



## From the Editor

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In this issue of the journal we are starting a series on evidence based medicine skills through four review papers on critical reading, thinking, and reviewing. These papers will appear subsequently in the coming issues of the journal.

The series is coming from the National Guard in Riyadh. The first paper elaborated on critical reading of an article about causation and harm. The authors stressed that by reading this paper, learners will be able to: Describe the cause and effect relationship; and Understand and apply Bradford Hill's criteria for establishing causality in the study of health problems

A descriptive cross-sectional was done on a non-probability convenient sample of 170 patients from Iraq attempted to estimate the percentage of Visceral Leishmaniasis in patients attending Central Child Teaching and Child Welfare hospitals in Baghdad during 2012-2013. In addition to assessing the validity of dipstick rapid test in the diagnosis of Visceral Leishmaniasis. The study was done on 170 patients. The study showed that almost all of the clinically suspected VL patients who were admitted to the hospitals had enlarged spleen, enlarged liver, prolonged fever, and anemia. In comparison with the result of bone marrow examination, the Validity of dipstick rapid test for diagnosis of Visceral Leishmaniasis was estimated by calculating the sensitivity of the test which was 80.35% and the specificity of it which was 86.84%. The accuracy of the dipstick rapid test in the diagnosis

of Visceral Leishmaniasis was equal to 84.7% while the Positive Predictive Value (P.P.V.) of the test was equal to 75% and its negative Predictive Value (P.P.V.) was equal to 90%. The authors concluded that Visceral Leishmaniasis is a significant child health problem as the percentage of cases diagnosed by bone marrow examination was 33% of the total number of patients included in the study. In addition that Dipstick rapid test which is the simplest, cheapest, and rapid test is proved to be a valid test in the diagnosis of Visceral Leishmaniasis as the sensitivity of this test was 80.35% and its specificity was 86.84%.

A paper from Iraq compared the sensitivity and specificity of FNA cytology, cell block histopathology and NCB in a consecutive series of 252 patients undergoing image guided sampling of abdominal masses in Welfare hospital in Erbil city during the period march 2010 till July 2015. The specificity & sensitivity of combined FNAC and cell block histopathology were 97.5% and 97% respectively while that for NCB were 91% and 75%. The authors concluded that combined FNAC, Cell block examination for abdominal masses are better than NCB.

A Laboratory study on rats look at the Hypolipidemic efficacy of Trigonella Foeniculum seeds in comparison with Rosuvastatin and Fenofibrate in hyperlipidemic rats. Forty two rats were divided into two groups. After six weeks of therapy, TGS of both concentrations (0.50% and 0.75% w/w) significantly reduced serum low density lipoprotein cholesterol (LDL-C), total cholesterol (TC) when compared with hyperlipidemic rats. The authors concluded that TGS has similar efficacy of Rosuvastatin and Fenofibrate in reducing TC. Whereas, TGS was non-significantly more effective than Rosuvastatin and Fenofibrate in changing serum HDL-C and LDL-C.

A paper from Bagdad looked at the Effect of sitagliptin on glycemic control in patients with type 2 diabetes. Sitagliptin is orally active selective DPP-4 inhibitor, its use results in 3-fold increase in postprandial levels of active GLP-1

compared with placebo. Eleven type 2 diabetic patients were initially screened for eligibility for the study. For all the patient a detailed history was taken followed by physical examination and full laboratory workup including fasting plasma glucose (FPG), postprandial glucose (PPG) 2 hours after breakfast, lunch, dinner with HbA1c. The use of sitagliptin as monotherapy or with metformin and or sulphonyluria was found to be fruitful in reducing FPG, PPG, and HbA1c with total absence of hypoglycemic episodes in those who are complaining from such sulphonyluria induced hypoglycemic episodes after substituting sulphonyluria with sitagliptin. The authors concluded that Sitagliptin use was found to be useful in reducing plasma glucose and HbA1c thus approaching the recommended targets, this was in harmony with Scott et al. (18)

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# Validity of Dipstick Rapid Test in the Diagnosis of Visceral Leishmaniasis in Two Hospitals in Baghdad City during two years (2012 -2013)

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## Abstract

**Background :** Visceral Leishmaniasis , commonly known as Kala Azar, is the most severe form of Leishmaniasis. It affects poor communities and has significant health, social and economic impact. Without treatment, it is fatal in almost all cases. The procedure of the gold standard tests for the diagnosis of Visceral Leishmaniasis, is cumbersome, time consuming, technically demanding, risky, and very difficult to apply in field conditions or remote places/Primary Health Care Centers. The dipstick rapid test based on the detection of antibodies to a recombinant antigen is used worldwide. The test requires only a drop of finger prick blood or serum, and the result can be read within 15 minutes.

## Objectives :

- 1- To estimate the percentage of Visceral Leishmaniasis in patients attending Central Child Teaching and Child Welfare hospitals in Baghdad during 2012-2013.
- 2- To assess the statistical relationship between the occurrence of Visceral Leishmaniasis and certain sociodemographic variables ( Gender and Age ) of studied patients.
- 3- To assess the validity of dipstick rapid test in the diagnosis of Visceral Leishmaniasis.

**Patients and Methods:** A descriptive cross-sectional study was done on a non-probability convenient sample of 170 patients to detect the validity of dipstick rapid test in the diagnosis of Visceral Leishmaniasis in comparison with bone marrow examination test (Gold standard test) among patients who attended two main specialized pediatric hospitals in Baghdad city, namely Central Child Teaching and Child Welfare Teaching hospitals during two years 2012 and 2013.

**Results:** The study was done on 170 patients and it was found that 60.58% of them were male, while only 39.42% of them were female. 60% of the males and 40% of the females were diagnosed as VL (by bone marrow examination). It was found that 65.29% of total number of surveyed patients were below 5 years and the disease appeared to affect mainly young children; 78.57% of patients with VL (as diagnosed by bone marrow examination) were below 5 years. The majority of the studied cases (75%) were found below the age of two years. The highest number of cases was recorded in Winter and Spring. The geographical distribution of VL showed that the highest percentage was from the central region of Iraq (26.78% from Baghdad governorate , 26.78% from Diyala governorate , 17.85% from Babylon governorate). The study showed that almost all of the clinically suspected VL patients who were admitted to the hospitals had enlarged spleen, enlarged liver, prolonged fever, and anemia. No statistically significant relation was found between the disease and the gender ( $P>0.05$ ) while there was a statistically significant relation between the disease and the age of patients ( $P<0.05$ ).

The percentage of the percentage of cases of Visceral Leishmaniasis diagnosed by bone marrow examination was 33%, and the percentage of the patients with negative bone marrow examination was 67%, while the percentage of positive dipstick rapid test results was 35%, and the percentage of the patients with negative dipstick rapid test was 65%.

In comparison with the result of bone marrow examination, the Validity of dipstick rapid test for diagnosis of Visceral Leishmaniasis was estimated by calculating the sensitivity of the test which was 80.35% and the specificity of it which was 86.84% .

The accuracy of the dipstick rapid test in the diagnosis of Visceral Leishmaniasis was equal to 84.7% while the Positive Predictive Value (P.P.V.) of the test was equal to 75% and its negative Predictive Value (P.P.V.) was equal to 90%.

### Conclusions:

- 1- Visceral Leishmaniasis is a significant child health problem as the percentage of cases diagnosed by bone marrow examination was 33% of the total number of patients included in the study.
- 2- Dipstick rapid test which is the simplest, cheapest, and most rapid test is proved to be a valid test in the diagnosis of Visceral Leishmaniasis as the sensitivity of this test was 80.35% and its specificity was 86.84%.

**Key words:** Visceral Leishmaniasis (VL), dipstick rapid test.

## Introduction

Visceral Leishmaniasis ( VL), commonly known as Kala Azar, the most severe form of Leishmaniasis, is a parasitic disease transmitted by the bite of infected female sandflies [1]. Months after this initial infection the disease can progress into a more severe form, called Visceral Leishmaniasis or Kala Azar [2]. It is characterized by prolonged high fever, substantial weight loss, swelling of the spleen and liver, and anaemia. It affects poor communities causing significant health, social and economic impact. Without treatment, it is fatal in almost all cases [1]. The disease is the second largest parasitic killer in the world, only malaria is more deadly [2] . In Iraq, VL is endemic and usually detected in infants and children [3]. It is usually caused by *Leishmania Donovanii* parasite and the sand fly vector

is the *Phlebotomus Alexandri* type [4] . Many of the cases are asymptomatic in Iraq [5] . The disease was known to be endemic since 1954 after reports from Baghdad [6], and it has been identified in Iraq for more than eight decades causing a serious public health problem with a high risk of morbidity, mortality and economical costs [7]. During the last 10 years, there was a marked increase in visceral leishmaniasis cases in southern Governorates (Baghdad areas, Myssan , Thi-qar, Al-Qadisiya, and Mothana) [8] . In Iraq it represents one of the serious public health problems and it is more in south and middle and lowest in the north, with highest frequency in Winter followed by Spring and lower in Summer and Autumn. [9-10] For decades, the detection of the parasite has been considered to be the definitive diagnosis of this disease. It requires invasive procedure to find the parasite in the tissue, i.e. spleen, bone

marrow, and lymph-node. Although it is the gold standard for the diagnosis of VL, the procedure is cumbersome, time consuming, technically demanding, risky, and very difficult to apply in field conditions or remote places/Primary Health Centers. To obviate these procedures, various serological tests have been developed, evaluated, and tried and one of these tests was The rapid immunochromatographic test based on the detection of antibodies to a recombinant antigen (rK39) consisting of 39 amino acids conserved in the kinesin region of *L. infantum* is used worldwide. The test requires only a drop of finger prick blood or serum, and the result can be read within 15 minutes [11].

## Objectives

- 1- To estimate the percentage of Visceral Leishmaniasis in patients attending Central Child Teaching hospital and Child Welfare hospital in Baghdad city during two years (2012 -2013).
- 2- To assess the statistical relationship between the occurrence of Visceral Leishmaniasis and certain sociodemographic variables ( Gender and Age ) of studied patients.
- 3- To assess the validity of dipstick rapid test in the diagnosis of Visceral Leishmaniasis.

## Patients and Methods

### 1-Study Design:

A descriptive cross-sectional study to detect the validity of dipstick rapid test in the diagnosis of Visceral Leishmaniasis and compare it with bone marrow examination among patients who attended Central Child Teaching and Child Welfare Teaching hospitals in Baghdad city during two years (2012 -2013).

### 2-Time of the Study:

This study was conducted from the second of December 2013 to the first of June 2014 including 2 days a week and 4 hours a day (9 a.m.- 1 p.m.).

### 3-Place of the Study:

This study was conducted in Central Child Teaching hospital and Child Welfare hospitals in Baghdad city. These two hospitals are referral centers providing specialized health care services to all attendees from all Iraqi governorates. These centers were chosen for convenience, after approving the necessary official agreement from the Ministry of Health to conduct the study.

### 4-Sampling Method:

The sampling design was a non-probability convenient sample.

### 5- Inclusion criteria:

The study population included all patients who attended the selected hospitals for any complaint depending on the following selection criteria:

- a- Both genders with age < 14 years.
- b- Patients who were from Iraq.

c- All the patients with the following laboratory investigations were included :

- a- Complete blood count.
- b- Bone marrow examination..
- c - Rapid dipstick test for Kala Azar.

### 6- Exclusion criteria:

Patients who did not complete all the required laboratory investigations in this study such as patients who did not have dipstick rapid test or bone marrow examination.

### 7-Sample size:

The sample size is convenient of 170 cases.

### 8-Data Collection tool:

The medical records which were surveyed and analyzed were the old medical records of the patients who attended two major secondary Child Health Care Centers in Baghdad city (Central Child teaching and Child Welfare hospitals) during two years (2012 -2013).

A specially designed questionnaire was filled from the medical records of each patient including general information on certain socio-demographic variables (like age, gender and residency), the date of diagnosis, the results of certain investigations and the main presenting clinical features of the Visceral Leishmaniasis.

### 9- Data analysis:

Data of all cases were checked for any error or inconsistency then transferred into a computerized database program; Microsoft Excel software was used.

Descriptive statistics were presented as frequency (number of cases) with percentages for the studied categorical variables. Then the percentage of cases diagnosed by dipstick rapid test and by bone marrow examination were found and compared with each other.

Chi-square test for independence was used to test the significance of association between certain sociodemographic variables and the frequency of cases of Visceral Leishmaniasis.

The validity of dipstick Rapid test was assessed by calculation of the Sensitivity and the Specificity of the test as follows:

1-Sensitivity: is the probability of the diseased people to give (+ve) results or it is the capacity to show positivity of the actually diseased people.

**Sensitivity = [True (+ve) / True (+ve) + False (-ve)]\*100%**

2-Specificity: is the probability of the non-diseased people to give (-ve) results.

**Specificity= [True (- ve) / True (- ve) + False (+ve)]\*100%**

The Accuracy of dipstick Rapid test was also assessed as follows:

Accuracy: is the proportion of true results among all results.

**Accuracy = [True (+ve) + True (-ve) / True (+ve) + False(+ve)+False (-ve)+True (-ve)]\*100%**

Finally the Positive Predictive Value (P.P.V.) and the Negative Predictive Value (N.P.V.) of dipstick Rapid test were also found as follows:

1-Positive Predictive Value (P.P.V.): is the probability of people with (+ve) result to be diseased truly.

**P.P.V.=[True(+ve) / True(+ve)+False(+ve)]\*100%**

2-Negative Predictive Value (N.P.V.): is the probability of people with (-ve ) result to be not diseased truly.

**N.P.V.=[True(-ve) / False(-ve)+True(-ve)]\*100%**

#### 10- Definition of Variables:

1- True (+ve) : Positive cases by dipstick rapid test and positive by bone marrow examination.

2- True (-ve) : Negative cases by dipstick rapid test and negative by bone marrow examination.

3- False (+ve) : Positive cases by dipstick rapid test and negative by bone marrow examination.

4- False(-ve): Negative cases by dipstick rapid test and positive by bone marrow examination.

## Results

The medical records of a total number of 170 patients aged 14 years old or less, were surveyed and the results include the following :

1- Description of the studied sample according to :

a- Gender : It was found that 60.58% of total number of surveyed patients were male, while only 39.42% of them were female. (Table 1).

**Table1: Distribution of the studied sample according to gender**

Gender	No. of patients	
	No.	%
male	103	60.58
female	67	39.42
Total	170	100

b- Age: It was found that 65.29% of total number of surveyed patients were below 5 years. (Table 2).

