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DISCOVERING THOUGHTS AND INVENTING FUTURE

Highlights

- Organic Dysfunctions
- Antibacterial Activity
- Anti Diabetic Therapy
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The Blood Plasma

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Outcomes of Counterfeit Drugs in the Prevention and Treatment of Diseases and Organic Dysfunctions in Africa

By Addis Demissie & Professor Yemane Berhane
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Abstract - I would like thank Prof. Yemane Berhane for his close guidance and technical assistances from inception to the end of this paper. My special thanks also go to my wife, W/ro Helen Bekele and my kids, Herma Addis and Michael Addis for their encouragements and family help to realize and complete my MPH studies.

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Outcomes of Counterfeit Drugs in the Prevention and Treatment of Diseases and Organic Dysfunctions in Africa

Addis Demissie [α] & Professor Yemane Berhane [σ]

I. Acknowledgements

I would like thank Prof. Yemane Berhane for his close guidance and technical assistances from inception to the end of this paper. My special thanks also go to my wife, W/ro Helen Bekele and my kids, Herma Addis and Michael Addis for their encouragements and family help to realize and complete my MPH studies.

II. Summary

The WHO defined counterfeit medicine as one which is deliberately and fraudulently mislabeled with respect to identity and/or source. There are many ways of presenting counterfeit drugs by the black marketers. Branded and generic drugs could be counterfeit and these products may contain the right or wrong ingredients; without active ingredients, with insufficient active ingredients or with fake packaging. The availability of counterfeit drugs in the market is considered by any country as problem. Published articles indicate that in developing countries a wide spectrum of types of counterfeit drugs, ranging from the precise copy of a genuine product to the extreme case of a drug product with none of the correct active ingredient exist.

Counterfeit drugs are a major threat for the treatment of deadly diseases, including malaria, tuberculosis, HIV/AIDS, and other chronic diseases in Africa. Development of drug resistant pathogenic organisms due to counterfeit drugs containing little quantities of the active ingredients has not only cause treatment failure and spread of drug resistant strain of the pathogenic organisms but also contribute to the death and disability of the patients who have taken the drug. Moreover, it incurs huge care and treatment cost while shifting the patient to the newer and costly drugs. This ultimately affects the country's health system and contributes to the loss of confidence on the health professionals who are giving the service to the patients in particular and the country's health system in general.

The widespread distribution of these drugs and troubling victimization rates added up to a long list of health and wellbeing challenges in the region. Apart from their role in compromising the prevention and treatment of diseases and organic dysfunctions, they are known to kill quite a huge number of people in African countries. Likewise, they are also known of depriving the revenue of these countries.

Countries should work in harmony to stop and control the manufacturing and distribution of counterfeit drugs. Above all, African countries should work hard to have a strong drug administration and control regulatory authorities and a strong Pharmacovigilance system which have a legal power to control and stop this rampant problem which is playing with the lives of citizens.

III. Background

The World Health Organization (WHO) defines counterfeit medicines as those that are deliberately and fraudulently mislabeled with respect to identity or source: their quality is unpredictable as they may contain the wrong amount of active ingredients, wrong ingredients or no active ingredients(1). They are usually manufactured in illegal and hidden laboratories which range from cottage to large factories that do not have any quality control system(1, 2). Their availability in the developing countries' market occurs in unregulated market and most of the vendors are not official or licensed (2).

Counterfeit drugs are predominantly available in countries where the custom procedure is not stringent and this usually results in loss of public confidence in the health care system. Among other drugs, life saving drugs are the principal target of counterfeiters(2). Moreover, among many methods of counterfeiting drugs, mimicking branded products in terms of their packaging and dosage forms would enable counterfeiter's to pass custom inspection easily. As these products are very similar to the original brands, they can be accepted by distribution and retails companies(3). Some studies showed that the pharmaceutical industry lost USD 30 billion as a result of counterfeit drugs in 2005 alone(4).

Though the overall death due to counterfeit drugs is not well known, it is a fact that its damage on

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the costs of public health is so huge. Among other direct impacts on individuals, resistance to medicine for curing diseases is considered to be the leading cause of mortality as a result of treatment failure. Counterfeit anti malarial drugs are also contributing a lot in the death of a million people around the world(5). The prevalence of counterfeit drugs in developing countries is very high and it is estimated to be 60% in countries which are found in African and Southeast Asia. Apart from this, most of the counterfeit drugs are those that are used to treat serious diseases such as malaria, tuberculosis and HIV/AID(6). In general, counterfeit drugs are made in various categories ranging from life-threatening diseases such as diabetes to simple pain killers and other lifestyle products such as drugs for erectile dysfunctions(2).

There are also some countries like India and china which are playing prominent role in the production of counterfeit drugs which is very much associated with the permissive legislation and inefficient judiciary system, absence of qualified supervising staff and widespread corruption(3, 7).

The counterfeit drug issue was first discussed in World Health Assembly in 1989 and launched the International Medical Products Anti-Counterfeiting Task Force (IMPACT) up to 2016 to enforce and tackle the counterfeit drugs problem in the world(5).

a) Objectives

The general and specific objectives are mentioned below:

i. *General objective*

To assess the health outcomes of counterfeit drugs on the prevention and treatment of diseases and organic dysfunctions.

ii. *Specific Objectives*

1. To review the health outcomes of counterfeit drugs on diseases and organic dysfunctions with special emphasis on Africa.
2. To indicate the magnitude, sources and distribution of counterfeit drugs in Africa in particular and the world in general.

IV. Methods

Published articles which are freely available from the PubMed were searched using the following key words and the followings were found: 121 published articles using 'counterfeit drugs' limiting the year below 10 years and in English language; 37 published articles using 'Counterfeit drugs AND Africa' limiting the years below 10 years and in English language; 19 published articles using ' Counterfeit drugs AND sources in Africa' limiting the years below 10 years and in English language; 11 published articles using 'Counterfeit drugs AND determinants in Africa limiting the years below 10 years and in English language. I also accessed DOAJ with the same key words; nevertheless, I could not find any published article on the subject.

The abstracts were then down loaded, read and selected 22 published articles which have direct relevance and which also comply with the objectives of the review. Twenty two articles were downloaded and kept them in a folder for review and queries which may be raised either by the readers or my advisor. All of them were read and tried to understand the core messages and findings which are indicated in the articles. To comply with the objectives, the review focused on:

- The sources of counterfeit drugs and the rationale behind why they are available in the market.
- The prevalence, magnitude and distribution of counterfeit drugs in Africa in particular and the world in general.
- The health effects they are bringing to the users in Africa in particular and the world in general.

The review gave special emphasis to the widespread and prevalent diseases in Africa such as malaria and HIV/AIDS, infections which require antibiotics for cure and other chronic non communicable diseases such as diabetes and dysfunctions which are very predominate on males, Erectile Dysfunction(ED).

Software called EndNote X5 was used to arrange the references in order using Vancouver style.

