for this type of disease, moreover the a linear equations was develop to predict the measurement of these variability.

5-2. Conclusion

Leishmaniasis is a parasitic disease caused by the Leishmania parasite. This parasite typically lives in infected sand flies. Patients can contract leishmaniasis from a bite from an infected sand fly. This study was intended to assess and evaluate the visceral leishmaniasis in Gadarif state using abdominal ultrasound scan in order to assess the feature changes in abdominal viscera due

to this parasitic infection, A retrospective study was conducted among 215 patients (male = 140 and female = 75), coming from kala-azar endemic areas (areas from where VL is regularly reported Gadarif state) with fever of more than 15 days and not responding to anti malarial and antibiotics during the period June 2013 to February 2017 at Omdurman Tropical Diseases Teaching Hospital, Gadarif state hospitals and centers in Sudan. The result showed the commonest ultrasound findings in VL participants were hepatomegaly and splenomegaly. While this study reveals that the most affected gender from this population were male (65.1%), the majority from south Gadarif state region accounted for (62.8%) where the most people experience distended bladder and vomiting in (39.5%) and (34.9%) respectively, lower corner of the liver appear to be rounded in (55.3%) which indicated the persistent hepatomegaly with hyperechogenic (46.5%) and homogenous (83.7%) liver texture. Same texture noted for spleen and both kidneys. Other complications such as focal liver lesions (4.7%) and dilated portal vein (2.3%) were detected. Conclusion: Ultrasound scanning presents an effective role in VL, because of its ability to detect the consequences of this disease in various abdominal organs such as liver and spleen as earlier, which in turn allowing the possibility to treat these complications and prevents the deterioration of a patient's health status.

5.3. Recommendations

- Ultrasound having a greater role in assessing such disease so this study should be carried in whole Sudan in order to assess the disease relative to its incidence and mortality in different regions
- Applications of blood flow indices to measure the changes in abdominal blood vessels e.g. hepatic and portal veins, splenic and abdominal aorta.
- The size of the vessels and organ should be correlated with duration of the disease.

References

Alvar J, Aparicio P, Aseffa A, et al. The relationship between leishmaniasis and AIDS: The second 10 years. *Clin Microbiol Rev.* 2008.

Alvar J, Cañabate C, Gutiérrez-Solar B, et al. Leishmania and human immunodeficiency virus coinfection: The first 10 years. *Clin Microbiol Rev.* 1997.

Alvar J, Velez ID, Burn C, et al. The WHO Leishmaniasis control team. Leishmaniasis worldwide and global estimates of its incidence. *PLoS One.* 2012.

Baba CS, Makharia GK, Mathur P, et al. Chronic diarrhea and malabsorption caused by Leishmaniadonovani. *Indian J Gastroenterol* 2006.

Bekaert ED, Dole E, Dubois DY, et al. Alterations in lipoprotein density classes in infantile visceral leishmaniasis: presence of apolipoproteins SAA. *Eur J Clin Invest.*1992.

Bekaert ED, Kallel R, Bouma M-E, et al. M. Plasma lipoproteins in infantile visceral leishmaniasis: deficiency of apolipoproteins A-I and A-II. *Clin Chim Acta.* 1989.

Bertoli A, Greco AV, Caputo S, et al. Visceral leishmaniasis presenting with hypertriglyceridaemia. *Lancet.* 1982.

Catalano O, Sandomenico F, Vallone P, et al. Contrast-enhanced sonography of the spleen. *Semin Ultrasound CT MR* 2006.

Collin S, Davidson R, Ritmeijer K, et al. Conflict and kala-azar: determination of adverse outcomes of kala-azar among patients in southern Sudan. *Clin Infect Dis.* 2004.

Das P, Samuels S, Desjeux P, et al. Annual incidence of visceral leishmaniasis in an endemic area of Bihar, India. *Trop Med Int Health.* 2010.

From Lamb PM, Lund A, Kanagasabay RR, et al. Spleen size: how well do linear ultrasound measurements correlate with three-dimensional CT volume assessments? *Br J Radiol*2002.