**Table 2: Distribution of the studied sample according to age**

Age of the patients	No. of patients	
	No.	%
< 5yrs	111	65.29
≥ 5yrs	59	34.71
Total	170	100

2- Description of cases of Visceral Leishmaniasis in the studied sample according to :

a- Seasonal distribution: The highest number of cases was recorded in Winter and Spring , less number of cases in Autumn and no cases were reported in Summer. (Table 3).

**Table 3: Seasonal distribution of Visceral Leishmaniasis in the studied sample**

Month of admission	No. of patients	%
Winter	31	55.36
Spring	22	39.22
Summer	0	0
Autumn	3	5.35
<b>Total</b>	<b>56</b>	<b>100</b>

b- Geographical distribution : showed that the highest percentage was from the central region of Iraq (26.78% from Baghdad governorate, 26.78% from Diyala governorate, 17.85% from Babylon governorate), while the lowest percentage was from Maysan governorate (3.5 %). (Table 4).

**Table 4: Geographical distribution of Visceral Leishmaniasis in the studied sample**

The Governorate	Number of patients with VL diagnosed by Bone Marrow Examination test	Percentage of cases %
Baghdad	15	26.78
Babylon	10	17.85
Diyala	15	26.78
Najaf	4	7.14
Thiqar	5	8.9
Maysan	2	3.5
Al-anbar	5	8.9
<b>Total</b>	<b>56</b>	<b>100</b>

c- Main presenting clinical features : The study showed that almost all of the clinically suspected VL patients who are admitted to the hospitals have enlarged spleen, enlarged liver, prolonged fever, and anemia. (Table 5).

**Table 5: Distribution of Visceral Leishmaniasis in the studied sample according to clinical features of the disease**

Bone marrow examination	Lymphadenopathy		Hepatosplenomegaly		Anemia		Fever	
	No.	%	No.	%	No.	%	No.	%
<b>+ve</b>	<b>2</b>	<b>3.5</b>	<b>53</b>	<b>94.6</b>	<b>53</b>	<b>94.6</b>	<b>56</b>	<b>100</b>

3- The Statistical relationship of occurrence of Visceral Leishmaniasis with certain sociodemographic variables which are Gender and Age of studied patients as follows :

a- The Statistical relationship with the gender of studied patients: 60% of the males and 40% of the females were diagnosed as VL (by bone marrow examination) but no statistically significant relation was found between the disease and the gender ( $P \geq 0.05$ ). (Table 6).



**Table 6: Relation of Visceral Leishmaniasis with gender**

VL as diagnosed by Bone Marrow Examination test	Positive		Negative		Total	
	No.	%	No.	%	No.	%
Male	33	60	70	61.4	103	60.58
Female	23	40	44	38.6	67	39.41
Total	56	100	114	100	170	100

\*P value >0.05

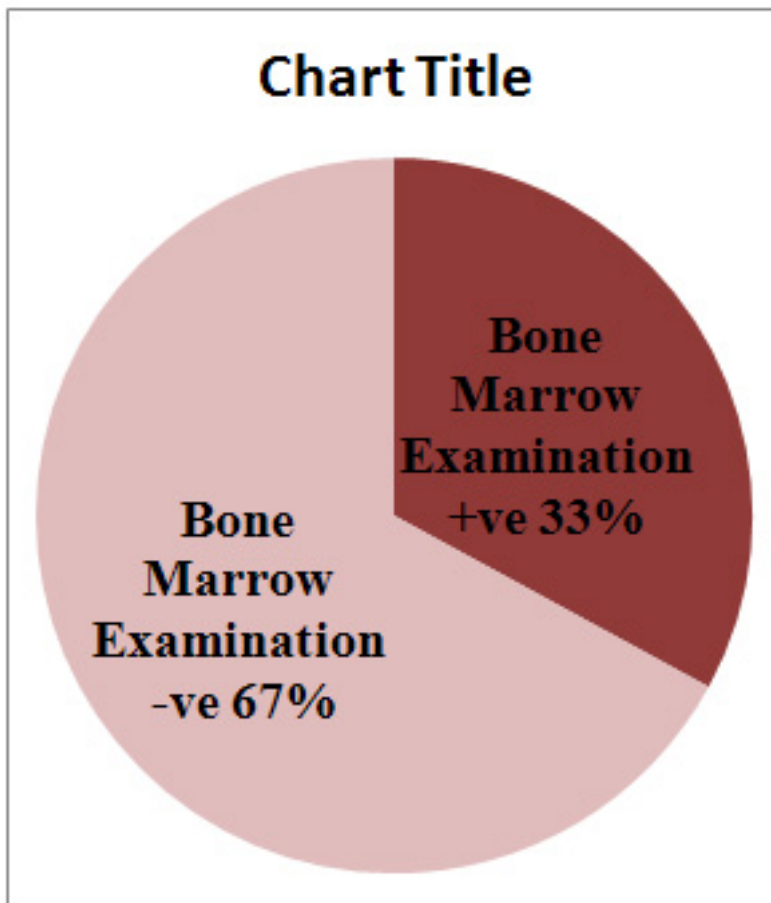
b- The Statistical relationship with age of studied patients: 78.57% of patients with VL ( as diagnosed by bone marrow examination) were below 5 years. There was a statistically significant relation between the disease and the age of patients (P<0.05). (Table 7).

**Table 7: Relation of Visceral Leishmaniasis with age**

Age	Number of cases of VL as diagnosed by Bone marrow examination test				Total	
	Positive		Negative		No.	%
	No.	%	No.	%	No.	%
<5	44	78.57	67	58.77	111	65.29
≥5	12	21.43	47	41.23	59	34.71
Total	56	100	114	100	170	100

\*P value >0.05

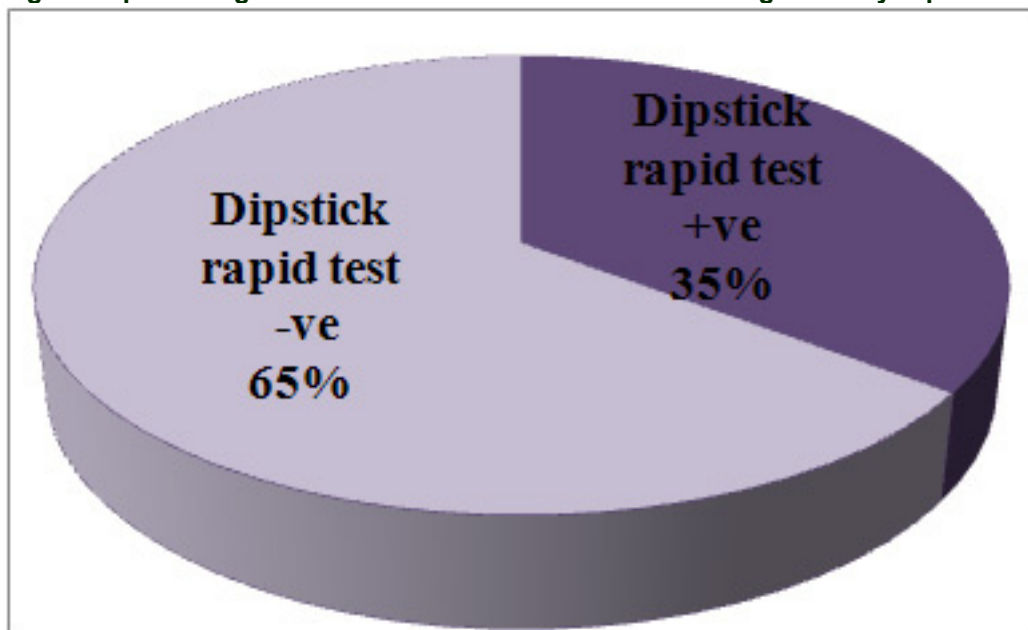
4- The percentage of the cases of Visceral Leishmaniasis diagnosed by bone marrow examination which was 33%, and the percentage of the patients with negative bone marrow examination was 67%. (Figure 1).



**Figure 1: percentage of cases of Visceral Leishmaniasis diagnosed by Bone marrow examination**

5- The percentage of the cases of Visceral Leishmaniasis diagnosed by dipstick rapid test results which was 35%, and the percentage of the patients with negative dipstick rapid test was 65%. ( Figure 2).

**Figure 2: percentage of cases of Visceral Leishmaniasis diagnosed by Dipstick rapid test**



6- Validity of dipstick rapid test for diagnosis of Visceral Leishmaniasis was estimated by calculating the sensitivity of the test which was 80.35% and the specificity of it which was 86.84% in comparison with the result of bone marrow examination. (Table 8).

**Table 8: Validity of dipstick test for diagnosis of Visceral Leishmaniasis was compared with results of Bone marrow examination**

Test		Bone marrow examination (Gold standard)				Total
		Bone marrow examination +ve		Bone marrow examination -ve		
		No.	%	No.	%	
Dipstick rapid Test	Dipstick rapid Test +ve	45	80.36	15	13.15	60
	Dipstick rapid Test -ve	11	19.64	99	86.84	110
	Total	56	100	114	100	170

7-The accuracy of the dipstick rapid test in the diagnosis of Visceral Leishmaniasis was equal to 84.7%. (Table 8).

8- Positive Predictive Value (P.P.V.) the dipstick rapid test was equal to 75% while the negative Predictive Value (P.P.V.) of the test was equal to 90% (Table 8).

## Discussion

Two tests were used in this study, dipstick rapid test and bone marrow aspiration (which is considered as a diagnostic test for diagnosis of Visceral Leishmaniasis)[1]. The present study shows that the majority of the patients were males (60.58%), while females accounted for 39.42%. 60% of the males and 40% of the females were diagnosed as VL (by bone marrow examination), but no statistically significant association was found ( $P>0.05$ ) between the disease and the gender as shown. This result is similar to that of other studies done in Iraq in Hilla[12]( Al-Marzoki ), Babylon[13] ( Al-Muhammadi ), Thiqr[14],[15] (Haidar A. et al), (Khlabus Kh. Raddam ) , Baghdad[16],[17],[18] ( Sukkar F. 1976), (Sukkar F. 1972),(Murad A.M, et al ) where they reported both genders equally affected, and the result is not compatible with that of other studies done in Iraq in Al-Anbar[19] (Zaid R Al-Ani, et al.), Basrah[20],[21]( Yousif S. K, et al),( Zainab H. G, et al), Iran [22]( Soleimanzadeh G, et al.), , India [23] (Kumar R, et al) in which the males were more affected than females.

It was found that 65.29% of the total number of surveyed patients were below 5 years and the disease appears to affect mainly young children as 78.57% of patients with VL (as diagnosed by bone marrow examination) were below 5 years (probably because of low immunity) and the results were in agreement with other Iraqi studies in Babylon[24]( Ahmed M. Al-Mosawy, et al), in Thi-Qar[15] (Khlabus Kh. Raddam ) and Basrah[21] ( Zainab H. G, et al) where they recorded cases down to the age of two months. In Wasit[25]( Qasim Dawood AL-Tammemi) in which the majority of cases of V.L.(52.41%) were between the age of (1-2) years and (32.72%) was < 1 year), Al-Anbar[19] ] (Zaid R Al-Ani, et al). The majority (75%) of the studied cases were found below the age of two years.

The highest number of cases was recorded in Winter and Spring, and this is in agreement with other Iraqi studies[9,10, 16] ( Qusay A et al),( Abid, Baqir K.), ( Sukkar F.), and differs from studies done in THI-QAR [15] (Khlabus Kh. Raddam).

The geographical distribution of VL showed that the highest percentage was from the central region of Iraq (26.78% from Baghdad governorate, 26.78% from Diyala governorate, 17.85% from Babylon governorate), while the lowest percentage was from Maysan governorate (3.5 %). This may be related to the location of the two referral hospitals that were involved in this study as both of them are located in Baghdad governorate (Center of Iraq). The result is in agreement with other Iraqi studies [26,27,28]( Abdulsadah A.Rahi, et al), (Sukker, F. 1983),( Sukker, F. 1985).

This study showed that almost all of the clinically suspected VL patients who were admitted to the hospitals have enlarged spleen, enlarged liver, prolonged fever, and anemia. These results are similar with the results of other studies in different countries, in Sudan[27] (Adler, S. et al.), Nepal[28]( Bern CS, et al), and India[29]( Tilak, N., et al).

In this study, the sensitivity of dipstick rapid test was 80.35% and this result is in agreement with studies done at Mid-Euphrate Region (Al-Qadisiya, Najaf and Karbala)[30] (Hashim Raheem Tarish1, et al.), sensitivity (88.23%), Venezuela [31] 88% ( Delgado O, et al.), but less than that in Wasit[25] ( Qasim Dawood AL-Tammemi, ), where the sensitivity of the rK39 Dipstick test was 90.39%, while in India[32] (Sundar,S.,et al.) and in Nepal[33]( S. N. Jha, et al.), there were the highest sensitivities which were equal to 100%. So in general, sensitivities of dipstick rapid test range from 67 to 100%; patients from India[32] and Nepal[33] show higher sensitivities 100% then Brazil with 90% sensitivity and Venezuela[34] (Silvo, F., et al.) in which the sensitivity is 88%.

The specificity of dipstick rapid test in this study was 86.84%, and this is less than that in the studies done in Baghdad and Wasit[7]( Jawad J, et al.) ; in Wasit[25] (Qasim Dawood AL-Tammemi, ), the specificity was 100%, and in India[32]( Sundar,S.,et al.),[33]( S. N. Jha, et al.), and higher than that in the studies in the Mid-Euphrates Region (Al-Qadisiya, Najaf and Karbala)[30] (Hashim Raheem Tarish1, et al.), in which the specificity is (60%), Sudan[27] (Adler, S. et al).

Regional variation in the results of the dipstick rapid test could be explained by the following explanations :

- 1- Differences in the test accuracy between subspecies of *L. donovani* complex as a result of variation in the recombinant antigen [35].
- 2- Age factor affecting the level of antibody response may explain the regional differences; Indian kala-azar occurs among individuals of all ages while in other parts of the world, such as Iraq and Iran VL is primarily of Mediterranean infantile type [36,37,38].

## Conclusion

- 1- Visceral Leishmaniasis is a significant child health problem as the percentage of cases diagnosed by bone marrow examination was 33% of the total number of patients included in the study.
- 2- Dipstick rapid test which is the simplest, cheapest, and most rapid test is proved to be a valid test in the diagnosis of Visceral Leishmaniasis as the sensitivity of this test was 80.35% and its specificity was 86.84%.

## Recommendations

- 1- Increased public awareness about this dangerous disease by encouraging family education about the prevention and control of VL including the necessity for the complete early diagnosis, treatment and follow up to save the affected children from otherwise fatal disease.
- 2- It is recommended the use of dipstick rapid test in the clinically suspected patients in remote areas where no expert hematologist is present, and to depend on the positive result of the test for diagnosis and treatment of VL, but if the patient has a past history of VL with a positive result of the test, or if the patient presented with negative result then the bone marrow examination is mandatory.