V. Synthesis

a) Magnitude of counterfeit drugs

According to WHO, "counterfeit drugs are defined as those medicines that are deliberately and fraudulently mislabeled with respect to identity and/or sources and include those products with correct ingredients or with wrong ingredients, without active ingredients, with insufficient or excessive amount of active ingredients or with false or misleading labeling"(1). In such regards, there are many types of counterfeit products classified based on their activities. There are products which contain the correct active ingredients and additives in the right amount and they are called perfect counterfeits(1). Likewise, there are also products which contain the right components with an incorrect concentration and/or formulation having defeggctive quality and they are called imperfect counterfeits. There are also products which are similar to the original product but containing non active ingredients or foreign substances and they are called apparent counterfeits(1). In the same way, products which are apparently similar to the original medicinal product but not having any active ingredients which cause harmful or toxic substances and they are called criminal counterfeits(1).

A study was conducted to know the prevalence of counterfeit drugs in some African and Latin American countries like Angola, Brazil, Cameroun, Central African Republic, Chad, Congo, Ethiopia, Guinea Bissau,

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Guinea Conakry, India, Kenya, Madagascar, Malawi, Rwanda, and Uganda. Samples of some drugs were purchased from both from licensed and unlicensed pharmacies and they were tested for quality(2). The following result was obtained as indicated in the table below:

Table 1 : Therapeutic classes of total and counterfeit samples.

Therapeutic classes	No. (%) of samples	
	Available for analysis	Counterfeit
Antibiotics	76 (34.4)	30 (29.7)
Antipyretics	24 (10.9)	9 (8.9)
Antimalarics	17 (7.7)	6 (5.9)
Antimycotics	13 (5.9)	9 (8.9)
Antihypertensives	8 (3.6)	1 (1.0)
Antianemics	5 (2.3)	4 (4.0)
Spasmolytics	5 (2.3)	2 (2.0)
Diuretics	5 (2.3)	1 (1.0)
Antiacids	5 (2.3)	2 (2.0)
Anti-inflammatories	44 (19.9)	22 (21.8)
Bronchodilators	4 (1.8)	5 (5.0)
Others	15 (6.8)	10 (9.9)

One study has also shown that, out that of 51 artusinate counterfeits which went through spectrometry analysis happened to have other drugs such as paracetamol, sulphadoxine, pyrimethamine, dimenhydrinate, erythromycine, and other active substances which are used for other purpose(7). Some of the counterfeit artusinates were also identified to contain banned substances which are very dangerous to health and some of them were identified as produced in southeast of People Republic of China(7). In another study done in Indonesia in 2006, one fifth of amoxicillin tables and 5 out of 22 samples obtained from pharmacies contained slightly less active substances than required; 50% of co-trimoxazole tablets had a trimethoprim content which was not meeting BP standards; deviations were up to 20% of the required amount (8).

i. *Sources of counterfeit drugs*

Highly priced and life-saving drugs, in general, are the target of the counterfeiters(2). Developing countries which do not have controls and sufficient Pharmacovigilance systems are the victims of counterfeit drugs(2). Moreover, provision of service by private sector on availability of anti malarial drugs with lower prices than the formal public health centers has also contributed to the widespread distribution of

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counterfeit drugs in Africa(9).The major driving force for counterfeiter to make available counterfeit drugs in the market is known to be for profit(7).

There are some countries like India and china which are playing prominent role in the production of counterfeit drugs which is very much associated with the permissive legislation and inefficient judiciary system, absence of qualified supervising staff and widespread corruption(3, 7). In accordance with WHO assessments, India was the first exporting country among those examined in the present study, with 61 samples (about 50% of which were counterfeits)(2).

ii. *Distribution of counterfeit drugs*

Counterfeit drugs are severely affecting African and other poorer countries and found to be a significant cause of morbidity, mortality and loss of public health confidence. Likewise, the scale of the problem is rising at alarming rate(10) . The prevalence of availability of counterfeit drugs in African and other pooper countries reached about 60%(6). In one study done in pharmacies found in Lagos Nigeria, the prevalence went up to 80%(6). In the same token, two third of anti malarial drugs which were supplied in Ghana in 2009 were counterfeit and 68% of the drug called 'CaortemR tablets are counterfeit in Laos, Burma, Vietnam and Cambodia(11). And one study also showed that 38% to 54% of oral artusinate collected, by convenience sampling, in 2000–2001 and 2002–2003, respectively, were counterfeit(12). Among other examples, counterfeit ARVs which were found in Ivory Cost and DRC can also show the spread, distribution and severity of the problem in African countries(11). But the world circulation of counterfeit drugs is estimated to be 15% and the figure went up to 50% in some part of Africa and Asia(13). A study done in 2006 indicated that the glucose test strips which were manufactured in China were found to give false readings which ultimately urge the patients to take high dose of insulin in 2006(6).

iii. *Health outcomes of counterfeit drugs on diseases and organic dysfunctions*

With regard to the health outcomes of counterfeit drugs, malaria is one of the targets of counterfeiters(7). And malaria is estimated to kill one million people per year and the majority are young children under five in Africa(10). Quality of anti malarial drugs in sub Saharan Africa is a concern as it plays crucial role in the control and management of the disease. Counterfeit or substandard drugs either produce toxic or adverse effects if they are found in higher quantity above the normal dose or result below therapeutic levels if they are found below the standard dose. The availability of lower doses of the drug in the blood stream will bring about resistance as the drug could not kill the parasite efficiently which thereby help the drug resistant parasite to flourish(13).

The effect of using these fake anti malarial drugs will facilitate resistance to malaria parasites and ultimately encourage the spread of drug resistant malaria (11, 12, 14). As a result of this, high-level pyrimethamine resistances seen in Africa which was believed to come from SE Asia(11, 12). In another study done on 30 anti malarial tablets samples containing drugs like chloroquine, quinine, mefloquine, sulphadoxine/pyrimethamine, many kinds of problems were observed such as poor dissolution(in about 50% of the samples) but among which low content of the active ingredients were the most important one from clinical point of view(15).

It is obvious that for a disease like malaria especially in young children, counterfeit drugs which have got either little or no active ingredient can be considered as intentional murder. The patient will not be cured of malaria and there will be a possibility of losing him/her due to death and there is also a danger of contracting disability(16). Though artusinate containing therapy(ACT therapy) was considered as hope to control malaria in Africa and Asia, the counterfeit artusinate are contribution a lot in wide distribution of resistant malaria in the regions(17). This has been persuading the drug to shorten the useful life of the same(18).Likewise, the drugs will develop resistance to the parasites which will urge the care taker to go for newer and more expensive drugs(12, 13, 19).

With regard to antibiotic usage for the treatment of diseases, resistance development is the most important determinant of treatment failure. Among other factors such as low-dose regimen, counterfeit products which do not contain antibiotic at all or containing low dose also played leading role which will finally end up with treatment failure(8). The drug failure is attributable to reduced adherence to therapy, suboptimal dosing, diagnostic and laboratory error, ineffective control and the most important of all is the usage counterfeit drugs(20). Price may be considered as one of the factors for the drugs to be counterfeited. The average price for the active ingredient of amoxicillin is about 26 Euro/kg and duly attracts counterfeits to a great extent(2). In some African countries like Nigeria, counterfeit antibiotics are well known to be available in the market which made the improper use and control of antibiotic difficult(21).