Gorg C. The forgotten organ: contrast-enhanced sonography of the spleen. *Eur J Radiol* 2007.

Gosink BB, Leymaster CE. Ultrasonic determination of hepatomegaly. *J Clin Ultrasound* 1981.

Jawhar NM. Visceral leishmaniasis with an unusual presentation in an HIV positive patient. *Sultan Qaboos Univ Med J*. 2011.

Kala-azar or Visceral Leishmaniasis. National Vector Borne Disease Control Programme, Ministry of Health and Family Welfare, Govt. of India. [2015](#).

Kallel R, Bekaert ED, Dubois DY, et al. Acute phase proteins and plasma lipoproteins during antimony treatment in infantile visceral leishmaniasis, *Clin Physiol Biochem*. 1993.

Lafortune M, Madore F, Patriquin H, Breton G. Segmental anatomy of the liver, a sonographic approach to the Couinaud nomenclature. *Radiology* 1991.

Lal CS, Kumar A, Kumar S, et al. Hypocholesterolemia and increased triglyceride in pediatric visceral leishmaniasis. *Clin Chim Acta*. 2007.

Leishmaniasis and HIV coinfection. 2015.

Liberopoulos E, Alexandridis G, Bairaktari E, et al. Severe hypocholesterolemia with reduced lipoprotein (a) in a patient with visceral leishmaniasis. *Ann Clin Lab Sci*. 2002.

Lukes J, Schorian G, Dujardin JC, et al. Evolutionary and Geographical history of the *Leishmania donovani* complex with a revision of current taxonomy. *Proc Natl Acad Sci USA*. 2011.

Malmendier CL, Lontie JF, Dubois DY. Mechanisms of hypocholesterolemia. *Adv Exp Med Biol*. 1991.

Mebazaa A, Kallel R, Boussen H, et al. Perturbations des lipides lipoproteins seriques au cours du kala-azar. *Tunis Med*. 1984.

Muniaraj M, Kumar S, Lal CS, et al. Biochemical profile of Milk of Buffalo (*Bubalus bubalis*), Cow (*Bos taurus*) and Goat (*Capra hircus*): potential

candidates for supporting the growth of *Leishmania donovani* promastigotes in culture medium as alternative to fetal bovine serum (FBS). *J Buffalo Sci.* 2012.

Niederau C, Sonnenberg A, Muller JE, et al. Sonographic measurements of the normal liver, spleen, pancreas, and portal vein. *Radiology* 1983.

Pintado V, Martín-Rabadán, Rivera ML, et al. Visceral leishmaniasis in human immunodeficiency virus (HIV)-infected and non-HIV infected patients. A comparative study. *Medicine (Baltimore)* 2001.

Pintado V, Martín-Rabadán, Rivera ML, et al. Visceral leishmaniasis in human immunodeficiency virus (HIV)-infected and non-HIV infected patients. A comparative study. *Medicine (Baltimore)* 2001.

Rosenberg HK, Markowitz RI, Kolberg H, et al. Normal splenic size in infants and children: sonographic measurements. *AJR Am J Roentgenol* 1991.

Rosenthal E, Marty P, Del Giudice P, et al. HIV and Leishmaniacoinfection: A review of 91 cases with focus on atypical locations of leishmania. *Clin Infec Dis* 2000.

Van Griensven J, Diro E. Visceral leishmaniasis. *Infect Dis Clin Clin North Am.* 2012, 2007.

WHO. Control of the Leishmaniasis. Geneva: WHO (Technil Report Series 949), 2010.

Yetter EM, Acosta KB, Olson MC, Blundell K. Estimating splenic volume, sonographic measurements correlated with helical CT determination. *AJR Am J Roentgenol* 2003.