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# Sensitivity and specificity of combined fine needle aspiration cytology and cell block biopsy versus needle core biopsy in the diagnosis of sonographically detected abdominal masses

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## Abstract

**Background:** The management of patients with suspected neoplastic disease involving abdominal sites are dependent on obtaining an accurate tissue diagnosis, usually via percutaneous sampling including Fine needle aspiration cytology (FNAC), cell block histopathology and needle core biopsy (NCB). The aim of this study is to identify which of the above mentioned procedures is better to be performed for patients with abdominal masses.

**Methods:** Therefore, we have compared the sensitivity and specificity of FNA cytology, cell block histopathology and NCB in a consecutive series of 252 patients undergoing image guided sampling of abdominal masses in Welfare hospital in Erbil city during the period March 2010 till July 2015.

**Result:** The specificity and sensitivity of combined FNAC and cell block histopathology were 97.5% and 97% respectively while those for NCB were 91% and 75%.

**Conclusion:** Combined FNAC ,Cell block examination for abdominal masses are better than NCB.

**Key words:** Abdominal masses, Sensitivity and specificity, FNAC, Cell block, NCB.

## Introduction

Patients with abdominal lesions may present with clinically evident tumour masses; at present the increasing use and sensitivity of radiological techniques has led to the identification of relatively small lesions, which require the use of image guidance for reliable targeting and obtaining samples for pathological study to reach the proper diagnosis to manage the patients correctly.

At present, there are two widely used and accepted methods for obtaining diagnostic material, namely fine needle aspiration (FNA) cytology and needle core biopsy (NCB). FNA specimens are usually acquired using 20-25 gauge needles and generally provide a sample for cytological examination, whereas NCB specimens are obtained using larger 14-18 gauge needles and primarily provide a tissue core for histological assessment. Because these are two different procedures and you have to put the needle in twice, we did a comparison study between FNAC and cell block histopathology with cases of needle core biopsy. In theory, each sampling method offers different advantages and limitations. Although both techniques are very safe, FNA is often preferred in sampling deeply placed lesions, sites adjacent to major vessels, or in situations in which needles are to be passed through the bowel wall. Cytological samples can be rapidly stained and examined. What remains from the sample is used for cell block histopathology examination. Cytology by itself is providing immediate assessment of adequacy, and in many cases a provisional diagnosis can be made while the patient remains in the radiology department(1). Furthermore, involvement by pathologists on site optimises clinical correlation and ensures that specimens are optimally handled and that appropriate samples are taken as required for ancillary investigations, such as microbiology or molecular studies. The advantages of NCB include the greater familiarity of histological preparations among some

pathologists, the preservation of tissue architecture, which may be important in the assessment and subtyping of some tumours, and the relative ease with which histochemical and immunohistochemical techniques can be applied to paraffin wax embedded biopsy material.

Therefore, although it might appear that cytological and histological examination would be complementary in the assessment of abdominal lesions, there are conflicting data in the literature regarding the accuracy and usefulness of these techniques. In particular, there are wide variations in the reported diagnostic sensitivities of FNA cytology, cell block biopsy and NCB(2) to the extent that some authors have suggested that core biopsy alone should be used or that FNA is the preferred technique with biopsy limited to cytologically indeterminate cases. These discrepancies may be partly explained by variations in the types of lesion subject to biopsy, and by the approach to cytological examination (such as the use of rapid staining techniques). Relatively few reports have evaluated FNA cytology and core biopsy obtained in combination in the investigation of patients with abdominal lesions.

## Patients and Methods

This is a prospective study performed in the pathology department of Welfare hospital where we searched for all patients undergoing combined, image guided percutaneous core biopsy and FNA cytology and all cases of cell block pathology sampling of abdominal mass lesions during the five year period from March 2010 to July 2015. All patients had one or more radiologically detected lesions and the initial clinical suspicion in each case was of neoplastic disease. In total, 252 cases were identified, comprising approximately 70% of all abdominal lesions subject to biopsy in the study period (in all cases FNA, cell block pathology performed and in 11 cases NCB was also performed). There were 114 men and 138 women with an age range 1-92 years (mean 46). Coagulation screen was checked before biopsy in each case. All patients provided written informed consent for the procedure.

The lesions were situated within the liver (n = 82), pancreas (n = 36), kidney (n = 10), retroperitoneum (n = 3), or miscellaneous abdominal and pelvic sites (n = 121). Sampling was usually performed with ultrasound guidance but computed tomography scanning was used in a few cases. The decision to use FNA and/or core biopsy as a sampling technique was at the discretion of the radiologist performing the procedure. In general, FNAC samples were taken first and subject to immediate assessment(1). Briefly, the samples were obtained following local anaesthesia using a standard 21 gauge needle attached to a 20 ml syringe. After localisation, the needle was passed gently through the lesion five to ten times with aspiration(2). The needle was withdrawn and passed to the cytopathologist. Direct smears were prepared from part of the sample and one or more slides were stained using the Diff-Quik method in the scanning room. The smears were examined by the cytopathologist and the radiologist was informed of specimen adequacy. The remaining sample of fine needle

aspiration is put in 10% neutral buffered formalin for cell block histopathology so all samples of FNA were also submitted for cell block pathology(3). The needle rinses were also submitted for microbiological study in those cases suspected to be of inflammatory or infective nature on rapid cytological assessment. After the FNA procedure, only 11 cases core biopsy samples were taken, 5 cases from liver masses and 6 cases from renal masses using an 18 gauge True-cut needle in those cases away from major vessels or away from bowels. In all cases after procedures a single injection of mesporin was injected; the patients were admitted to hospital for few hours and then discharged with stable good conditions. The adequacy of the specimens was judged visually and up to three separate core samples were taken as required. The core biopsies were fixed in 10% neutral buffered formalin, processed routinely, and stained with haematoxylin and eosin. The specimens were examined and reported by cytopathologist and histopathologist. Then the specificity and sensitivity were measured according to the following equations(2);

Specificity=number of true -ve/number of true -ve+number of false +veX100

Sensitivity=number of true +ve/number of true+ve+number of false -veX100

**Statistical Analysis Used:** Categorical and continuous variables were compared using independent t -test and identified standard deviation using Microsoft SSPS version 19 to measure. P value. P value less than 0.05 is regarded as significant, less than 0.01 highly significant and above 0.05 non significant.

## Result

A total of 252 cases of patients presented with abdominal masses with M:F ratio 0.85:1 nearly equal, and the age distribution of different cases studied as shown in Table 1; the mean age ( $\pm$ SD) for cases of abdominal mass was 47.7 years. Out of 252 cases the majority of cases (54%) were above 60 years age group.

Among these diseases the majority of cases were malignant lesions especially carcinoma either primary or metastatic which constituted about 50.3%.

The relation between age group and types of diseases indicated that the majority of cases were metastatic carcinoma and most of them above 40 years with P value =0.008 highly significant.

The relation between age groups and site of lesion indicated that the majority of lesions occur above 40 years of age with P value =0.005 highly significant and also indicate that the majority of cases belong to the miscellaneous group most of them are malignant.

**Table 1: Number and percentage of age group distribution of patients with abdominal mass**

Age groups	Numbers	%
0-10	7	2.8
11-20	6	2.4
21-30	33	11.9
31-40	37	14.7
41-50	36	14.3
51-60	25	9.9
Above 60 years	108	44
Total	252	100

**Table 2: Number and percentage of different types of neoplastic and non neoplastic diseases**

Type of lesion	number	percentage
Inflammations (acute & chronic	78	30.9
Adenoma	7	2.7
Benign cystic lesions	17	6.7
Hemangioma	17	6.7
Lymphoma	7	2.7
Carcinoma	127	50.3
Total	252	100

**Table 3: Relation between age group & site of lesions**

Site of lesion	Age ≤40 years	Age >40 years	Total
Liver	27	55	82
Pancreas	11	25	36
Renal	4	6	10
Retroperitoneal	0	3	3
Miscellaneous	53	68	121
Total	95	157	252

Regarding specificity and sensitivity of procedures are as follows:

**Specificity:**

Specificity of FNAC=96%

Specificity of Cell block=99%

Specificity of NCB=91%

Specificity of both FNAC+cell block=97.5%

**Sensitivity:**

Sensitivity of FNAC=96%

Sensitivity of cell block=98%

Sensitivity of NCB=75%

Sensitivity of both FNAC+cell block=97%

Figure 1: Sonography of liver showing echogenic mass in the liver in A and showing True cut needle in B.

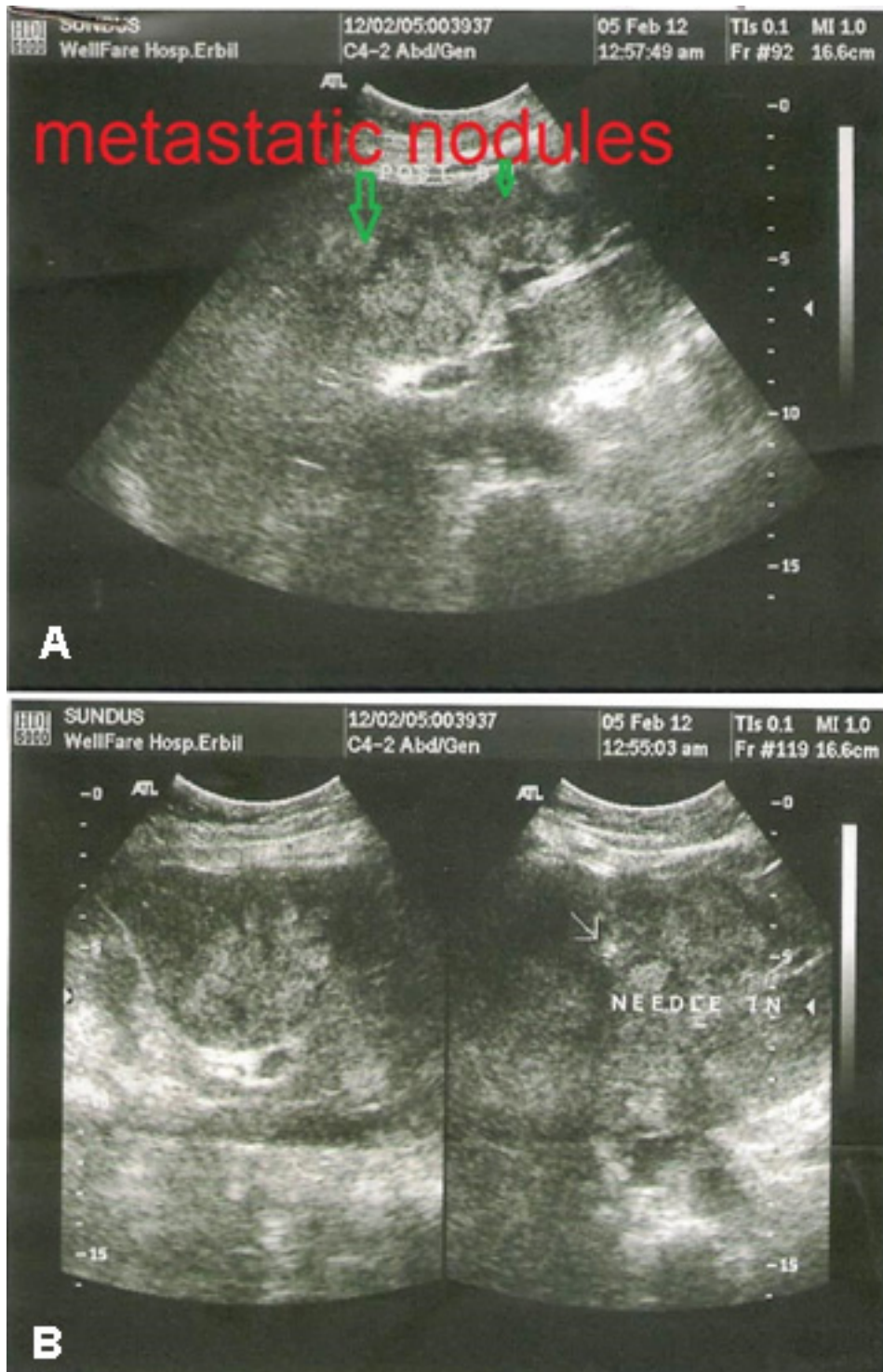




Figure 2: Sonography of liver showing site of needle (FNAC).