ARVs are also a target for counterfeiters. As most poor countries do not have or insufficient capacity for pharmacological control, it is very difficult for them to halt the health damages that would result due to using these sub standard drugs(22). Though sufficient prevalence data is not available, ARVs which are smuggled from other countries illegally are used in some African countries. As ARVs do have high unit cost and long-term and sustained demand, they are now the major target for the counterfeiters. Some patients are using these counterfeited ARVs to avoid stigma and fear

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related to keeping their cases confidential(3). Some studies have shown that there were counterfeit ARVs in Ivory Cost and DRC which signaled the spread, distribution and severity of the problem in African countries(13). With regard to ART, adherence to the treatment is expected to be strictly followed and counterfeit drugs are known to break the chain so that the patient will be liable either to switch to other combinations of ARVs such as second line regimen or death(22).

Among other examples, counterfeit ARVs which were found in Ivory Cost and DRC can also show the spread, distribution and severity of the problem in African countries(11). But the world circulation of counterfeit drugs is estimated to be 15% and the figure went up to 50% in some part of Africa and Asia(13). There was an alert from WHO in 2006 that there appeared counterfeit ARV which was supposed to have three ARVs in fixed dose combinations (zidovudine/lamuvidine/indinavir) and which were finally identified to have only one ARV, zidovudine(3). Recently, a counterfeit ARV triple fixed dose combination of stavudine/lamuvidine/nevirapine and a dual fixed dose combination of lamivudine/zidovudine have been found in central Africa which is a dangerous hurdle to the treatment of AIDS in sub sharan Africa(10).

Among chronic non communicable diseases, diabetes is one them and it is also the target of counterfeiters. There are many episodes of the dangers of these counterfeit drugs on diabetic drugs. One study done in Singapore on 150 non diabetic patients who were admitted in hospital indicated that seven patients were in coma due to neuroglycopenia which resulted in the death of four patient(1). In relation to diabetes, the blood glucose test strips that are used by most patients to check their blood glucose were also identified to be a target(7).

Drugs which are used for the treatment of erectile dysfunction(ED) are among others where counterfeiters are interested. Between 2004 to 2008 alone, 35.8 million tablets of sildinafil were found in the markets of European countries. As the disease is embarrassing for the patients and the cost of the drug is very high, counterfeiters were very much attracted to the business. Accordingly, the patients who were using these counterfeit sildinafil tables were exposed to direct and indirect risks. Among direct risks stated in the study is failures of the treatment is predominant. The indirect effects could be seen in the patients that they may lose confidence on their physicians and thereby resulting in not disclosing their case anymore.(1).

As it can be seen in the above data, counterfeit drugs are currently a threat to the management of diseases and organic dysfunctions in particular and the public health system in general. Many health outcomes which range from development of drug resistances to the susceptible to pathogenic agent to deaths are occurring. Besides, they are also compromising the health programs and policies laid down by countries and other international stakeholders.

VI. Limitations of The Review

The review was supposed to be done to show the health outcomes of counterfeit drugs on many types of diseases and organic dysfunction in Africa. However, the focus is narrowed only to few types of diseases and organic dysfunctions as a result insufficient availability of published articles in freely available websites such as PubMed and others. Even if the problem is rampant and hot issue for Africa and the world, it seems that only few researches were conducted. But this review has vividly indicated to the researchers as an opportunity to conduct many researches which are targeting on the prevalence, magnitude, determinants, sources distribution and health outcomes of these counterfeit drugs and counterfeiters.

VII. Conclusions and Recommendations

Among the major health outcomes of counterfeit drugs, development of resistance and toxic action to the body which will ultimately end up with death were mentioned. Studies done on the area also indicated that there are some individuals and countries which are involved in such kind of unethical business. Even if the major driving force to be engaged in this counterfeiting business is known to be for profit, the countries' loose legislation on the registration, import, distribution, manufacturing and retail activities have been identified to play a pivotal role.

The problem is not only confined to one country but it is currently becoming the problem of all nations. Being Africa very poor, the prevalence of the problem seems very huge and therefore African governments do have responsibility to protect its people from being victims of such counterfeit drugs.

As general recommendations, the followings could be laid:

- As the problem is rampant and crosses boarder, there shall be partnership among countries which are responsible for manufacturing and distribution these counterfeit drugs. In such regards, WHO's effort called International Medical Products Anti-Counterfeiting Task Force (IMPACT) should be strengthened.
- Countries should build stringent regulatory authority to control and administer drug and drug related substances in collaboration with custom authorities.
- There should be a clear post market surveillance system that will aid the fast identification of counterfeit and other substandard drugs.

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- A strong Pharmacovigilance system which goes from bottom to top should be established so that any adverse/unwanted effects and other drug related problems will be identified.
- Public awareness raising activities should be carried out so that citizens could have a capacity to protect their health from these counterfeit drugs...

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Influence of Anti Diabetic Therapy on Plasma Lipid Profile and its Relation to Erythrocyte Membrane Lipid Levels in Type 2 Diabetic Subjects

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Abstract - The diabetes induced dyslipidemia may lead to an alteration in RBC membrane cholesterol/phospholipids ratio in diabetic subjects resulting in an alteration in RBC membrane properties. It has been observed in our laboratory that diabetes induced dyslipidemia causes a change in RBC membrane lipid composition in type 2 diabetic subjects. The effect of various oral anti diabetic drugs and or Insulin therapy on diabetes induced RBC membrane lipid alteration is not established. Hence the present work was undertaken to study the influence of anti diabetic drugs and or Insulin on RBC membrane lipid composition in type 2 diabetic subjects. Blood samples from randomly selected type 2 diabetic subjects were collected after obtaining written consent. The plasma lipids as well as RBC membrane lipids were estimated. The study group include normal subjects (group-1), control diabetics diabetic subjects (group-2), diabetic subjects receiving oral drugs (group-3), diabetic subjects receiving insulin (group-4) and diabetic subjects receiving both oral drugs and insulin (group-5).

Keywords : *RBC membrane lipids, anti diabetic drugs, plasma Lipids.*

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Influence of Anti Diabetic Therapy on Plasma Lipid Profile and its Relation to Erythrocyte Membrane Lipid Levels in Type 2 Diabetic Subjects

Basavaraj S. Aski [α], B. B. Devarnavadagi [σ], G. Rudrappa [ρ] & R. T. Kashinath [ω]

Abstract - The diabetes induced dyslipidemia may lead to an alteration in RBC membrane cholesterol/phospholipids ratio in diabetic subjects resulting in an alteration in RBC membrane properties. It has been observed in our laboratory that diabetes induced dyslipidemia causes a change in RBC membrane lipid composition in type 2 diabetic subjects. The effect of various oral anti diabetic drugs and or Insulin therapy on diabetes induced RBC membrane lipid alteration is not established. Hence the present work was undertaken to study the influence of anti diabetic drugs and or Insulin on RBC membrane lipid composition in type 2 diabetic subjects. Blood samples from randomly selected type 2 diabetic subjects were collected after obtaining written consent. The plasma lipids as well as RBC membrane lipids were estimated. The study group include normal subjects (group-1), control diabetics diabetic subjects (group-2), diabetic subjects receiving oral drugs (group-3), diabetic subjects receiving insulin (group-4) and diabetic subjects receiving both oral drugs and insulin (group-5). The study suggest an increase in plasma lipid levels with a parallel raise in RBC membrane lipid composition in diabetic subjects and hypoglycemic drugs -insulin combined therapy regime may help to control the diabetic dyslipidemia induced erythrocyte membrane lipid alterations.