Appendix (A) Data sheet

Cases #	Age	Gender	Region	Disease duration	Signs	Symptoms	Family history	Laboratory tests				Lower corner of liver
								Liver F	Kidney F	TWB	Hp%	

Liver								Gall bladder				Aorta			C	
o	Texture	Size (Rt lobe)	size (Lt lobe)	Mas s	Cyst	Fatty stat	Shap e	shap e	Wall thick	Lume n L	W	shap e	Caliber	thick ness	shap e	C

Case s #	kidney	Spleen	Portal vein

	echog y		Textur e		position		size		Mass		Cyst		Shap e	siz e	textur e	ech o	Shap e	Caliber	Thi ne
	Rt	Lt	R t	Lt	Rt	Lt	Rt	Lt	Rt	Lt	Rt	Lt							

Appendix (B)
Ultrasound images



Image (B1) liver for patient with visceral leishmaniasis

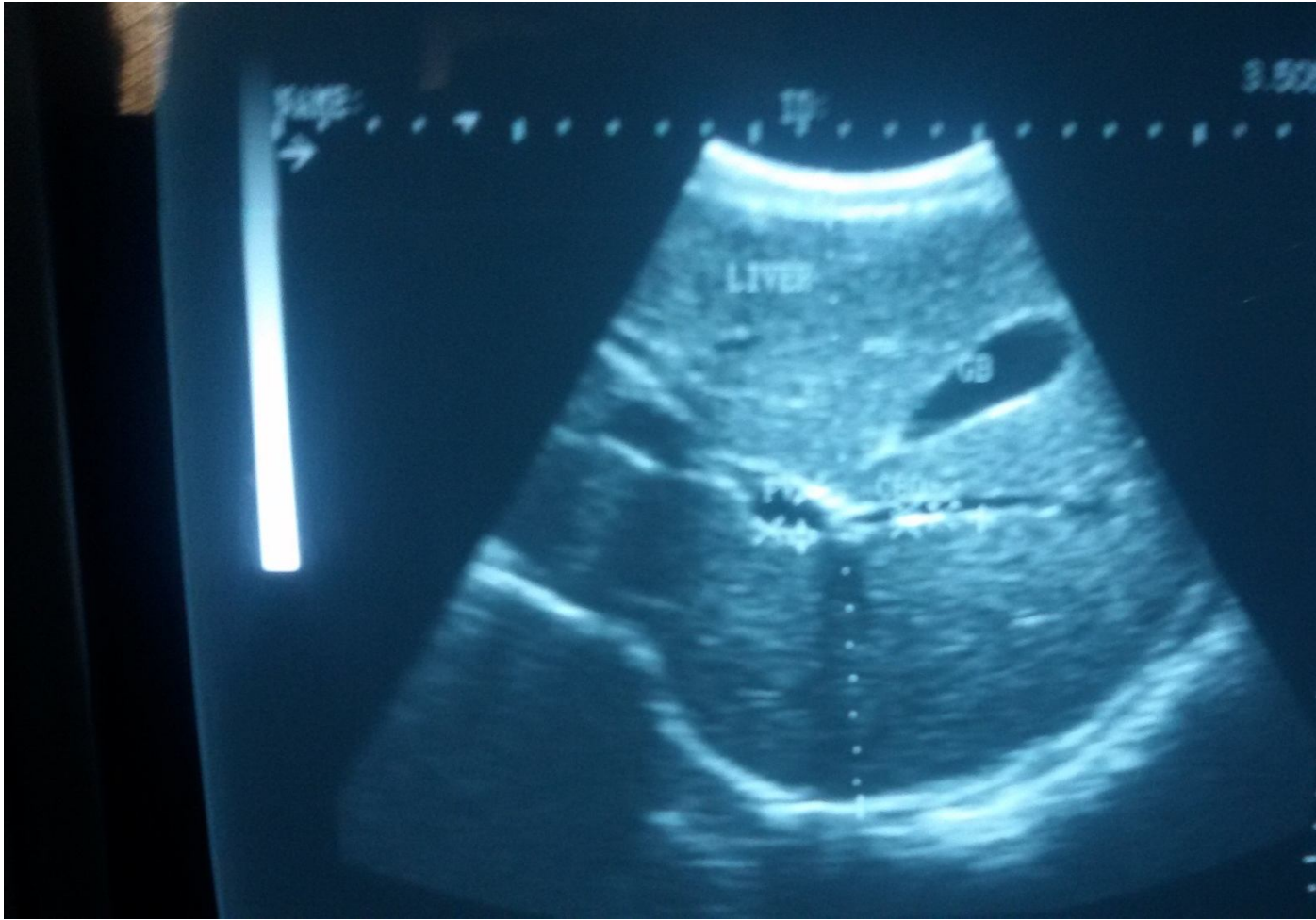


Image (B2) liver with GB for patient with visceral leishmaniasis

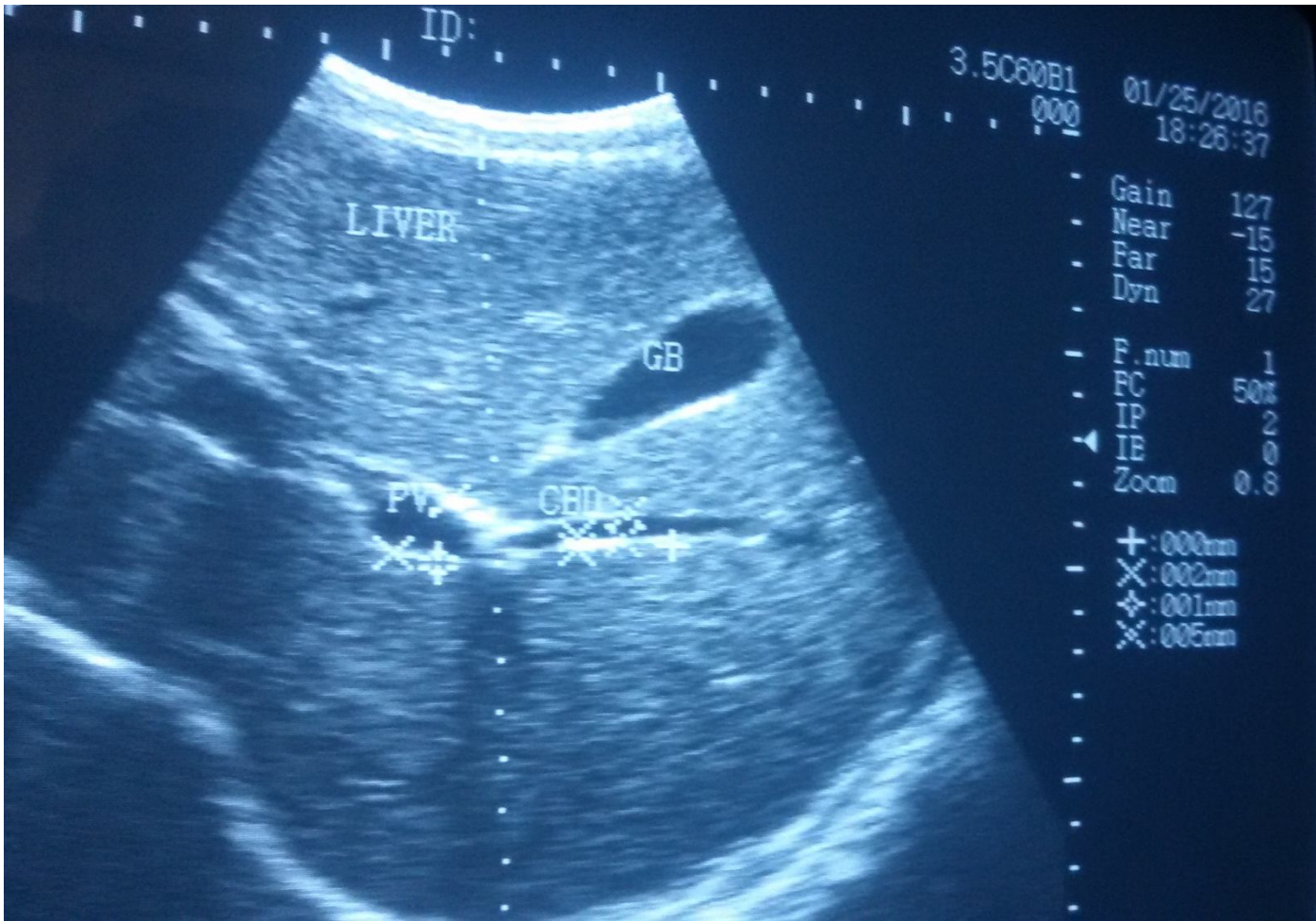


Image (B3) liver with GB and portal vein for patient with visceral leishmaniasis