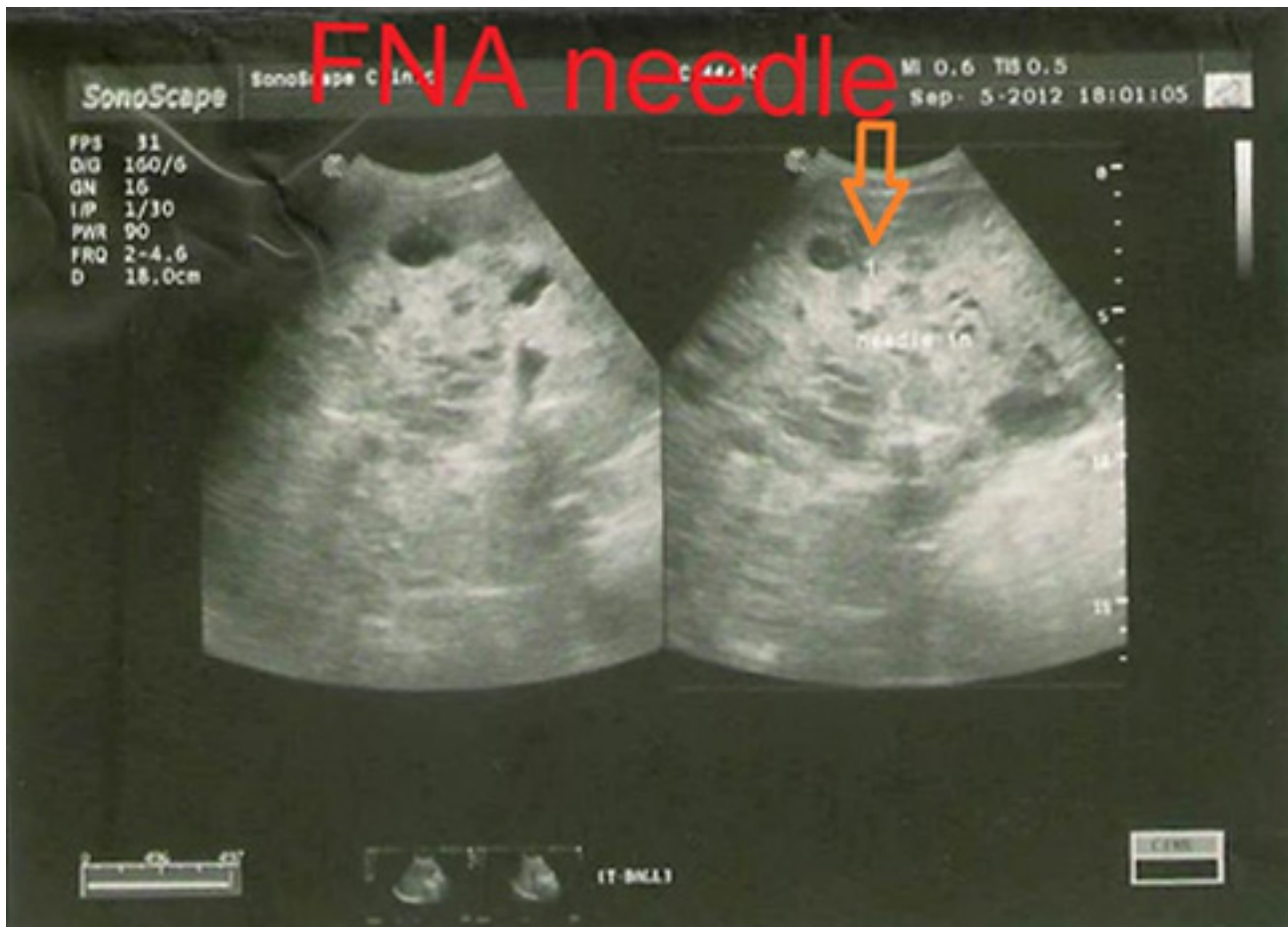
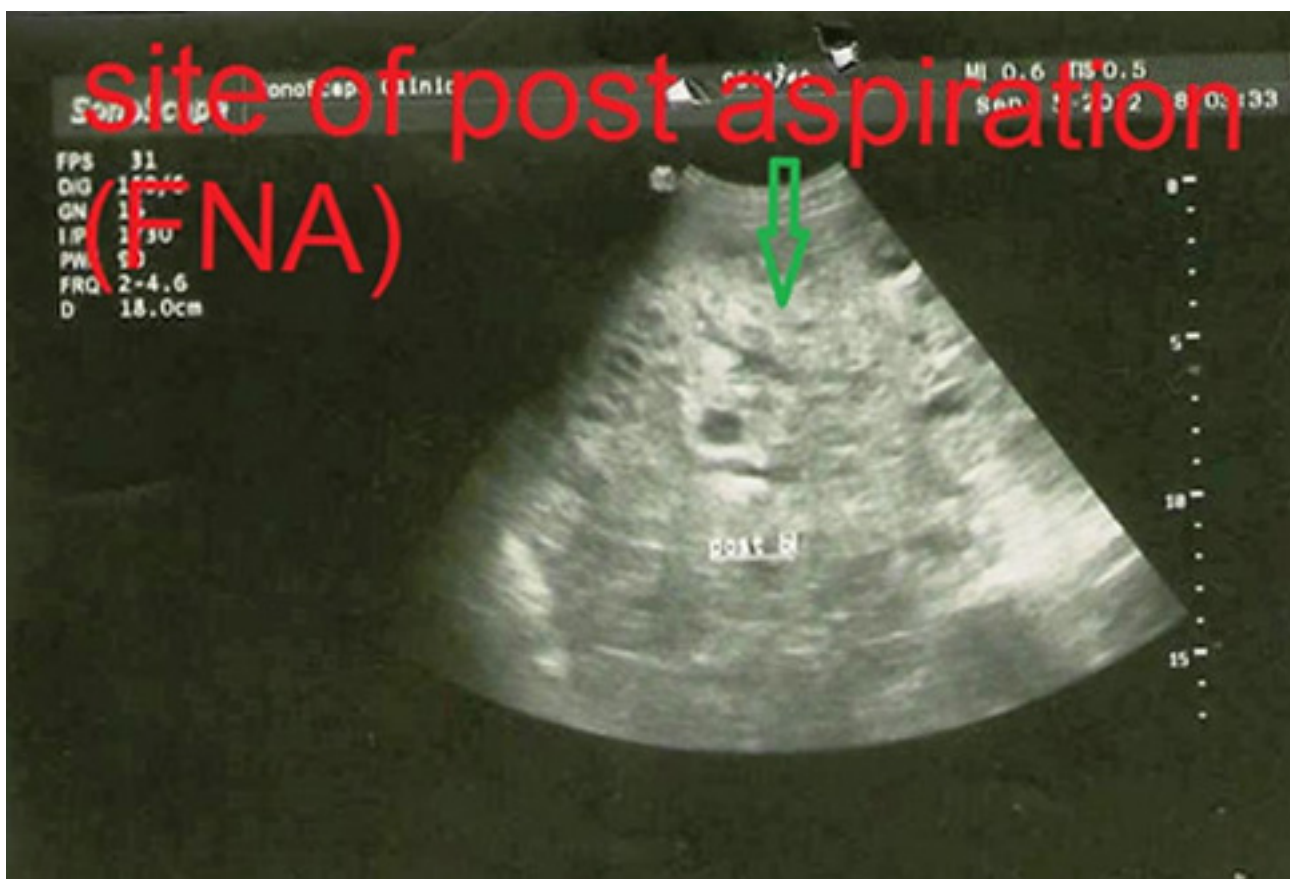
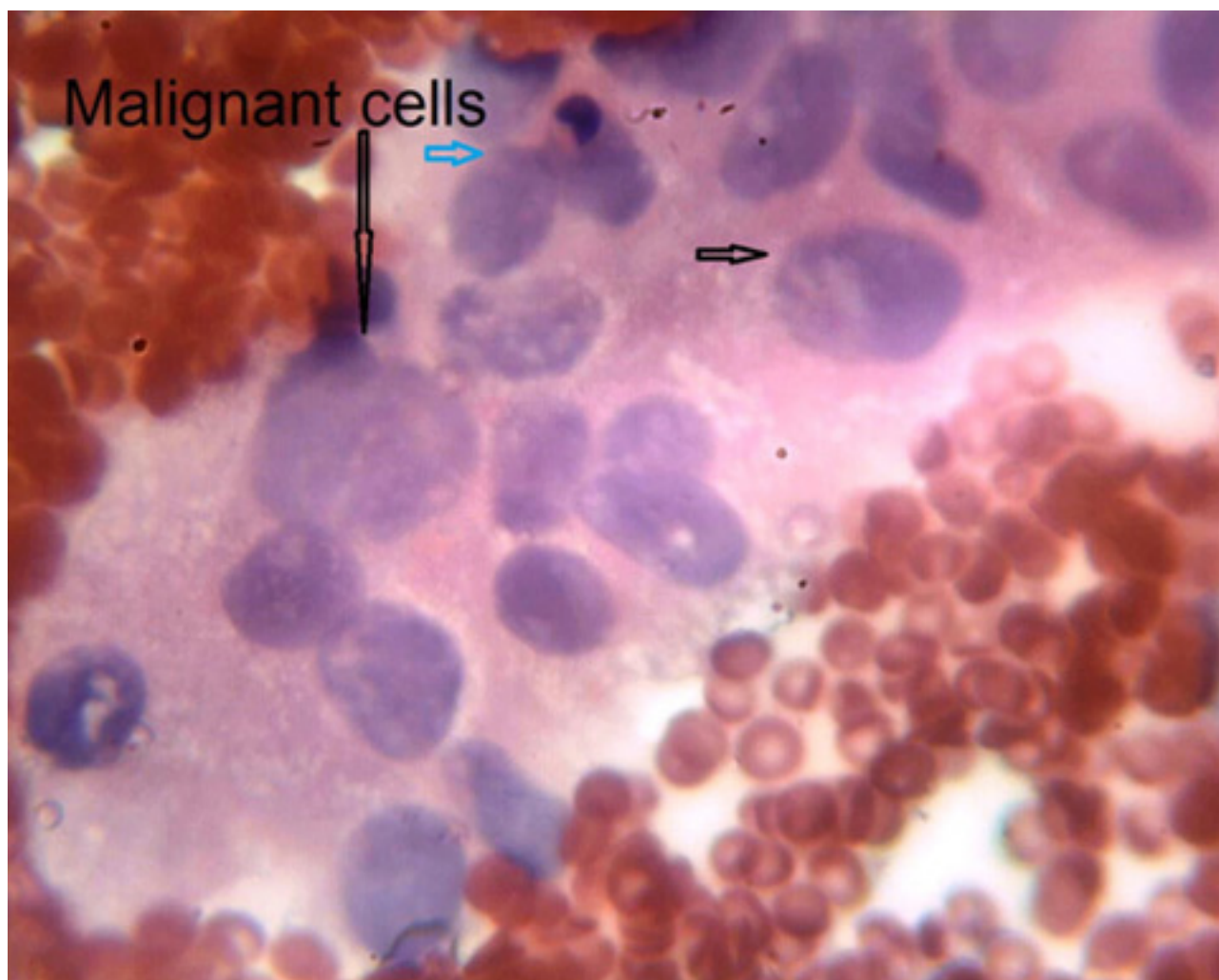


Figure 3: Sonography of liver is showing site of post FNAC cytology there is no any complication.



**Figure 4: FNAC of liver (H&E)X1000 is showing metastatic carcinoma.**



## Discussion

Nowadays most cases of abdominal masses should undergo needle biopsy to establish the diagnosis in all conditions whether the treatment is surgical or medical(4) and most surgeons will not do an operation for any patient without preoperative cytopathology or histopathological diagnosis.

The decision to use FNAC, cell block histopathology and/or NCB as sampling techniques depends on many factors including the size, site of the lesion, the suspected likely diagnosis, and the risk of complications. Because most biopsies are performed using image guidance, the experience of individual radiologists is an important factor, and the preferred technique may be influenced by the availability of cytopathologists for on site specimen assessment. The sensitivity and specificity of FNAC, cell block histopathology and NCB should also be considered in choosing the optimal technique.

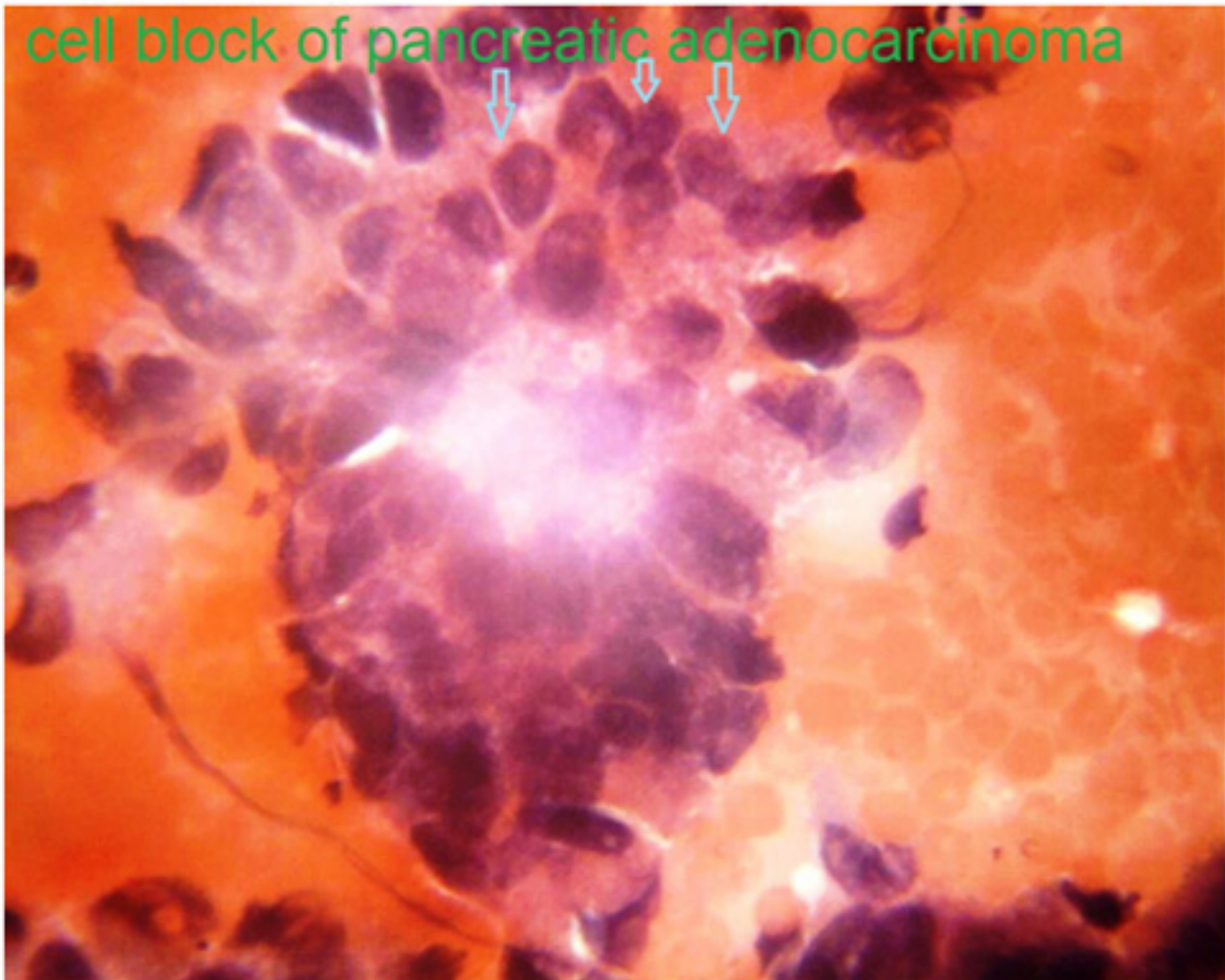
In this study because most of the lesions were located in the miscellaneous group which includes para aortic lymph nodes, pelvic organs and pancreatic masses which were located behind stomach and bowels (Table 3) that is why we preferred FNAC together with cell block pathology and we added NCB biopsy in liver and kidneys when lesions are located away from dangerous areas.

In this study we identified that sensitivity and specificity of FNAC and cell block pathology together were (97%, 97.5%) were higher than NCB (75%, 91%) which is similar to other studies in which sensitivity and specificity of even FNAC alone were higher than NCB (5,6,7); their sensitivity and specificity were (86% v80.6%, respectively) especially when the lesion was located near large vessels such as pancreatic masses (8,9,10) para aortic lymph nodes(11) and in pelvic organs or retroperitoneal (4) areas, even in safe anatomical sites such as liver(13) or kidney(14).