Keywords : RBC membrane lipids, anti diabetic drugs, plasma Lipids.

I. Introduction

Diabetes Mellitus is a metabolic syndrome with disturbances principally in carbohydrate ,protein and lipid metabolism due to insulin deficiency or subnormal insulin functions. In diabetic subjects overproduction of FFA and impaired lipoprotein metabolism induces an increase in plasma lipid components.(1). The long-standing diabetes induces micro vascular complications due to oxidative damage of membrane poly unsaturated fatty acids (2,3). The membrane fluidity is directly related to the membrane phospholipids and cholesterol which are asymmetrically arranged in the membrane lipid bilayer. The relative amounts of phospholipids and cholesterol are responsible for basic structural integrity of the red cell membrane. There are conflicting results in the literature regarding variations of red cell membrane lipid levels and their relevance to plama lipid alterations in diabetic subjects (4). Our earlier report clearly indicates a direct relationship between plasma lipid profile and the erythrocyte membrane lipid composition (5). However no reports available to show the influence of plasma lipid changes on erythrocyte membrane lipid levels and its relationship with anti diabetic therapy. Hence an attempt being made in the present study to investigate the influence of anti diabetic therapy on the diabetes induced plasma lipid alterations and its effect on red blood cell membrane lipid levels in type2 diabetic subjects receiving various anti diabetic drugs and or insulin.

II. Materials & Methods

The type 2 diabetic subjects attending the medical OPD of Sri B M Patil Medical College and Hospital Bijapur were randomly selected and a brief diabetic history with the anti diabetic therapy was collected from each of these selected diabetic subjects. An informed consent was also taken from these subjects. Blood sample in fasting state was collected from these diabetic subjects using heparin as an anticoagulant. The blood samples were centrifuged at 3500 rpm for 8 minutes to separate plasma which was employed for estimation of glucose (6), total lipids (7), total cholesterol (8),triacyl glycerol (9), HDL cholesterol (10) & free fatty acids (FFA) (11). The RBCs were washed three times with 4 ml aliquot of normal saline and the washed erythrocytes were lysed by adding 3ml distilled water and stirring with a clean glass rod. The resultant mixture was centrifuged at 3500 rpm for 5 minutes. The supernatant was discarded and the membranes were washed three times with 3 ml aliquots of normal saline. One part of the erythrocyte membranes were homogenized with 9 parts of chloroform-methanol

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mixture (1: 1, v/v) for 7 minutes in a Potter-Elvejham tissue homogenizer. The resultant mixture was centrifuged at 3500 rpm for 5 mins and the clear supernatant was employed for the estimation of lipid profile:- total lipids (7), total cholesterol (8) and total phospholipids (12).

The results were statistically evaluated with student "t"test. The diabetic subjects (Group 2) are compared with normal subjects (Group 1) and groups 3, group 4 and group 5 were compared with one another for statistical evaluation.

III. Results

The present study included a total number of 166 subjects consisting 36 normal subjects (Group 1) and 130 type 2 diabetic subjects (Group 2). These diabetic subjects included 86 diabetics receiving oral anti diabetic drugs (Group 3), 28 diabetics receiving insulin (Group 4) and 16 diabetics receiving both oral anti diabetic drugs and insulin (Group 5).

The results obtained in the present study are given table 1- 3. Table 1 narrates the plasma levels of glucose and lipid profile levels in normal subjects(Group 1), in diabetic subjects (Group 2), in diabetic subjects receiving oral anti diabetic drugs (Group 3), in diabetic subjects receiving insulin alone (Group 4) and in diabetic subjects receiving both oral anti diabetic drugs and insulin (Group 5). It is seen from the table that the parameters included in the lipid profile (TL, TAG, PL and FFA) are significantly elevated in group 2 as compared to group 1 whereas the TL and FFA are significantly lowered in group 3, group 4 and in group 5 as compared to group 2.

Table 2 depicts the plasma cholesterol profile – total cholesterol, HDL cholesterol, LDL cholesterol and VLDL cholesterol in group1, group 2, group3, group 4 and in group 5 subjects. It is evident from the table that total cholesterol , LDL cholesterol and VLDL cholesterol levels were significantly raised in group 2 as compared to group 1 whereas the HDL cholesterol is significantly lowered. Further it is evident from the table that there is no much change in the parameters studied in group 3 and group 5 as compared to group 2 but a significant decrease is seen in total cholesterol , LDL cholesterol and in VLDL cholesterol as well as a significant raise in HDL cholesterol is seen in group 4 as compared to group 2.

Table 3 shows the erythrocyte membrane lipid levels – total lipids (mTL), total cholesterol (mTC), phospholipids (mPL) and the ratio mPL/mTC in group 1, group 2, group 3, group 4 and in group 5. As it evident from the table mTL and mPL were significantly raised whereas the ratio mPL/mTC is significantly lowered in group 2 as compared to group 1. No much alterations observed in group 3, group 4 and in group 5 as compared to group 2 whereas a significant raise seen in group 4 as compared to group 2.

IV. Discussion

Diabetes mellitus is a chronic syndrome involving not only disturbance in glucose metabolism, protein but also there is disturbances in lipid and purin metabolism, resulting in varied life threatening complications like nephropathy, cardiopathy, retinopathy etc. (**3, 13, 14**). Apart from hyperglycemia and glucoseuria in diabetes mellitus, lipid alteration has been observed by many workers (**15 -17, 19**). A significant raise was observed in serum total lipids ($p<0.001$), serum total cholesterol ($p<0.001$), serum phosholipids. ($p<0.001$) and in serum total free fatty acids. ($p<0.001$) in diabetic subjects as compared to normal subjects. This in agreement with earlier studies (**10, 18 – 26**). As well as with our earlier report (5).

The observed elevation in TL, TC, may be due to an increase in availability of more acetyl CoA, the starting substance for the synthesis of fatty acids and cholesterol (**22**). This is in part due to non availability of glucose for energy purpose and tissues do depend on fatty acid oxidation and increased fatty acid oxidation is responsible to increase cellular acetyl CoA concentration, hence favoring fatty acid, and cholesterol synthesis. The elevated serum TL, and serum TC in diabetic subjects as compared to normal subjects (Ref Table 1 and Table 2), may be in part due to decreased suppression of tissue lipolysis in diabetes mellitus, due to lack of Insulin. As insulin is known to suppresses tissue lipolysis (**23, 24, 25**).