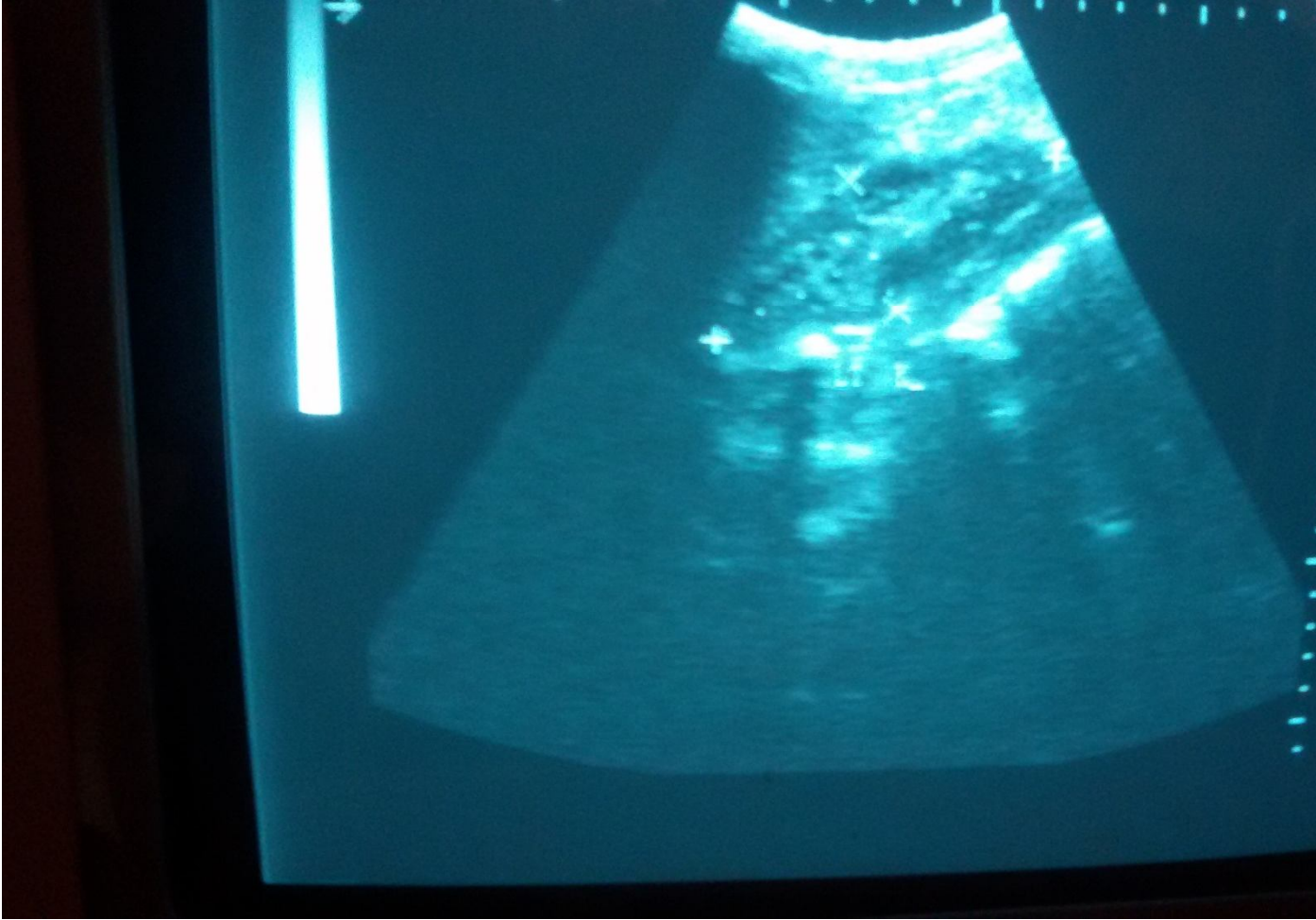


Image (B4 - A) kidney for patient with visceral leishmaniasis

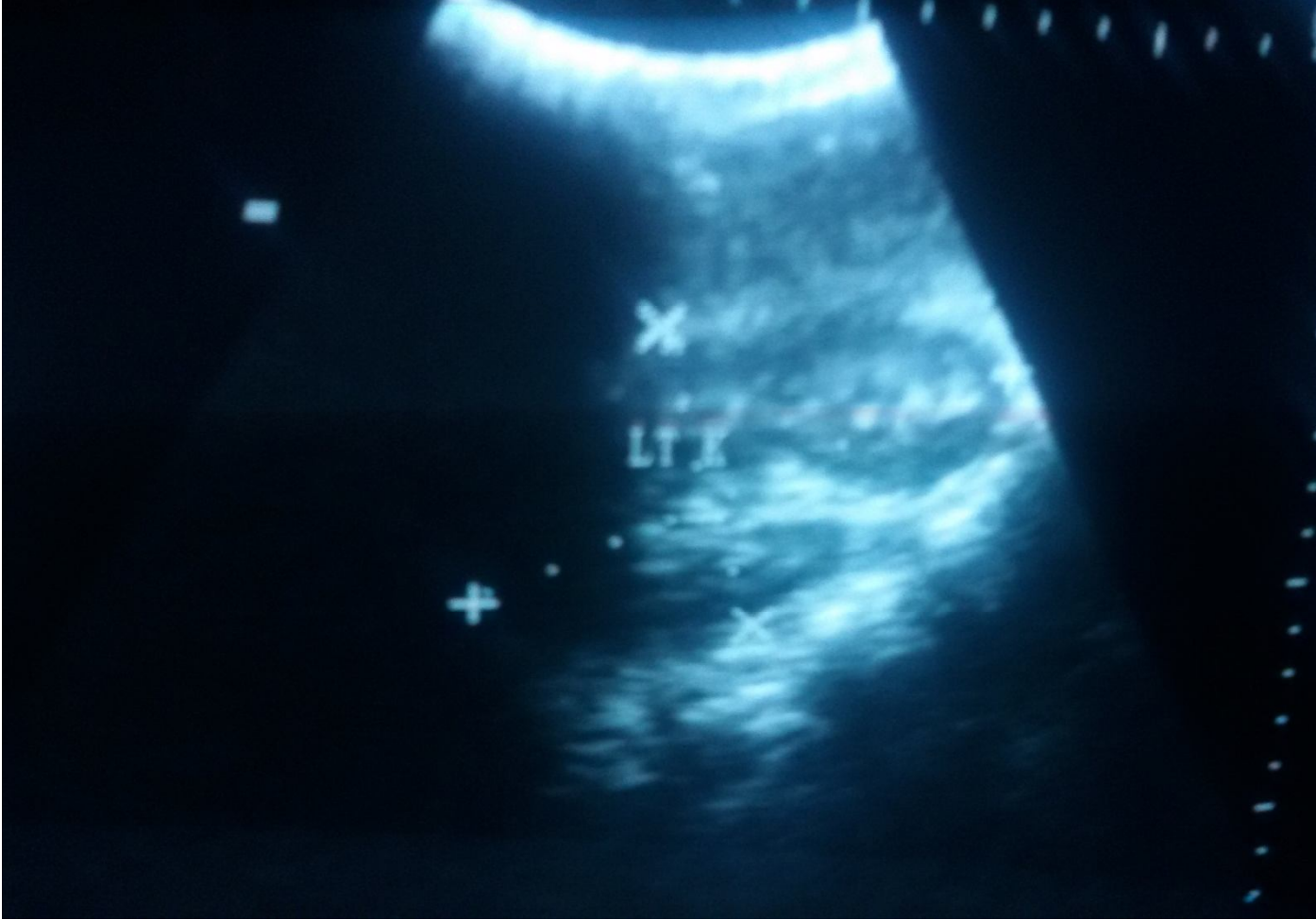


Image (B4 – B) kidney for patient with visceral leishmaniasis