## Conclusion

- 1-** Fine needle aspiration cytology( FNAC) combined with cell block histopathology are more sensitive and more accurate than needle core biopsy (NCB) in diagnosis of abdominal lesion.
- 2-** FNAC is more rapid for giving professional diagnosis and more useful for obtaining sufficient amount of tissue for assessment.
- 3-** FNAC and cell block pathology are less dangerous with no complication, and more cost effective than NCB for evaluation of abdominal lesions.

**Figure 5: Cell block of pancreas primary adenocarcinoma (H&E)X1000.**



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# Effect of sitagliptin on glycemic control in patients with type 2 diabetes

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## Abstract

Eleven type 2 diabetic patients were enrolled in a study at National Diabetes Center Al-Mustansyria University.

### The inclusion criteria were:

- 1- Patients with poor glycemic control having high fasting and postprandial glucose, With a glycosylated hemoglobin (HbA1c) more than 7% while on modified diet or on metformin or metformin plus sulphonylurea.
- 2- Acceptable glycemic control but frequent hypoglycemic episodes while on the lowest dose of sulphonylurea.

To their regimen of therapy sitagliptin 100 mg was given once daily as additional drug on metformin or a substituent of sulphonylurea in those with sulphonylurea induced hypoglycemic episodes.

**Result:** The use of sitagliptin as monotherapy or with metformin and or sulphonylurea, was found to be fruitful in reducing FPG, PPG, and HbA1c with total absence of hypoglycemic episodes in those who are complaining from such sulphonylurea induced hypoglycemic episodes after substituting sulphonylurea with sitagliptin.

**Key words:** sitagliptin, metformin, diabetes, type 2.

## Introduction

Despite the health benefit associated with good glycemic control, many patients with diabetes fail to achieve recommended glycosylated hemoglobin (HbA1c) treatment goals (1).

The majority of diabetic patients maintain HbA1c levels above 8% and less than one third achieve the target HbA1c levels set forth by organizations such as the American Diabetes Association (ADA; HbA1c target L 7%). (1,2)

These reports of suboptimal glycemic control in the vast majority of patients with diabetes suggest that there is an urgent need for new approaches to anti-diabetic therapy.

Normal glucose homeostasis is maintained through a highly complex mechanism involving interaction between pancreatic islet-cell hormones (e.g. insulin and glucagon), end organs and tissues. (3)

Insulin suppresses hepatic glucose output (HGO) and increases glucose uptake in muscle and fat resulting in lower blood glucose concentration. (4)

Glucagon also plays an important role in regulating blood glucose levels. (5)

When blood glucose levels are low, the alpha cells of the pancreas release glucagon which will increase HGO, thereby raising blood glucose concentration. (6)

Incretin hormones are important glucoregulatory hormones produced by the gut. (7)

Incretin hormones are released in response to ingestion of oral nutrients, including glucose, fatty acids, and dietary fibre.

These hormones are released from the bowel in minutes after a meal to reach the blood stream where they potentiate insulin secretion from pancreatic beta cells in response to elevated blood glucose and inhibit glucagon release from pancreatic alpha cells under conditions of hyperglycemia.

These incretion hormones are: glucagon like peptide 1 (GLP1), and glucose dependent inhibitory peptide (GLP).

After their release incretion hormones are rapidly degraded by dipeptidyl peptidase - 4 (DPP4) and cleared (8,9,10,11).

GLP1 is secreted by L-cells in the distal gut (ileum and colon); it stimulates glucose - dependent insulin release from beta cells and suppresses HGO by inhibiting glucagon response from alpha cells in a glucose dependent manner. (12)

Glucose-dependent inhibitory peptide (GLP) is secreted by K cells in the proximal gut (duodenum and proximal Jejunum), it stimulates glucose-dependent insulin release from beta cells. (13)

Enhancing circulating levels of incretin hormones is a new approach to treat type 2 diabetes. Sitagliptin is a new therapeutic agent in the treatment of type 2 diabetes. It is a member of a new class of dipeptidyl peptidase (DPP4) inhibitors, thereby enhancing levels of intact incretin hormones, increasing the insulin - to glucagon ratio, and improving pancreatic beta cell function, including significant improvement in homeostasis model assessment (HOMA).

In placebo-controlled clinical trials the incidence of hypoglycemia in patients taking sitagliptin was comparable to patients taking placebo (1.2% vs 0.9%). (14)

Incretin hormones increase the release of insulin from pancreatic cells and suppress the release of glucagon from pancreatic alpha cells in a glucose dependent manner; these incretins have a very short half life time.

Sitagliptin is an orally active selective DPP-4 inhibitor; its use results in 3-fold increase in postprandial levels of active GLP-1 compared with placebo. (15)

The first oral DPP-4 inhibitor, sitagliptin, was approved by the Food and drug Administration in October 2006 for use as monotherapy or in combination with metformin or thiazolidendiones.

Another DPP4- inhibitor, vildagliptin, was approved in Europe in February 2008, and several other compounds are under development. In clinical trials performed to date, DPP4 inhibitors lower levels by 0.6-0.9 percentage points and are weight neutral and relatively well tolerated . (16)

## Patients and methods

Eleven type 2 diabetic patients were initially screened for eligibility for the study.

All of them met the inclusion criteria and were recruited to participate in the study.

Written informed consent was obtained from all patients.

The enrolled type 2 diabetic patients are 8 males and 3 females, their age ranged from 40 to 70 years ( $51.8 \pm 8.18$ ) and their body mass index is ( $29 \pm 2$ ).

The inclusion criteria were:

**1-** Patients with poor glycemic control having high fasting and postprandial glucose, With a glycosylated hemoglobin (HbA1c) more than 7% while on modified diet or on metformin or metformin plus sulphonylurea.

**2-** Acceptable glycemic control but frequent hypoglycemic episodes while on the lowest dose of sulphonylurea.

To their regimen of therapy sitagliptin 100 mg was given once daily as additional drug on metformin or a substituent of sulphonylurea in those with sulphonylurea induced hypoglycemic episodes.

The study was conducted in the National Diabetes Center Al-Mustansyria University over a period of 8 weeks.

For all the patients a detailed history was taken followed by physical examination and full laboratory workup including fasting plasma glucose (FPG), postprandial glucose (PPG) 2 hours after breakfast, lunch, and dinner with HbA1c.

## Results

The use of sitagliptin as monotherapy or with metformin and or sulphonylurea, was found to be fruitful in reducing FPG, PPG, and HbA1c with total absence of hypoglycemic episodes in those who are complaining from such sulphonylurea induced hypoglycemic episodes after substituting sulphonylurea with sitagliptin.

As shown in Figure 1 (next page) FPG before using sitagliptin is 156.18 mg/dl and drops to 129.2 mg/dl after 2 weeks. This decrement is not significant  $P > 0.05$  but after 12 weeks it drops to 109 mg/dl which is statistically significant ( $p < 0.01$ ).

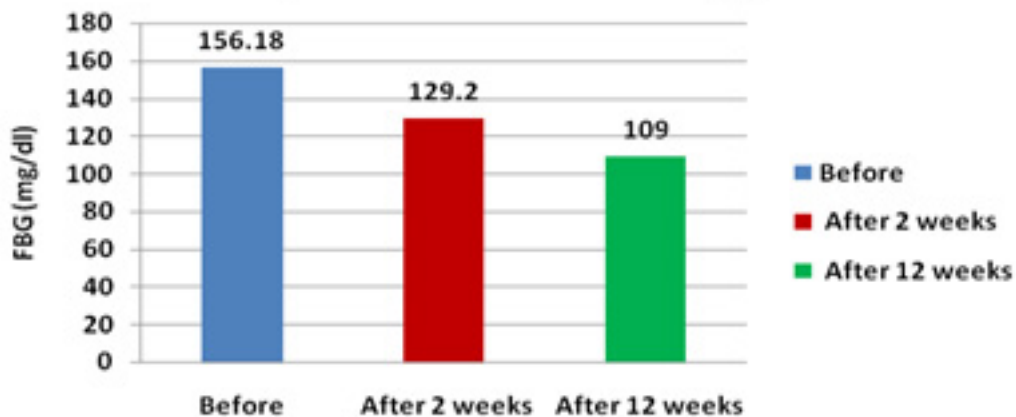
Before using sitagliptin the mean postprandial glucose was 237.1 mg/dl. It drops to 170.6 mg/dl after 2 weeks of taking sitagliptin; this reduction was found to be highly significant ( $p = 0.001$ ).

At the final visit after 12 weeks of taking sitagliptin, postprandial glucose reaches a mean of 163.6 mg/dl which is a highly significant reduction when compared with the initial level before taking this medication ( $p = 0.001$ ) as shown in Figure 2 (next page).

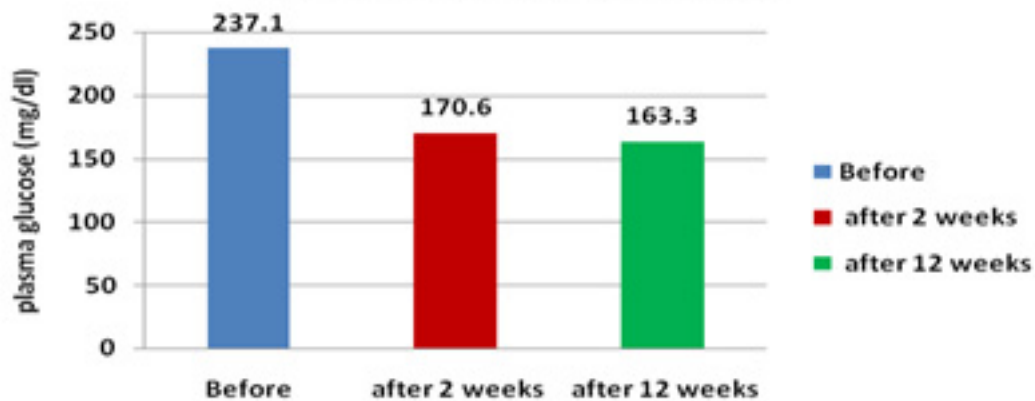
Postprandial plasma glucose after dinner drops from 201 to 162.2 mg/dl which is significant ( $p < 0.05$ ) after using sitagliptin for 2 weeks, but after 12 weeks of use it drops from 162.2 to 148 mg/dl. This reduction is statistically highly significant ( $p < 0.001$ ) as shown in Figure 3 (next page)

The mean HbA1c of the studied subjects before being enrolled is 8.06% while after using sitagliptin for 12 weeks it drops to a mean of 7.26% with a decrement of 1.17%. This was found to be statistically significant ( $p < 0.05$ ) as shown in Figure 4.(page 23).

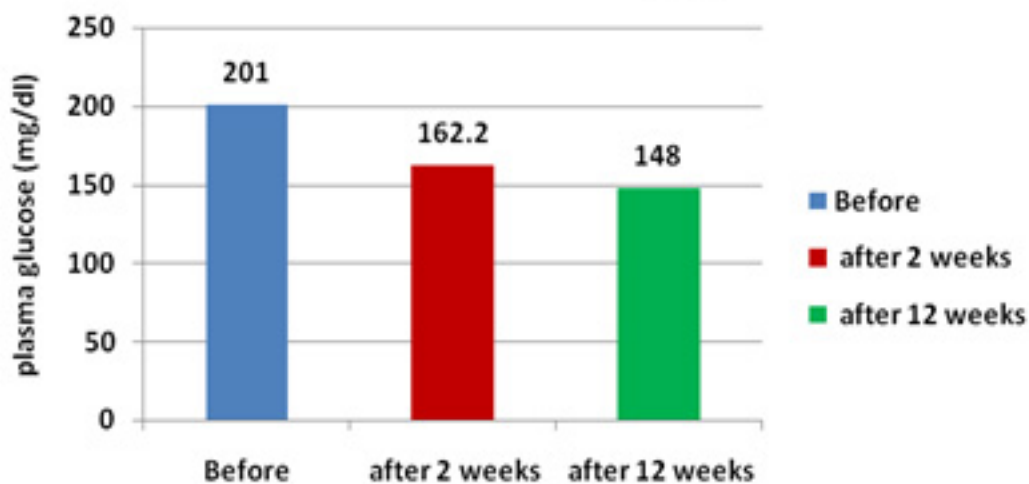
**Figure 1: Fasting plasma glucose before, 2 weeks, and 12 weeks of sitagliptin.**



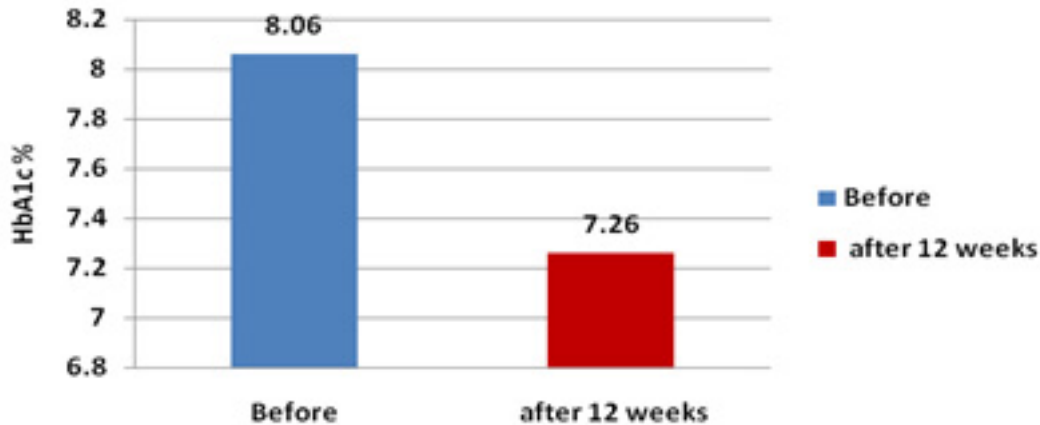
**Figure 2: Plasma glucose after lunch before and after sitagliptin**



**Figure 3 : Plasma glucose after dinner before and after sitagliptin**

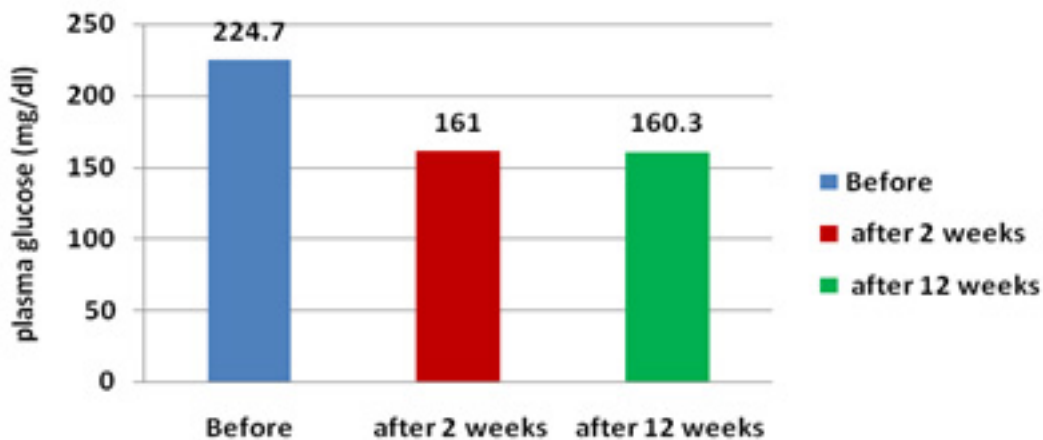


**Figure 4: HbA1c before and after the use of sitagliptin**



After breakfast the mean postprandial plasma glucose was 224.7 mg/dl. It drops to 161 mg/dl 2 weeks after using sitagliptin which is a significant reduction ( $p=0.02$ ) while 12 weeks after using this drug plasma glucose reaches a mean 160.3 mg/dl which is not significant when compared with that achieved 2 weeks after using sitagliptin ( $p>0.05$ ) but highly significant when compared with the basal value before using sitagliptin ( $p=0.001$ ) as shown in Figure 5.