Cholesterol is the principle sterol present in human plasma and its concentration in fasting serum amounts to 150-200 mg/dl in adults. This cholesterol is principally transported in plasma by lipoproteins. It is evident from the Table 2 total cholesterol ($p< 0.001$) VLDL-C ($p<0.01$) and LDL-C ($p< 0.001$) are significantly raised in diabetic subjects as compared to normal subjects, suggesting cholesterol synthesis as well as transport may be abnormal in diabetes mellitus. Lipoprotein lipase, a lipase different from other lipases, catalyses hydrolysis of triacylglycerol (TAG) part of lipoproteins. TAG are transported in plasma mainly in the form of chylomicron and VLDL, these circulatory chylomicron, VLDL are acted by lipoprotein lipase, which also known as clearing factor. The plasma enzyme, LP lipase, is insulin sensitive and activity enhanced by insulin favoring the clearance of chylomicron, VLDL from circulating plasma. The result observed as shown in table 1 clearly indicates a elevation in serum TAG levels in diabetes as compared to normal subjects ($P < 0.001$) may be in part due to non availability of insulin as insulin is essential for lipoprotein lipase activity.

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Lipoprotein in addition to the transport function of non polar lipid, particularly cholesterol, recently has been shown to impart an important role in the metabolism of the four major categories of lipo proteins (28). The low density lipo protein (LDL) and high density lipo protein (HDL) along with transport function cholesterol are also known to be involved in exchange of certain protein, apo protein and phospholipids with VLDL as well as it favors conversion of VLDL to LDL (29). The results of serum lipid profile levels in diabetic subjects receiving oral drugs (Group 3), receiving insulin (Group 4) or receiving both oral drug and insulin (Group 5) are depicted in (table 1 & 2). It is evident from tables no much difference is seen between group 3, 4 and 5 except TAG and TL T PL and HDLC, LDLC levels ($P < 0.001$) where as a significant alteration was observed in group 3, 4, 5 as compared to group 2 in the levels of TL, TFA, T PL, ($P < 0.001$) and HDLC, LDLC ($P < 0.001$). This may be in part due to alterations in the lipoprotein or its apo protein metabolism as diabetes mellitus may induce changes in the synthesis of apo proteins or over all metabolism of lipoproteins (30). It is also known that there exists a symmetrical bilayer distribution of lipid in biological membrane including erythrocyte membrane. Normally amine- rich lipids are on the inner side (cytoplasmic side) of the membrane where as choline-rich spingolipid is on outer surface.

In diabetes mellitus high incident of micro-vascular atherosclerotic disease has been associated with abnormality of erythrocyte composition and rheological function and with increased oxidative stress among many other factors. The increased blood viscosity seen in diabetes mellitus (31) is more in patients with established complications (32) and has been ascribed to decrease in erythrocyte deformability (31) and changes in erythrocyte membrane fluidity.

It is now well established that phospholipids distribution across erythrocyte membrane, bilayer is asymmetrical (32) Sphingomyelin and phosphatedyl-cholin, and most phosphatidylethhanolamine are present in inner side of the bilayer membrane. The presence of phosphatidylserine and phosphatidylethhanolamine on the inner side of the erythrocyte membrane has a biological significance. Phosphatidylserine plays a very significant role as a rate enhancing cofactor in blood coagulation cascade (33, 34, 37). And alteration in the levels of lipid components specifically cholesterol and phospholipids do effect the transport of glucose thus causing a subnormal glucose utilization leading to hyperglycemia (25).

An increase in the erythrocyte membrane lipid levels as well as the mPL/mTC ratio as evident from the table --- in group 2 as compared to group 1 is in agreement with our earlier findings(5) and may be due to diabetes induced dislipidemia. Any such alteration in the erythrocyte membrane lipid composition may alter the glucose transport by altering the orientation of the membrane transport particles thereby affecting glucose uptake and utilization (39). A significant decrease in the mPL/mTC ratio is observed in Group 4 and Group 5 as compared to group 3 (ref table 3) . Such an alteration in group 4 and in group 5 diabetics may be assumed due to a lipoprotein mediated exchange of lipid components from the plasma on to the erythrocyte membrane which may be due to insulin induced altered lipoprotein function and metabolism as it is known that insulin has a role in lipoprotein metabolism (30). Altered mPL/mTC in part which may be due to an exchange of fatty acids between plasma and erythrocyte membrane lipids. The plasma fatty acid levels as well as plasma lipid levels is under the influence of not only dietary fats but also on insulin amount and action. A change in fatty acid type and content of erythrocyte membrane lipid may alter the fluidity of membrane, hence may bring about an alteration in cell surface receptors (37).

The present study suggests that there is a definite change in the erythrocyte membrane lipid composition in type 2 diabetic subjects inducing a change in the cholesterol-phospholipids composition thereby inducing changes in the membrane behaviors whereas the insulin therapy or oral ant diabetic drugs-insulin combined therapy has a definite beneficial effect in controlling the diabetes induced lipid alterations in erythrocyte membranes thus controls any possible changes in the membrane properties. The present study suggests that insulin may have a role in phospholipids addition on to the membrane inducing more flexibility in the membrane as well as suggests that the oral hypoglycemic drugs – insulin combined protocol therapy may help regulation of normalcy of erythrocyte membrane lipid composition favoring better glucose utilization by the cells.

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Table : 1

Table showing plasma Glucose and Lipid Levels in normal subjects (Group 1) and diabetic subjects (Group 2), diabetic subjects receiving hypoglycemic drugs (Group 3), diabetic subject receiving Insulin (Group 4) and in diabetic subjects receiving both insulin and hypoglycemic drugs (Group 5).

Parameters	Group 1 Normal Subjects (36)	Group 2 Diabetic Subjects (130)	Group 3 Diabetics Receiving Oral Drugs alone (86)	Group 4 Diabetics Receiving Insulin alone (28)	Group 5 Diabetics Receiving Both Oral Drugs & Insulin (16)
Fasting plasma Glucose mg/dl	85.54 ± 13.65	**156.20 ± 35.31*** **	126.58 ± 22.32	116.63 ± 8.98	112.38 ± 10.82
Total Lipids mg/dl	705.62 ± 128.80	**1348.96 ± 103.58*** **	1114.28 ± 92.50	**1226.60 ± 151.20∂∂∂**	**1268.53 ± 112.60 βββ**
Triacylglycerol mg/dl	108.95 ± 20.14	**235.29 ± 31.66*** **	228.51 ± 18.80	**242.31 ± 16.60∂∂∂**	234.51 ± 22.20
Total Phopholipids mg/dl	16.62 ± 3.18	**23.31 ± 3.16*** **	24.12 ± 4.10	26.36 ± 6.16	25.52 ± 3.17
Total Fatty Acids mg/dl	166.51 ± 8.28	**205.59 ± 7.68*** **	**194.60 ± 7.28βββ**	**199.93 ± 5.65 ααα**	189.28 ± 3.28

Note : 1) The number in parenthesis shows the number of subjects.
2) Values are expressed as their Mean ± SD
3) P value * P<0.02** P<0.01 *** P<0.001
4) p value */∂ /α/β/ p<0.02

/∂ ∂/ **αα/ββ p<0.01

***/∂∂∂ **ααα/ βββ** p<0.001

Statistical Significance between – Gp 1 & 2 mentioned by *
Gp 3 & 4 by - ∂
Gp 3 & 5 by - **α**
Gp 5 & 3 by - **β**

Table : 2

Table showing serum cholesterol profile Levels in Normal and Diabetic subjects in G-1&2 also diabetic subjects receiving hypoglycemic drug and or Insulin in G-3, G-4 & G-5.