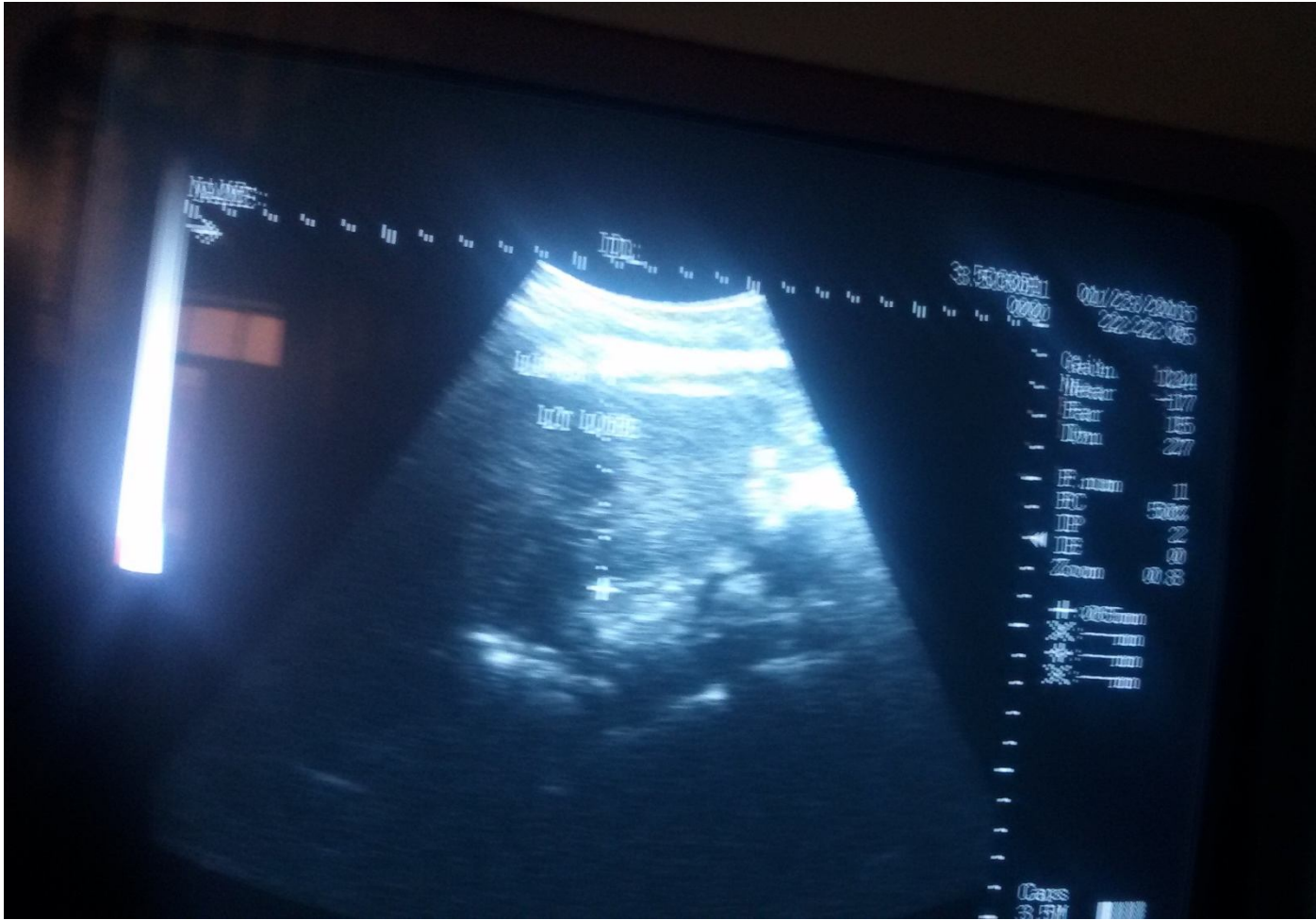


Image (B5) spleen for patient with visceral leishmaniasis

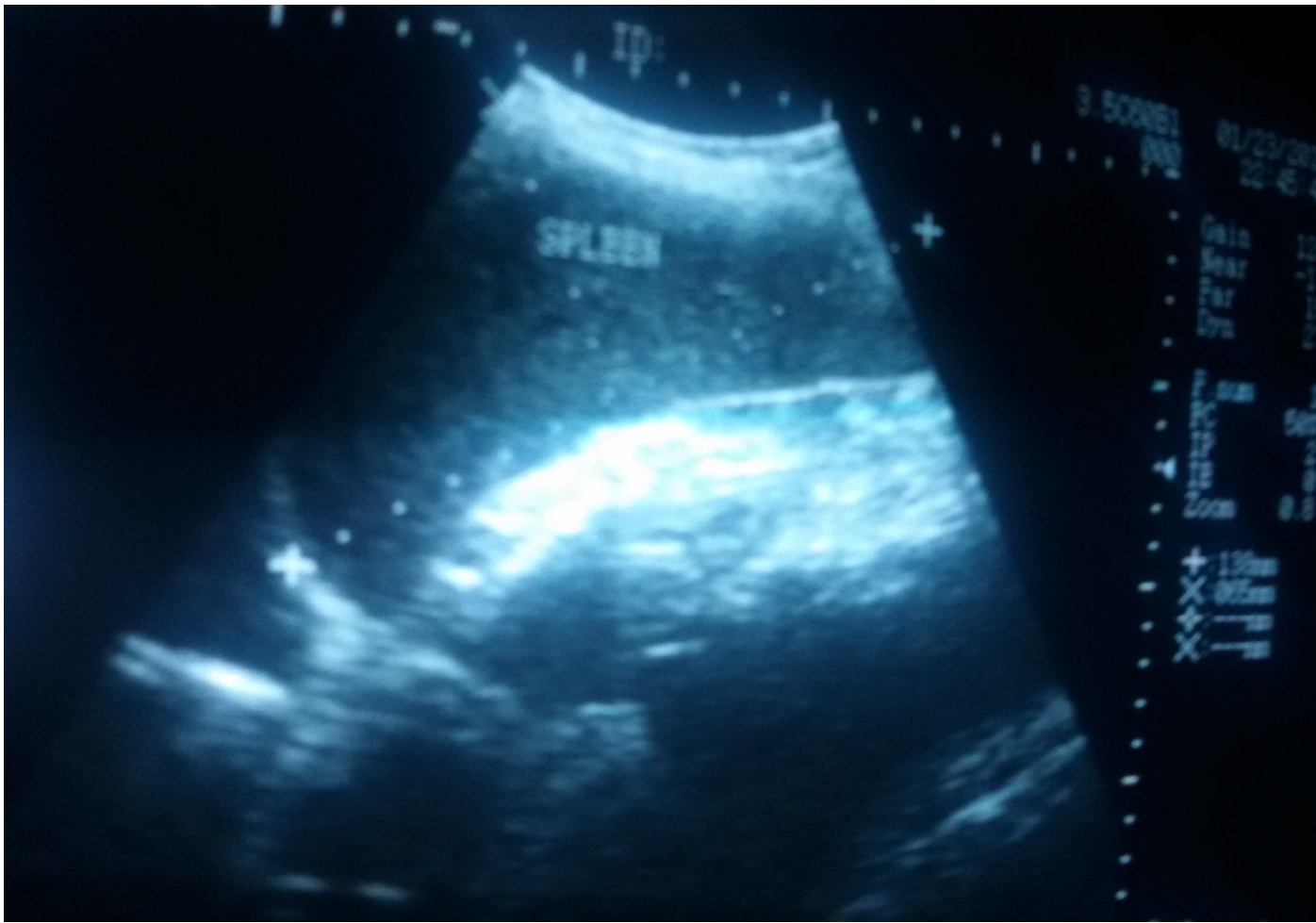


Image (B6) spleen for patient with visceral leishmaniasis