**Figure 5 : Plasma glucose after breakfast before and after sitagliptin**



## Discussion

Diabetes looms in its devastating complications. United Kingdom Diabetes prospective study (UKPDS) proved that tight glycemic control prevents and reduces the expected complications. (17)

Sitagliptin use was found to be useful in reducing plasma glucose and HbA1c thus approaching the recommended targets; this was in harmony with Scott et al. (18)

In treating type 2 diabetes there are two major concerns; they are weight gain, and hypoglycemic episodes.

As the pharmacokinetics and pharmacodynamics of sitagliptin are glucose dependent so patients in whom

hypoglycemia induced by sulphonylurea is a major concern found sitagliptin as a good substituent and they were free from any hypoglycemia. (19,20).

Its properties that allow oral administration in a once daily regimen are favorable.

The initial combination of sitagliptin and metformin provided substantial and additive glycemic control and was generally well tolerated in patients with type 2 diabetes; this was in harmony with Barry J et al. (21)

In summary, initial combination therapy with sitagliptin and metformin provided improvement in glycemic control, suggesting that the marked benefits of this combination is the product of the complementary action of these two agents.

This combination was also generally well tolerated, with a tolerability profile similar to metformin alone.

The initial combination of sitagliptin and metformin provided substantial and additive glycemetic improvement and was generally well tolerated in patients with type 2 diabetes. (22)

The full effect of sitagliptin is achieved over a 24-week treatment period. (23)

DeFronzo suggests that a combination of sitagliptin and metformin improves pathological defects associated with type 2 diabetes, diminished Beta-cell function with reduced insulin release, increased insulin resistance, and increased hepatic glucose output (24,25).

This combination improved markers of insulin resistance - HOMA - IR - Quantitative insulin sensitivity check index with significant reduction of hepatic glucose output and fasting plasma glucose (24).

Metformin based therapy results in significant reduction of body weight, while there is no change in body weight in sitagliptin treated patients. These data were confirmed by good number of workers.

If both drugs are co-administered the weight loss is similar to that induced by metformin monotherapy (26).

Both metformin and sitagliptin are well tolerated when used as monotherapy or in combination. The addition of sitagliptin to metformin is not associated with increased incidence of gastrointestinal side effects.

Despite marked improvement in glycemetic control, there was low incidence of hypoglycemia related to sitagliptin. This is consistent with the glucose dependent effect of incretins.

Similarly metformin has been associated with low incidence of hypoglycemia. (26,27)

Efficacy and safety of sitagliptin were studied extensively by Razz in Haddasah University Hospital - Israel and by M. Honefeld in Dresden, Technical University, Germany. It is a multinational randomized, double blind, placebo controlled, parallel- group study, over a period of 18 weeks; the patients were divided into 3 groups; group one on placebo, group 2 on sitagliptin 100 mg/day and group 3 on sitagliptin 200 mg/day.

The eligible patients were randomized in 1:2:2 ratio.

There was no meaningful difference among the three treatments groups in the incidence of overall serious drug - related adverse events or experiences including those that lead to discontinuation of the drug.

There was no statistically significant difference in the incidence of hypoglycemia between the placebo and sitagliptin groups.

There was no statistically significant difference in the incidence of selected gastrointestinal adverse experiences of abdominal pain, diarrhea, nausea and vomiting between the placebo and sitagliptin groups.

Few specific adverse experiences occurred at more than a minimally higher incidence with sitagliptin compared with placebo. These included nasopharyngitis, back pain, osteoarthritis and pain in the extremities.

Similarly no meaningful differences were observed between treatment groups in mean change in vital signs or ECG data. (28,29)

## Conclusion

Sitagliptin significantly improved glycemetic control and was well tolerated in patients with type 2 diabetes who had inadequate glycemetic control on diet and metformin.

It was found to be good substituent of for sulphonylureas in patients complaining of hypoglycemic events.

In the current study no side effect was recognized but the main limitation of the study is the small sample of enrolled patients as the medication is expensive and not available in the general health sector.

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# Critical Reading of an Article about Causation and Harm

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## Objectives

By reading this chapter, readers will be able to:

Describe the cause and effect relationship; and Understand and apply Bradford Hill's criteria for establishing causality in the study of health problems.

## What is causation?

Causation is defined as the relationship between an exposure or cause and an outcome or effect. The cause may be a risk factor resulting in a disease, an exposure, or a treatment that helps alleviate suffering. The effect is defined as a particular outcome that is measurable. Most biomedical research studies try to prove a relationship between a particular cause and a specified effect.

The stronger the design of a study, the more likely it is to prove a relationship between cause and effect. Not all study designs are capable of proving a cause-and-effect relationship. The cause is the independent variable and is set by the researcher (e.g., Rosiglitazone medication as treatment for diabetes) or the environment (e.g., asbestos).

However, the effect is the dependent variable. It can be an outcome, such as death or survival, or the degree of improvement on a clinical score.

**It is not always easy to establish an association or link!**

You may think that hyperlipidemia is a cause for cardiovascular disease; but, how can we be sure that this is a cause and not just a related factor (confounder)? Perhaps hyperlipidemia is caused by the lack of exercise, which actually causes both!

## Case Study

You are the Family Physician who is treating a 55 years old diabetic male nurse, for the last 5 years, who is on Metformin and Gliclazide full dose. His HbA1c is > 9.0 for the last 6 months. You added Pioglitazone as a third medication according to guidelines. After a few days, he called up and requested to see you urgently, as he read an article about Pioglitazone use and the risk of urinary bladder cancer. You advised him to visit you the next day.

You formulated a question in PICO format to guide your search:

P : T2 diabetic patient  
I : Pioglitazone  
C : No Pioglitazone  
O : Incidence of bladder cancer

You searched PubMed and found a systematic review that addresses your question. The systematic review is of accepted quality and shows that the incidence of bladder cancer increases with increased dose and/or duration of Pioglitazone use.

The question you asked yourself: what to tell your patient who is coming to see you next day?

### The Bradford Hill Criteria

In 1965, Sir Austin Bradford Hill developed the Causality Criteria. Hill's criteria are considered flexible guidelines to assess the association between a causal factor and the outcome (effect). He stated that he didn't intend for these "viewpoints" to be used as "hard and fast rules". The following are the Bradford Hill Criteria:(2)

## I. Strength of Association

There is a direct relationship between the magnitude of an effect and the causative factor, i.e. the higher the magnitude of an effect, the stronger the association between the proposed risk factor and the outcome.

Effect measures are expressed as Relative Risk (Risk ratio) (RR) and /or Odds ratio (OR), both RR & OR can be used in cohort studies; however RR can't be used in case-control studies, because the number of cases and controls is pre-determined by the study authors. Hazard ratio is another effect measure, where it's calculation and interpretation is similar to RR except that time intervals are taken into account.

The degree of "strong" association may be understood by the following "rule-of-thumb" as depicted in Table 1.

**Table 1: "Rule of Thumb" Degree of Association**

Risk Ratio (RR)	Interpretation
1.1 – 1.3	Weak
1.4 – 1.7	Modest
1.8 – 3.0	Moderate
3.0 – 8.0	Strong
8.0 – 16.0	Very strong
16.0 – 40.0	Dramatic
40 +	Overwhelming

Why this criterion is important: When there is a high magnitude of the effect measure (e.g. RR > 3.0), it is less likely to be due to other etiologic factors (confounders).

### Example 1:

RR for lung cancer and cigarette smoking from various studies are around 10.0 while RR for breast cancer and cigarette smoking from various studies are between 1-1.5; which suggests that the association between smoking and lung cancer is more likely to be causal than smoking and breast cancer.

### Example 2:

Palomaki et al., (1991) studied the relationship between sleep apnea including snoring and stroke.

Risk of stroke is 1.22 times in patients who snore (i.e. a single symptom); while, it is 8.00 times in patients having the full picture of obstructive sleep apnea syndrome. There is a stronger association between obstructive sleep apnea and stroke compared to snoring alone and stroke.

### Example 3:

Rosiglitazone (Avandia) is an oral medication for treatment of diabetes; the published systematic reviews show that it moderately increases cardiovascular risk (RR 1.43).

Relatively weak association is common but one can rely on proper study designs that minimize bias (selection, information, and confounding). One rule to remember is: Absence of a strong association does not rule out a causal effect.

## II. Consistency

When a casual relationship is observed repeatedly across studies.

When there are similar results from different studies which were conducted in different populations, in different methods by different researchers in different centers, if this happened, it increases our confidence of the causal association.

### Examples

Many RCTs were published with a consistent result of the relationship between Rosiglitazone and cardiovascular events (RR 1.38 - 1.44).

Another example; the three published studies show a relationship between sleep apnea and stroke with similar results.

Conversely, studies addressing the same question may have different results. This may be due to:

- (1) Different study designs and conduct (error-prone versus error-free methods);
- (2) Presence of a confounder, which is an independent, unintended to study variable that affects the outcome; and,
- (3) The role of chance. If inconsistency is present, look for reasons, e.g., different population, methods and exposure.

Consistency is not always necessary to explain the cause and effect relationship. Sometimes, inconsistency provides useful insights of the causal components of an outcome.

## III. Specificity of the Association

A factor influences specifically a particular outcome or population

Specificity of the association suggests that one exposure is specific to one disease,(8) i.e., Rubella virus causes rubella. This criterion is best evidenced among infectious diseases.

This criterion is not applicable to all exposure-disease associations because a disease can be caused by several exposures, and an exposure may cause several diseases.

### Example:

Diabetes is associated with end stage renal disease (ESRD) and other micro and macro vascular complications. On the other hand, ESRD is caused by diabetes and other diseases.

An exposure is likely to have a harmful effect on a specific mechanism (i.e., at a cellular or molecular level) that may then lead to one or more diseases. For example, an exposure such as smoke from cigarette smoking is comprised of many smaller chemical components.

The value of this rule lies in its combination with the strength of an association. For instance, among smokers, the risk of death from lung cancer should be elevated to a higher degree as compared to the risk of other causes of death.

When present high specificity does provide evidence of causality, low specificity value does not exclude causation.

#### IV. Temporal Relationship

The cause must precede the effect in time. This is the only one among Hill's criteria that everyone agrees upon.

Prospective studies clearly establish the correct temporal relationship between an exposure and a disease.

Temporal direction might be difficult to establish if a disease developed slowly and initial forms of a disease were difficult to measure (e.g., the egg first or chicken argument).

##### Example 1:

The reports of increased suicidal ideation associated with the use of anti-depressant fluoxetine illustrate the importance of this question. However, one must realize that the reason for using fluoxetine is depression, which is the actual cause of suicide.(9)

**Depression ⇒ Fluoxetine ⇒ Suicidal ideation**

##### Example 2:

Children given antibiotics have greater incidence of asthma. But this ignores the fact that they were given antibiotics because of previous chest infections.(10)

**Chest infection ⇒ Antibiotics ⇒ Bronchial Asthma**

#### V. Dose Response Gradient (Biological gradient)

As quantity or the duration of exposure to harmful exposure increases, the risk of the adverse effect also increases. If risk increases with increasing exposure, it supports the notion of a causal association. However, the absence of dose-response does not preclude causal association.

##### Example 1:

The risk of dying from lung cancer in male physician smokers is dose dependent. The risk increases by 50%, 132% and 220% for 1-14, 15-24 and 25 or more cigarettes smoked per day, respectively.

##### Example 2:

Effect of neuraminidase inhibitors (Oseltamivir) compared with placebo on prophylaxis against laboratory confirmed influenza. RR is 0.39 with Oseltamivir 75 mg reduced to 0.27 with 150 mg.(11)

##### Example 3:

A meta-analysis of the association of pioglitazone use and risk of bladder cancer among diabetics showed that those who used pioglitazone for more than two years or used a

dose higher than 28,000 mg have higher risk of bladder cancer (RR 1.44 CI 95% 1.19 - 1.74) compared with those who used it for less than one year (RR 1.03) or less than 10,500 mg (RR 1.13).(1)

#### VI. Biological Plausibility and Coherence

Does the association make biological sense? If there is plausible biological or pathological mechanism that could explain the relationship, the possibility of causation is increased.

##### Example:

The association between cigarette smoking and lung cancer can be explained by presence of many carcinogens in cigarettes like polycyclic aromatic hydrocarbons (PAH).

At the same time, research that disagrees with established biological theory is not necessarily false; it may, in fact, force a reconsideration of accepted beliefs and principles.

##### Coherence:

The cause-and-effect interpretation for an association does not conflict with the current knowledge of the natural history and biology of the disease.

#### VII. Reversibility and Experimental Evidence

All or none rule: All subjects will be vulnerable to disease when exposure present, however, all subjects will not be affected when exposure is removed. Best example is vaccination.

Well-designed experiments may give strong reason to believe that causation is at work. RCTs reduce the likelihood that there may be a systematic difference between the treatment and control groups.

##### Are the comparison groups similar?

Only RCT design gives two balanced groups (intervention and control groups), while other designs (Cohort or Case control) don't.

##### Example

Clinical trials have shown that diabetes can be prevented through lifestyle modification programs, with reduced cumulative incidence of 58% compared to placebo.(12)

#### VIII. Analogy

Judgment from analogy, observes what effects a similar drug has on a disease.

##### Example:

If one COX-2 inhibitor has a certain side effect, then it is more plausible that another one will cause the same side effects too.

##### Another example:

Glitazone group causes heart failure.