Parameters	Group 1 Normal Subjects (36)	Group 2 Diabetic Subjects (130)	Group 3 Diabetics Receiving Oral Drugs alone (86)	Group 4 Diabetics Receiving Insulin alone (28)	Group 5 Diabetics Receiving Both Oral Drugs & Insulin (16)
Total Cholesterol mg/dl	144.22 ± 26.12	**253.58 ± 35.90. *** **	228.81 ± 20.62	**257.72 ± 22.18∂∂∂**	**242.48 ± 19.90 ββ**
HDL Cholesterol mg/dl	41.38 ± 9.36	37.37 ± 5.65	37.18 ± 4.40	**41.21 ± 5.50∂∂∂**	38.25 ± 6.23

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LDL Cholesterol mg/dl	117.41 ± 23.90	**123.83 ± 39.20. *****	120.61 ± 12.81	**136.23 ±** 16.58∂∂∂ **αα**	**130.30 ± 15.18β**
VLDL Cholesterol mg/dl	22.13 ± 5.61	**46.25 ± 8.51. *****	45.50 ± 6.16	47.33 ± 8.18	44.38 ± 6.78

Note: 1) The number in parenthesis shows the number of subjects.
2) Values are expressed as their Mean ± SD
3) P value * P<0.02** P<0.01 *** P<0.001
4) p value */∂ /**α/β**/ p<0.02

/∂ ∂/ **αα/ββ p<0.01

***/∂∂∂ **ααα/ βββ** p<0.001

Statistical Significance between – Gp 1 & 2 mentioned by *
Gp 3 & 4 by - ∂
Gp 3 & 5 by - **α**
Gp 5 & 3 by - **β**

Table : 3

Table Showing m TL,m TC ,m PL ,m TC/m PL ratio in Normal and Diabetic Subjects in G-1 &G-2 also diabetic subjects receiving hypoglycemic drugs and or Insulin in G-3, G-4 & G-5.

Parameters	Group-1 Normal subjects (36)	Group-2 Diabetic Subjects (130)	Group 3 Diabetics Receiving Oral Drugs alone (86)	Group 4 Diabetics Receiving Insulin alone (28)	Group5 Diabetics Receiving Both Oral Drugs & Insulin (16)
Membrane Total Lipid mg/dl (mTL)	5.02 ± 1.62	5.35 ± 1.53	5.08 ± 0.88	5.22 ± 0.76	**5.64 ± 0.94 βββ**
Membrane Total Cholesterol mg/dl (mTC)	1.16 ± 0.32	**1.72 ± 0.10*****	1.68 ± 0.63	1.76 ± 0.36	1.69 ± 0.48
Membrane Total Phospholipids mg/dl (mPL)	7.36 ± 1.78	**8.18 ± 0.88****	7.61 ± 0.66	**7.94 ± 0.71∂ ααα**	7.38 ± 0.44

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Membrane Phospholipid/Cholesterol Ratio (mPL/mTC)	6.40 ± 0.64	**4.73 ± 0.28*** **	**5.09 ± 0.53∂∂∂ βββ**	4.45 ± 0.2	**4.66 ± 0.31αα**

Note : 1) The number in parenthesis shows the number of subjects.
2) Values are expressed as their Mean ± SD
3) P value * P<0.02** P<0.01 *** P<0.001
4) p value */∂ /**α/β**/ p<0.02

/∂ ∂/ **αα/ββ p<0.01

***/∂∂∂ **ααα/ βββ** p<0.001

Statistical Significance between – Gp 1 & 2 mentioned by *
Gp 3 & 4 by - ∂
Gp 3 & 5 by - **α**
Gp 5 & 3 by - **β**

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A Ten Year Study of Management of Ischemic Heart Disease in a Tertiary Hospital in South West Nigeria

By Omole, Moses Kayode Pharm. D. & Ma'aji, Hadiza Usman M. Pharm
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Abstract - The Pharmacotherapy of drugs used in the management of ischemic heart disease at the University College Hospital (UCH) in Ibadan between June 1998 and May 2007, was studied retrospectively. The objective was to assess the rational use of the prescribed drugs and to determine the tolerability and benefits of using nitrates, beta-blockers, calcium channel blockers and acetylsalicylic acid (aspirin). A total of 52 case files of patients with ischemic heart disease were randomly selected from the central medical record department and used for the study. Information extracted includes demographic data, the prescribed drugs and side effects.

Keywords : *ischemic heart disease, pharmacotherapy, management, prescriptions, patients.*

GJMR-L Classification : *NLMC Code : WG 240, WC 220*

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A Ten Year Study of Management of Ischemic Heart Disease in a Tertiary Hospital in South West Nigeria

Omole, Moses Kayode Pharm. D. [α] & Ma'aji, Hadiza Usman M.Pharm [σ]

Abstract - The Pharmacotherapy of drugs used in the management of ischemic heart disease at the University College Hospital (UCH) in Ibadan between June 1998 and May 2007, was studied retrospectively. The objective was to assess the rational use of the prescribed drugs and to determine the tolerability and benefits of using nitrates, beta-blockers, calcium channel blockers and acetylsalicylic acid (aspirin). A total of 52 case files of patients with ischemic heart disease were randomly selected from the central medical record department and used for the study. Information extracted includes demographic data, the prescribed drugs and side effects.

Results showed that males 27(51.9%) were more affected with ischemic heart disease than females 25(48.1%). Age range between 51- 60 years were 15(28.8%) and constituted the highest percentage with mean 54.1 years. Nitrates 36 (30.5%) were the highest prescribed drugs followed by acetylsalicylic acid 29 (24.5%), angiotensin converting enzyme inhibitors 14 (11.9%), beta blockers 13(10.9%), sedatives 11(9.3%), calcium channel blockers 8 (6.7%), morphine sulphate 4 (3.3%) and dopamine 3 (2.5%). Side effects documented were headache 8(19.2%), dizziness 5(12.2%), flushing 5(12.2%), tachycardia 4(9.7%), broncho-constriction 2(4.8%), and depression 7(17.0%).

There is a need for proper education of patients on dietary pattern, smoking ceasation, moderate exercise and drug compliance.

Keywords : ischemic heart disease, pharmacotherapy, management, prescriptions, patients.

I. Introduction

Coronary heart disease (CHD) also known as coronary artery disease (CAD) is a condition in which the vascular supply to the heart is impeded by artheroma, thrombosis or spasm of coronary arteries. This may impair the supply of oxygenated blood to the cardiac tissue sufficiently to cause myocardial ischemia which if severe or prolonged may cause death resulting from myocardial infarction[1].