### Were the exposures and outcomes measured in the same way as the groups being compared?

- In case-controlled ascertainment of exposure: Recall bias and interviewers bias
- Example: Patients with leukemia, when asked about prior exposure to solvents, may be more likely to recall exposure than would a control group; either because of increased patient motivation (recall bias) or greater probing by an interviewer (interviewer bias).
- In RCT and Cohort ascertainment of outcome: When intervention group is not blinded, investigators diligently search for outcome.

### Results:

The association between exposure and outcome can be presented as follows: Ratios for RCT and RR for cohort and case control = OR for case control.

The RR is the risk (or incidence) of the adverse effect in the exposed group divided by the risk of the adverse effect in the unexposed group. Values >1 represent an increase in risk associated with the exposure; while values <1 represent a reduction in risk; and, values = 1 means both have similar effects.

### Example:

In a cohort study assessing in-hospital mortality following non-cardiac surgery in males, 23/289 patients with a history of hypertension died, compared with 3/185 patients without.

Risk with HTN =  $23/289 = 0.07958$

Risk without HTN =  $3/185 = 0.01622$

RR = 4.9

### Interpretation:

The relative risk tells us that death occurs almost 5 times more often in the hypertensive patients than in normotensive patients.

Very large values of RR or OR represent strong associations that are less likely to be due to confounding or bias.

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# Hypolipidemic efficacy of *Trigonella Foenum* seeds in comparison with Rosuvastatin and Fenofibrate in hyperlipidemic rats

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## Abstract

**Background:** *Trigonella foenum-graecum* seeds (TGS) have been historically used for the treatment of various chronic human diseases and studies concerned with application of this plant in diabetes and dyslipidemia support this hypothesis. The present study was designed to evaluate and compare the effect of different doses of TGS powder with Rosuvastatin and Fenofibrate on lipid profile, liver function enzymes, body weight and malondialdehyde (MDA) in hyperlipidemic rats.

**Method:** Forty two rats were divided into two groups. The first group included 12 rats and received a standard diet throughout the experimental period and were subdivided into two subgroups of 6 rats each. The first subgroup served as a control group. The second subgroup received a standard diet containing TGS Powder at a concentration of 0.75% (w/w). The second group included 30 induced hyperlipidemic rats by feeding them with high cholesterol diet. They were subdivided into five subgroups each of 6 rats. First subgroup served as a positive control. The second subgroup received atherogenic diet containing TFSP at a concentration of 0.50% w/w, the third subgroup received the same diet containing TGS at a higher concentration of 0.75% w/w. The fourth and fifth subgroups received a daily dose of Rosuvastatin and Fenofibrate respectively. At the end of the treatment period (six weeks) all of these groups were subjected to various biochemical analyses of blood.

**Results:** After six weeks of therapy, TGS of both concentrations (0.50% and 0.75% w/w) significantly reduced serum low density lipoprotein cholesterol (LDL-C), total cholesterol (TC) when compared with hyperlipidemic rats. Both concentrations of TFS (0.50%, 0.75% w/w) increased serum high density lipoprotein cholesterol (HDL-C) significantly for both normal and hyperlipidemic rats. Daily administration of Rosuvastatin of (10mg/kg) for six weeks reduced serum TC, LDL-C and triglycerides (TG) level significantly when compared with hyperlipidemic rats. Administration of Fenofibrate for six weeks markedly and significantly reduced serum TG when compared with hyperlipidemic animals. Daily use of TFS (0.75% w/w) for six weeks increased both serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) significantly for both normal and hyperlipidemic rats.

Daily administration of TGS of both concentrations (0.50% and 0.75% w/w) significantly decreased serum MDA level of hyperlipidemic rats. There was a significant increase in body weight of normal rats taking diets containing TGS for six weeks.

**Conclusion:** TGS has similar efficacy of Rosuvastatin and Fenofibrate in reducing TC. whereas, TGS was non-significantly more effective than Rosuvastatin and Fenofibrate in changing serum HDL-C and LDL-C.

**Key words:** Hyperlipidemia, *Trigonella Foenum*, Rosuvastatin, Fenofibrate

## Introduction

Medicinal plant products have been highlighted as an alternative to current management of dyslipidemia to some extent (1). There are many types of medicinal herbals which are useful in treatment of hyperlipidemia such as *Avena Sativa* Which has been shown to decrease serum cholesterol (2). The lipid-lowering effects of Garlic (*Allium sativum*) may occur via inhibition of HMG-CoA reductase or other enzymes, possibly by diallyl di- and trisulphide components of garlic (3). The decoction of the (*Coriandrum sativum*) is effective in lowering blood lipid levels (4).

*Trigonella foenum-graecum* (TGSP), an annual medicinal plant of the Fabaceae family is extensively cultivated in most regions of the world for its medicinal value (5). TGSP seeds have been historically used for the treatment of various chronic human diseases and studies concerned with application of TGSP seeds in diabetes and dyslipidemia support this hypothesis (6, 7).

TGSP is full of 4-hydroxyisoleucine, which directly induces insulin secretion from pancreatic  $\beta$  cells (8). It's reported to have restorative and nutritive properties and to stimulate digestive processes, useful in healing of different ulcers in the digestive tract (9). TGSP has also been reported to exhibit pharmacological properties such as antitumor, anti-inflammatory, hypotensive and antioxidant (10, 11).

TGSP has been used to treat peptic ulcers and inflamed conditions of the stomach and bowel; it absorbs toxic material and eliminate it. The healing and soothing action creates a protective coating, like a lubricant, over inflamed areas (12).

TGSP seed is widely used as a milk producing agent by nursing mothers to increase inadequate breast milk supply (13). Flavonoids of TGS extract have been observed to possess anti-oxidant activity (14).

The present study was designed to evaluate and compare the effectiveness of different doses of Fenugreek seeds, Rosuvastatin at 10mg/kg and Fenofibrate at 30mg/kg on the lipid profile, liver function tests, malondialdehyde level and weight in hyperlipidemic rats after six weeks of administration.

## Materials and Methods

### Animals:

Adult female rats weighing between 100-250 g were used throughout the study. The rats were obtained from Mosul and Abu ghreb city. All animals were kept in the animal house at the College of Medicine under controlled conditions of 12 hours light and 12 hours dark cycles in a room temperature of 25 °C. The rats were allowed to acclimatize to these conditions for one week.

### Experimental design:

Forty two rats were divided into two groups. The first group included 12 rats which received standard diet throughout the experimental period. They were subdivided subsequently into two subgroups each of 6 rats. The first subgroup served as a control group. The second subgroup received a standard diet containing (TFSP) at a concentration of 0.75% (w/w).

The second group included 30 hyperlipidemic rats. Hyperlipidemia was induced by feeding the rats with high cholesterol diet; they received atherogenic diet (79% standard diet and 21% butter fat) for six weeks (15). The hyperlipidemic rats were subsequently subdivided into five subgroups of 6 rats. The first subgroup, served as a positive control (hyperlipidemic rats). The second subgroup, received atherogenic diet containing TGS Powder at a concentration of 0.50% (w/w) every day. The third subgroup, received atherogenic diet containing TGS powder at a concentration of 0.75% (w/w) every day. The fourth and fifth subgroups received a daily dose of Rosuvastatin (10mg/kg) and Fenofibrate (30mg/kg) respectively.

The solutions of two drugs Rosuvastatin (10mg/kg), and Fenofibrate (30mg/kg) were freshly prepared in normal saline and given to animals by oral gavage every day. (16, 17)

At the end of the treatment period (six weeks), the animals were subjected to various biochemical analysis of blood. They were fasted overnight and the following day blood samples were taken. The procedure started by anaesthetizing the rats by giving them a combination of ketamine in a dose of 35 mg/kg with xylazine in a dose of 5 mg/kg (18) which was followed by a cardiac puncture by a sterile disposable plastic syringe which was then put into a specified numerically labeled blood tube.

Blood samples were collected from rats for determination of serum lipid and lipoprotein profile (T.Ch, TGs, HDL-C and LDL-C), serum malondialdehyde (MDA) and liver function tests (serum alanine aminotransferase S.ALT, serum aspartate aminotransferase S.AST and serum alkaline phosphatase ALP).

During the experimental period, body weight was individually recorded for each rat before and after treatment.

### Statistical analysis:

All data are expressed as means  $\pm$  standard error of means (M  $\pm$  SEM) and Statistical analysis was carried out using statistically available software (SPSS Version 19). Data analysis was made using one-way analysis of variables (ANOVA). Comparisons between groups were done using Duncan test and unpaired student t-test. P  $\leq$  0.05 was considered as statistically significant.

## Results

### Effects of *Trigonella foenum graecum* on lipid profile:

Daily administration of TGS at a concentration of (0.75% w/w) had no significant effect on serum levels of total cholesterol, TG, LDL-C of normal rats (Table 1). The same dose of TGS has produced a statistically significant rise in serum HDL-C as seen in Table 1.

**Table 1: Effects of TGS 0.75% (w/w) on the lipid profile of normal rats (n=12).**

Parameters	Control group	TGS
S. Total Cholesterol mg/100ml	58.31 ± 9.703	66.83 ± 4.46
S. Triglyceride mg/100ml	57.25 ± 17.45	58.83 ± 8.33
S.HDL mg/100ml	35.33 ± 6	47.16 ± 0.79 *
S.LDL mg/100ml	6.51 ± 1.71	7.16 ± 0.60

\* P < 0.05

### Effects of *Trigonella foenum graecum* on lipid profiles of hyperlipidemic rats:

There was a marked increase in the level of serum triglyceride, serum cholesterol and serum low density lipoprotein in the rats treated with atherogenic diet compared to the control group indicating the induction of hyperlipidemia as shown in Table 2.

**Table 2: Effects of different doses of TGS Rosuvastatin and Fenofibrate on lipid profile of hyperlipidemic rats (n=36)**

Parameters	Control group	Hyperlipidemic group	TGS 0.50%	TGS 0.75%	Rosuvastatin 10 mg/kg	Fenofibrate 30 mg/kg
S. Cholesterol mg/100ml	58.31±9.70 a	104.33±23.91 b	63.33±3.26 a	57.33 ±4.17 a	51.33 ± 7.85 a	79 ± 5.23 ab
S. TG mg/100ml	57.25±17.45 a	174.83 ±53.8 b	150.33±11.99 b	148.50±9.76 b	40.33 ±3.95 a	42.83 ±3.97 a
S. HDL mg/100ml	35.33 ± 6 ab	32.66 ± 5.77 a	44 ± 2.39 b	44.66 ±3.16 b	35.50 ± 3.64 ab	37 ± 2.59 ab
S. LDL mg/100ml	6.51 ± 1.71 a	29 ± 11.51 b	9.43 ± 1.08 a	6 ± 0.47 a	17.60 ± 3.60 a	30.83 ±3.42 b

The same letters mean that there is no significant difference

The different letters mean there is a significant difference at P < 0.05

There was a statistically significant reduction in both serum LDL and TC by both concentrations of TGS (0.50% and 0.75% w/w). While the same concentrations of TGS reduced serum TG when compared with hyperlipidemic rats, but the result was not statistically significant, Table 2.

Both concentrations of TGS (0.50%, 0.75% (w/w)) increased serum HDL-C significantly, when compared with hyperlipidemic rats.



Daily administration of Rosuvastatin of (10mg/kg) for six weeks reduced serum TC, LDL-C and TG level significantly when compared with hyperlipidemic rats (Table 2).

Administration of Fenofibrate at a dose (30 mg/kg) for six weeks significantly reduced serum TG when compared with hyperlipidemic animals. However the same dose had no significant effects on serum LDL-C and TC level in hyperlipidemic rats (Table 2).

Effects of TGS (0.75% w/w) on liver enzymes of normal rats (n= 12):

Daily use of TGS (0.75% w/w) for six weeks increased both serum AST and ALT significantly when compared with control group as seen in table 3. While the same dose of TGS (0.75% w/w) had no significant effect on serum ALP when compared with control group (Table 3).

**Table 3: Effects of TGS (0.75% w/w) on liver enzymes of normal rats (n= 12).**

Parameter	Control group	TGS 0.75%	Statistical evaluation
S. AST	106 ± 3.66	134.83 ± 7.58*	0.007
S. ALT	37.66 ± 1.17	44.16 ± 2.18*	0.025
S. ALP	25.26 ± 2.62	21.5 ± 1.54	0.244

\* compared to the control.

**Effects of different doses of TGS (0.50% and 0.75% (w/w)), Rosuvastatin and Fenofibrate on liver enzymes of hyperlipidemic rats (n=36):**

Table 4 shows that the serum levels of AST, ALT and ALP did not change significantly in rats treated with atherogenic diet when compared with control group. As shown in Table 4 no significant change in liver enzymes (S. AST, S. ALT and S. ALP) was also observed in hyperlipidemic rats treated with TGS (0.50% w/w), while high concentration of TGS significantly affected both serum AST and ALP of hyperlipidemic animals.

Both Rosuvastatin (10mg/kg) and Fenofibrate (30mg/kg) significantly increased the serum level of ALP (alkaline phosphatase) of hyperlipidemic rats.

**Table 4: Effects of different concentration of TGS (0.50%, 0.75%w/w), Rosuvastatin and Fenofibrate on liver enzymes of hyperlipidemic rats (n=36)**

Parameter	Control group	Hyperlipidemic group	TGS 0.5%	TGS 0.75%	Rosuvastatin (10mg/kg)	Fenofibrate (30mg/kg)
AST	106± 3.66 ab	94 ± 2.55 a	107.5±5.69 ab	126.33±20.46 b	114.50±4.07 ab	114.8±3.94 ab
ALT	37.66±1.17 a	41.66±2.13 a	41 ± 3.10 a	43.33±1.20 ab	45.16±4.36 ab	51.50±3.37 b
ALP	25.26±2.62 ab	34.50±2.68 b	23.4±3.37 ab	19.93 ± 1.33 a	38.5±5.55 c	41.66±6.76 c

**Effects of TGS on MDA of normal rats (n=12).**

No significant changes in the serum of malondialdehyde were observed between the normal rats taking TGS (0.75% w/w) and control group as shown in Table 5.

**Table 5: Effects of TGSP on MDA in normal rats (n=12)**

Groups	Serum MDA $\mu\text{mol/L}$
Control	$3.35 \pm 0.16$
TGS 0 (0.75% w/w)	$3.36 \pm 0.22$

**Effects of TGS on MDA of hyperlipidemic rats (n=24):**

Serum MDA level of rats fed with atherogenic diets increased significantly when compared with control group as shown in (Table 6).

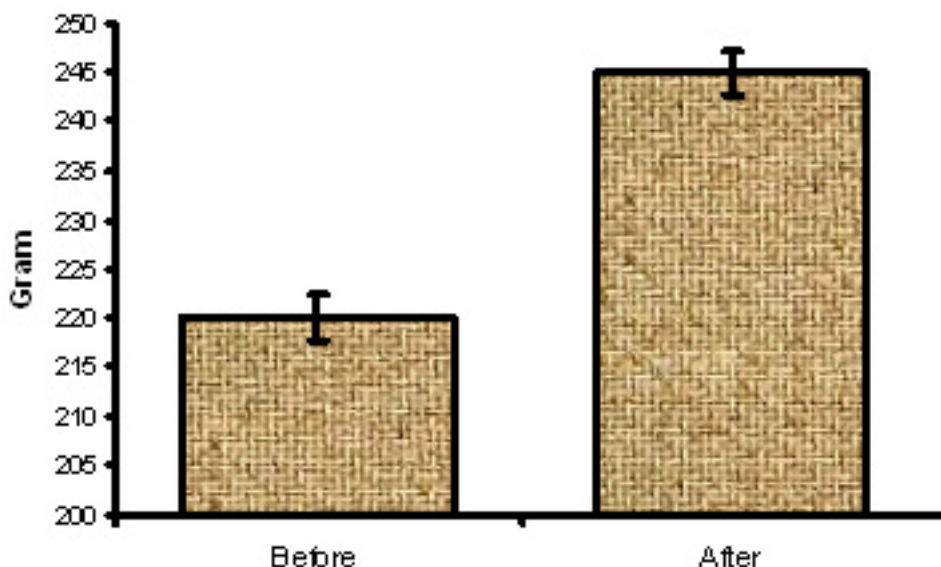
Daily administration of TGS of both concentrations (0.50% and 0.75% w/w) significantly decreased serum MDA level of hyperlipidemic rats.

**Table 6. Effects of TGSP on MDA of hyperlipidemic rats (n=24)**

Groups	Serum MDA $\mu\text{mol/L}$
Control	$3.35 \pm 0.16$
Hyperlipidemia	$6.39 \pm 0.87^*$
TGS 0.50% (w/w)	$2.3 \pm 0.1$
TGS 0.75% (w/w)	$2.07 \pm 0.12$

**Effects of TGS (0.75% w/w) on body weight of normal rats (n=12) after six weeks.**

There was a significant increase in body weight of rats taking diets containing TGS as shown in Figure 1.

**Figure 1: The effects of TGS (0.75 %w/w) on the body weight of normal rats****Comparison of hypolipidemic efficacy of TGS with Rosuvastatin and Fenofibrate of hyperlipidemic rats:**

As shown in Table 2 no significant differences in hypolipidemic efficacy were found in serum TC between rats taking both concentrations of TGS (0.50% and 0.75% (w/w)) when compared with each of Rosuvastatin (10mg/kg) and Fenofibrate (30mg/kg) group.

Unlike Rosuvastatin and Fenofibrate, both concentrations of TGS (0.50% and 0.75% (w/w)) did not significantly decrease serum TG of hyperlipidemic rats.

Effect of TGS of both concentrations (0.50% and 0.75% (w/w)) on serum HDL-C was non-significantly higher than that of Rosuvastatin (10mg/kg) and Fenofibrate (30mg/kg), Table 2.

As shown in Table 2. TGS (0.75% w/w) non-significantly was more effective in reducing serum LDL-C than that of Rosuvastatin.

#### **Comparison of TGS effects on liver enzymes with Rosuvastatin and Fenofibrate of hyperlipidemic rats:**

Unlike both Rosuvastatin and Fenofibrate, which caused a significant rise in ALP level, *Trigonella foenum graecum* seeds (0.75% w/w) significantly decreased serum ALP of hyperlipidemic rats.

Table 4 shows no significant difference in the effect of the TGS at (0.75% w/w) with Rosuvastatin (10mg/kg) and Fenofibrate (30mg/kg) on each of serum AST and ALT of hyperlipidemic rats.

## Discussion

Dyslipidemia is a major cause of atherosclerosis and atherosclerosis associated with conditions such as ischemic cerebrovascular disease, coronary heart disease and peripheral vascular disease (19, 20). Animal and human studies have established the role of cholesterol in the development and progression of atherosclerosis. Low density lipoprotein cholesterol (LDL) constitutes approximately 60-70% of serum total cholesterol (TC). Epidemiological studies directly implicated LDL to the development of atherosclerosis and coronary heart disease (21).

The result of the present study, showed that both concentrations of TGS (0.50% and 0.75% w/w) had reduced serum TC and LDL significantly and reduced TG level of hyperlipidemic rats. This result was similar to the findings of Kumar and Bhandari, (2013) who reported that giving aqueous extract of *Trigonella foenum graecum* (0.5 and 1g/kg orally) to hyperlipidemic rats for 28 days produced a significant reduction in serum TC and TGs (22). In another study Saxena and Saxena (2009) observed that administration of aqueous seed extract of *Trigonella foenum graecum* (120mg/kg, p.o.) for seven weeks to high fat diet and triton induced hyperlipidemic models of albino rats showed a 20.42% reduction in plasma cholesterol level and significantly attenuated the elevated plasma triglycerides and LDL level (23). Similar findings were reported by Patel et al., (2011) who showed that administration of *Trigonella foenum graecum* ethanol extract at a dose of 250 mg/kg for one week induced a significant reduction in serum LDL, TC and TG and significantly increased HDL level of hyperlipidemic rats (24).

In this study, TGS (0.75% w/w) significantly increased serum HDL level of normal and hyperlipidemic rats. These result were in agreement with the results of another study by Xue et al., (2007) who found that rats treated with *Trigonella foenum graecum* extracts of different doses (0.44

g/kg/day, 0.87g/kg/day and 1.74g/kg/day for 6 weeks) had lower blood glucose, serum TG and TC and higher serum HDL in a dose dependent way of diabetic rats (25).

Elmnan et al., (2012) also observed that TGS added to experimental diets at concentrations of 0.25%, 0.50% and 0.75%w/w have significantly decreased plasma total lipid and TG (26).

Other studies reported that the seeds of *Trigonella foenum-graecum* contain an unusual amino acid, which significantly decreased the plasma TG levels and TC, and free fatty acids, accompanied by an increase in HDL /TC ratio in the dyslipidemic hamster model ( 27, 28).

The reduction in TG and TC level in hyperlipidemic rats induced by TGS could be due to presence of bioactive fibers which act to decrease the rate of gastric emptying thereby delaying the absorption of lipid from the small intestine (29) or it directly inhibits the absorption of cholesterol by enterocytes of small intestine as ezetimibe (30), or it binds to bile acids and increases excretion of bile acids and neutral sterols in faeces (31). This action prevents the enterohepatic cycling of acids and obligates the liver to synthesize replacement of bile acids from cholesterol (32).

Rosuvastatin (10mg/kg) caused a significant decrease in serum TC, TG and LDL of hyperlipidemic rats. This result was in accordance with the observations of Ansari et al., (2012) who found that oral administration of Rosuvastatin (10mg/kg/day) for 21 days along with high fat diet had reduced serum TC, TG and LDL significantly when compared with hyperlipidemic rats (17).

This reduction of serum TC of Rosuvastatin is due to inhibition of HMG-CoA reductase which catalyzes the conversion of HMG-CoA to mevalonate which decreases the cholesterol synthesis (33, 34).

The results of the present study showed that Fenofibrate (30mg/kg/day) significantly reduced serum TG when compared with hyperlipidemic rats and this is similar to the findings of Santiago et al., (2013) who found that administration of 10mg/kg of Fenofibrate in hyperlipidemic mice significantly decreased TG by 54.87% (35). The remarkable decrease in TG levels by Fenofibrate supports the literature stating that it increases the expression of genes for lipoprotein lipase, and decreases the expression of apolipoprotein CIII. Apolipoprotein CIII is a known potent inhibitor of lipoprotein lipase while apolipoprotein CII activates the same enzyme. An imbalance in apo CIII/ CII ratio due to increase in plasma apolipoprotein CIII may cause inactivation of lipoprotein lipase (36 Moberly et al., 1999).

In this study Fenofibrate did not reduce serum LDL of rats fed with atherogenic diet and this is in contrast with the study of Li et al, (2010) who found that hyperlipidemic rats treated with a high dose of Fenofibrate of 80mg/kg/day for 12 weeks reduced serum TG ,TC and LDL significantly. This indicates that the beneficial effects of Fenofibrate on

serum LDL probably needs more time and higher doses would have been more informative in our study (37).

Serum transaminases (ALT, AST) in this study, increased significantly in hyperlipidemic rats treated with TGS of both concentrations (0.50% and 0.75% w/w). This result was incompatible with another study reported by Kumar and Bhandari (2013) who observe that aqueous extract of TGS of (0.5 and 1g/kg, orally) for 28 days caused a significant reduction in serum transaminases (22). This discrepancy with our findings could probably be due to the higher doses of TGS and longer duration of the study. Conversely, Toppo et al., (2009) reported that TGS powder did not alter ALT, AST and alkaline phosphatase (ALP) levels maintained on (1%, 5%, 10%) up to 90 days (38). In another study Haeri et al., (2009) found that unusual amino acid (4-hydroxyisoleucine) isolated from the plant did not affect liver damage markers but it significantly improved HDL cholesterol levels (31% increase) in diabetic rats (30).

Serum alkaline phosphatase (ALP) of hyperlipidemic rats was significantly increased by daily administration of Rosuvastatin (10mg/kg), this result was similar to a study reported by Dodiya et al., (2013) who found that orally administration of Rosuvastatin (40mg, 80mg/kg) for 21 days to rats significantly increased AST, ALP and total bilirubin levels. The serum ALP elevation might be in response to direct irritant effect of Rosuvastatin on hepatic cells (40).

In the present study Fenofibrate (30mg/kg) increased serum ALP and ALT significantly when compared with hyperlipidemic rats. It has been reported that Fenofibrate activates the aminotransferase gene expression, thus leading to a mild and transient elevation of aminotransferase via PPAR $\alpha$  through mechanisms involving increased levels of reactive oxygen species and intracellular glutathion depletion, thus leading to mitochondrial dysfunction and a perturbation of intracellular Ca<sup>++</sup> homeostasis and also cell death (41); (37).

In this study, Malondialdehyde an end product of polyunsaturated fatty acid peroxidation, was increased significantly in the hyperlipidemic group (taking atherogenic diet 21% w/w) when compared with control group.

Hypertriglyceridemia and hypercholesterolemia were associated with oxidative modification of LDL, protein glycation, glucose-auto oxidation, thus leading to excess production of lipid peroxidation products which may cause elevation of oxidative stress in higher lipid and hyperlipidemic subjects. Clinical and epidemiological studies have proven that individuals with elevated LDL showed an increased risk for cardiovascular diseases (42). HDL may be protective by reversing cholesterol transport, inhibiting the oxidation of LDL and by neutralizing the atherogenic effects of oxidized LDL (43).

Increased lipid peroxidation is thought to be a consequence of oxidative stress which occurs when the dynamic balance between pro oxidant and antioxidant mechanism is impaired (44). It is known that hyperlipidemic states

are associated with altered physical properties of cellular membranes (45), which may facilitate the escape of free radicals from the mitochondrial electron transport chain or the activation of NADPH oxidase (46).

The present study demonstrated that TGS significantly reduced malondialdehyde level of hyperlipidemic rats. However the same dose of the plant did not show a significant reduction in malondialdehyde level of normal rats. These results are in agreement with the study of Kumar and Bhandari, (2013) who detected that aqueous extract of TGS (0.5 and 1g/kg, orally) for 28 days produced a significant reduction in serum malondialdehyde of hyperlipidemic rats (22). This could be suggestive of an antioxidative effect during oxidative stress induced by hyperlipidemia and increased lipid peroxidation. Myhrstad et al (2002) found the antioxidant activity of flavonoids isolated from the seed of *Trigonella foenum graecum*, as it exhibited scavenging of hydroxyl radicals (OH) and inhibition of hydrogen peroxide-induced lipid peroxidation in rat liver mitochondria (47).

In this study, TGS (0.75% w/w) increased the body weight of normal rats significantly. This outcome is in agreement with the results of Elman et al., (2012) who observed that TGS added to experimental diets at concentrations of 0.25%, 0.50% and 0.75%w/w have significantly increased the body weight of normal rats (26). This effect on body weight could be attributed to the appetizing activity of steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin) which are the major constituents of TGS (44, 45). Therefore, it is possible that the presence of saponins in the plant is responsible for the antioxidant and appetizing activity.

According to the above results on the effects of TGS, Rosuvastatin and Fenofibrate on the lipid profile and liver enzymes of hyperlipidemic rats, it can be observed that TGS has similar efficacy of Rosuvastatin and Fenofibrate in reducing TC. Whereas the seed powder was non-significantly more efficient than Rosuvastatin and Fenofibrate in changing serum HDL and LDL. Moreover, unlike to Rosuvastatin and Fenofibrate it significantly decreased serum ALP of hyperlipidemic rats.

## Conclusion

TGS has similar efficacy of Rosuvastatin and Fenofibrate in reducing TC. Whereas, TGS was non-significantly more effective than Rosuvastatin and Fenofibrate in changing serum HDL-C and LDL-C.

Fenofibrate and Rosuvastatin significantly increased serum alkaline phosphatase (ALP), while *Trigonella foenum graecum* seeds significantly decreased ALP for hyperlipidemic rats.

Rats that received TGS exhibited a significant decrease in malondialdehyde level when compared to hyperlipidemic rats.

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Our cover this month features Dr Manzoor and Mrs Rahila Butt of Rawalpindi, Pakistan with their firstborn grandson Mohammad Abdur Rafay.

Both are great humanitarians and tireless workers and contributors to their community with a particular focus on women's and girl's health and the health and practical needs of the impoverished.

We have featured the many humanitarian community projects of Dr Butt, a family physician, in our journals over the past years and this time we would like to also acknowledge the work of Rahila who has assisted Dr Butt in his clinic and in his community projects.

Rahila is a locally trained Lady Health Technician capable of assisting in general physical examination of children and women. She can assist in labour and ante natal care. She is an integral part of all breast and pelvic examinations performed in the clinic. It is also one of her important duties to inform women patients about details of breast and pelvic examination before the check ups.

Dr Manzoor advises that the most important property that Rahila possesses is her readiness, intention and will to help deprived children and women.

In the clinic, she acts as assistant, social counsellor and psychotherapist.

Rahila has a strong rapport with our female population who trust her in their breast and reproductive problems.

She guides interested women in how to improve family income through home based income generation activities.

Rahila plays a vital role in our family life as wife, mother, mother in law and now as grandmother. We take this opportunity to congratulate both Dr Butt and Rahila on the birth of their grandson and to thank them for their tireless efforts to make the lives of those in their community, happier and healthier.