Ischemic heart disease (IHD) or myocardial ischemia is a disease characterized by ischemia (reduced blood supply) to the heart muscle usually due to coronary artery disease (CAD). Angina pectoris is one of the primary manifestations of ischemic heart disease. Other manifestations include myocardial infarction (MI), heart failure (HF), arrhythmias, and sudden cardiac death.[1] In 1977, Ladipo and colleagues[2] documented coronary artery disease to be nonexistent in Zaria, Northern Nigeria. Abengowe in 1979 also studied 4,456 medical admissions at Ahmadu Bello University Teaching Hospital, Kaduna Nigeria. These include 354 cardiovascular patients. He concluded that coronary heart disease occurred only among non-Africans.[3] Sani MU et al (2006) in Kano studied the case notes of 1347 patients with CVD over a period of five (5) years. The study which showed 46 cases of IHD, with 41(89.1%) of whom were Nigerians suggested a change of epidemiology of this disease over the last three decades[4]. Several authors have alluded to the factors that contribute to the increase in the incidence of coronary artery disease in our environment[5]. These factors include urbanization, low level of physical activities, acquisition of unhealthy habits and diets of westernized population. World heart federation reports that global burden of cardiovascular disease is on the increase especially in the developing world. It is estimated that CVD will claim 30 million lives by the year 2020, 18.5 million of whom will be in the developing countries[6].

Anti-angina drugs provide prophylactic or symptomatic treatment for IHD patients with angina symptoms. Beta adrenergic receptor antagonist reduces mortality apparently by decreasing the incidence of sudden cardiac death associated with myocardial ischemia and infarction7. Acetylsalicylic acid (ASA) also called aspirin is used at a homeopathic dose of 75mg to 300mg daily to reduce or prevent platelet aggregation and myocardial ischemia. The treatment of cardiac risk factors by aspirin reduces the progression and regression of atherosclerosis[7].

The rational use of drugs used in the patients with ischemic heart disease has not been documented. This led to this study which assessed the rational use of drugs in the management of ischemic heart disease over a period of 10 years at the University College

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Hospital (UCH), Ibadan with the goal of providing and promoting pharmaceutical care.

II. Patients and Methods

This is a retrospective study in which 52 case notes of patients with ischemic heart disease who registered at UCH between June 1998 and May 2007 were thoroughly studied during the 3-month period of February and April 2009. Information obtained included patients' age, sex, family history, diagnosis, prescribed drugs and side effects. Site of the study was the Medical Outpatient Unit (MOP) of University College Hospital Ibadan, in Southwestern Nigeria.

Inpatients and outpatients from cardiology unit with symptoms of ischemic heart disease were included in the study while inpatients and outpatients without symptoms of ischemic heart disease were excluded. A total of all 52 patients who were treated for ischemic heart disease over a period of ten years were used for the study. The sample size was small because the whole population was used. Statistical analysis was done using SPSS version 15.0 software programme for frequency distribution and cross tabulation.

The UI/UCH institutional review committee of the institute for advanced medical research and training (IMRAT) gave the ethical approval to conduct the study. The ethical approval was dated 31/03/2009 with IRC protocol no UI/EC/09/0049.

III. Results

Table 1 shows the age and sex distribution of patients with ischemic heart disease. Nine (17.3%) patients aged between 31 and 40 years, 7 (13.5%) aged between 41 and 50 years, while 15, 10 and 11 patients aged between 51 and 60 years, 61 and 70 years and 70 years and above respectively. There were 27 (51.9%) males and 25 (48.1%) females. Table 2 shows that there was statistical significant association between the age and sex of patients with ischemic heart disease. $P < 0.05$.

Table 3 indicates drugs prescribed for patients with ischemic heart disease. Nineteen (16.1%) patients were prescribed sublingual glyceride trinitrate, 17 (14.4%) were prescribed oral isosorbide dinitrate, 4 (3.3%) on propranolol, 2 (1.7%) on acebutol, 5 (4.2%) on atenolol, 2(1.7%) on metoprolol, 1 (2.8%) on dilitiazem, 4(3.5%) on nifedipine, 3 (2.5%) on amlodipine, 29 (24.5%) on aspirin, 11 (9.8%) on sedatives, 4 (3.3%) on morphine sulphate, while 14 (11.9%) and 3 (2.5%) were on angiotensine converting enzyme inhibitors (ACEI) and dopamine respectively.

Table 4 shows that aspirin was used as a combination drug in patients with ischemic heart disease. Twenty (38.5%) patients were on aspirin + sublingual nitroglycerine tablet, 8 (15.4%) on aspirin + sublingual nitroglycerin + beta blockers, 4 (7.7%) on aspirin + sublingual nitroglycerin + calcium channel blocker, 11 (21.1%) on aspirin + sublingual nitroglycerin + ACEI, 1 (1.9%) on aspirin + sublingual nitroglycerin + lipitol, while 8 (15.4%) were on aspirin + glyceride trinitrate + ACEI + calcium channel blocker.

Table 5 shows side effect of prescribed drugs. Eight (19.2%) patients had headache, 5 (12.1%) had dizziness, 5 (12.1%) had flushing, 4 (9.7%) had tachycardia, 2 (4.8%) had cough (bronchoconstriction), 7 (17.0%) had depression while 10 (24.3%) patients had hypoglycemia. In 11 (21.2%) patients, there was no side effect documented.

IV. Discussion

Table 1 show that males 27(51.2%) were more prone to ischemic heart disease than females 25(48.1%). This result was supported by the findings of Sani in Kano where incidence in males was found to be higher than in females[4]. This high incidence may be attributed to hypertension which occurs more commonly in males than females as important risk factors for IHD[7]. In addition males have been suggested to likely utilize healthcare services than females.[1, 7, 8].

Table 1 also indicates that patients within the age range of 50-59 years constituted the highest percentage while those within the age range of 40-49 years constituted the least percentage of IHD. This agrees with the findings that risk of IHD increases with an advance age. It was stated that approximately half of all deaths of persons older than 65 years of age was as a result of IHD, and 80% of all coronary deaths occur in this age group [8, 9, 10]. Table 2 shows that there is a correlation between age and sex of patients having IHD. This is in agreement with the study which shows that IHD is a major health issue in the elderly [8, 9, 10]. Table 3 shows classes of drugs used in the management of IHD and their frequency of administration. Nitrates were the most commonly administered drugs for the symptomatic relief of chest pain and discomforts associated with angina[11]. Thirty six (30.5%) patients were on nitrates. Nitrates had been established to dilate both veins and arteries thereby reducing preload to the heart and filling pressure in the ventricles. This in turn reduces myocardial oxygen demand and increase myocardial oxygen supply thereby reducing angina[12,13]. Thirty six (30.5%) were prescribed both short acting and long acting nitrates. The study showed that there was benefit in the use of the nitrates since low incidence of side effect had been reported with the use of nitrates and tolerability in the use of nitrate is high[11, 12, 13].

Thirteen (10.9%) patients were prescribed β-blockers. This is in agreement with current guidelines which recommend that beta-blockers should be administered before nitrates or calcium channel blockers when long term therapy is indicated[14, 15]. β-adrenergic antagonists have been shown to prevent

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angina and also reduce mortality following myocardial infarction. Cardio selective beta-blockers are used for patients in order to minimize adverse effects such as bronchospasm in asthmatic or chronic obstructive pulmonary disease, intermittent claudication and sexual dysfunction. It is rational to use bêta blockers to treat patients with angina pectoris to prevent acute myocardial infarction[14, 15, 16].

Eight (6.7%) were administered calcium channel blockers (CCBs). The documented side effects of the drugs showed that most of the patients tolerated CCBs which indicates the benefits of the drug. The benefits provided by calcium channel antagonists is related to reduced myocardial oxygen demand and improved oxygen supply[16, 17, 18].

Acetylsalicylic acid 29(24.5%), administered at homeopathic dose of 75-325mg are effective in the treatment of angina and post myocardial infarction. Studies have been conducted evaluating the efficacy of aspirin in cardiovascular disease in the second international study of infarction survival (ISIS-2) trials[19]. The study randomized 1,718 patients with suspected MI to receive a double blind version IV SK (Streptokinase) for 1 month, both aspirin and SK for 1 month compared with placebo for 1 month. The use of aspirin for 35 days in the study was associated with a highly significant 23% reduction in mortality rate. Therefore early administration of aspirin helps reduce the incidence of IHD and prevent myocardial infarction when used indefinitely[19, 20, 21, 22].

Table 3 further explains the individual drugs used within each class. Nineteen (16.1%) patients were prescribed sublingual glyceride trinitrate while 17(14.4%) were on oral isosorbide dinitrates. In table 4, eleven (21.1%) were administered a combination of Aspirin + Sublingual Nitrates + ACEI. Angiotensin converting enzyme inhibitors have a prominent role in the overall treatment of patients with CAD. It has demonstrated significant benefits in morbidity and mortality in a number of patients with heart failure (HF), acute MI and Diabetes mellitus[20, 21, 22, 23].

Table 5 shows the side effects documented when drugs in table 3 and 4 were administered in the management of ischemic heart disease. Adverse effects seen with the sublingual nitroglycerine include dizziness, tachycardia and head ache. Side effects seen in dihydropyridines calcium channel blockers such as nifedipine include head ache, bradycardia, flushing and dizziness. Bronchoconstriction (cough) is a major side effect of ACEI[22]. Hypoglycemia can result from administration of insulin and oral hypoglycemic agents when diabetes is treated in patients with ischemic heart disease[7, 24, 25, 26].

V. Conclusion

The administration of both short acting and long acting nitrates for symptomatic relief of chest discomfort and angina symptoms have benefits and tolerability. This aids compliance because of few side effects. A combination therapy of beta-blockers or calcium channels blockers with nitrates and with daily aspirin dose have shown great improvement in angina patients. Administration of aspirin daily has been found to be of great benefit in the management of patients with ischemic heart disease and to prevent acute mycordial infraction.

VI. Acknowledgement

We acknowledge the technical support and cooperation of staff in Cardiology unit Medical Outpatient (MOP) Department of the University College Hospital (UCH) Ibadan.

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Table 1 : Age and Sex distribution of patients with ischemic heart disease.

Age group (years)	Frequency	Percentage (%)
31 – 40	9	17.3
41 – 50	7	13.5
51 – 60	15	28.8
61 – 70	10	19.2
Above 70	11	21.2
Total	52	100
Sex		
Males	27	51.9
Females	25	48.1
Total	52	100

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Table 2 : Age group* SEX Cross tabulation.

			SEX		
			Male	Female	Total
Agegrp	<40	Count	5	4	9
		% within Agegrp	55.6%	44.4%	100.0%
		% within SEX	18.5%	16.0%	17.3%
	40-49	Count	4	3	7
		% within Agegrp	57.1%	42.9%	100.0%
		% within SEX	14.8%	12.0%	13.5%
	50-59	Count	10	10	20
		% within Agegrp	50.0%	50.0%	100.0%
		% within SEX	37.0%	40.0%	38.5%
	60-69	Count	5	5	10
		% within Agegrp	50.0%	50.0%	100.0%
		% within SEX	18.5%	20.0%	19.2%
	70+	Count	3	3	6
		% within Agegrp	50.0%	50.0%	100.0%
		% within SEX	11.1%	12.0%	11.5%
Total		Count	27	25	52
		% within Agegrp	51.9%	48.1%	100.0%
		% within SEX	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	.177[a]	4	.996
Likelihood Ratio	.178	4	.996
Linear-by-Linear Association	.106	1	.744
N of Valid Cases	52		

a. 7 cells (70.0%) have expected count less then 5. The minimum expected count is 2.88.

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Table 3 : * Drugs prescribed for patients with IHD.

Drugs	Frequency	Percentage (%)
Sublingual glyceride trinate	19	16.1
Oral isosorbide dinatrate	17	14.4
Propanol	4	3.3
Acebutolol	2	1.7
Atenolol	5	4.2
Metoprolol	2	1.7
Diltiazem	1	0.8
Nifedipine	4	3.4
Amlodipine	3	2.5
Aspirin	29	24.5
Sedatives	11	9.3
Morphine sulphate	4	3.3
Angiotensin converting enzyme inhibitors	14	11.9
Dopamine	3	2.5
Total	**118**	**100**

**multiple responses*

Table 4 : Combination of drugs used in the treatment of patients with ischemic heart disease.

Drugs	Frequency	Percentage (%)
Aspirin + Sublingual tablets	20	38
Aspirin + Subl. Nitroglycerin + Beta blockers	8	15
Aspirin + ubl. Nitrates + Calcium channel blockers	4	7.8
Spirin + Subl. Nitrates + ACEI	11	21.1
Aspirin + Subl. Nitrates + Liptor	1	1.92
Aspirin + glyceride trinitrate + antihypertensive + others	8	15.4
Total	52	100

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Table 5 : Side effects documented.

Side effects	Frequency	Percentage
Headache	8	19.2
Dizziness	5	12.1
Flushing	5	12.1
Tachycardia	4	9.7
Bronchoconstriction	2	4.8
Depression	7	17.0
Making of hypoglycemia	10	24.3
Total	**41**	**100**

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Antibacterial Activity of *Raphanus Sativus Linn.* Seed Extract

By Faiyaz Ahmad, Izharul Hasan, Danish Kamal Chishti & Haqeeq Ahmad
Govt. Nizamia Tibbi College

Abstract - *Raphanus sativus Linn.* (Radish) is an annual herb of family Cruciferae or Brassicaceae and grown as an edible root.

Objectives : The aim of the study is to test the potentiality of different solvent extracts (Ethanol, Methanol, Ethyl Acetate, Chloroform, Benzene, Aqueous hot and Aqueous cold) against various pathogenic bacterial strains *E.coli* (ATCC-25922), *Klebsiella pneumonia* (ATCC-27736), *Proteus vulgaris* (ATCC-6380), *Pseudomonas aeruginosa* (ATCC-27853), *Staphalococcus aureus* (ATCC-25923), *Shigella sonnie* (ATCC-25931), *Salmonella typhi* (ATCC-25241) and *Salmonella paratyphi* (ATCC-9150).

Methods : The antibacterial activity was performed in vitro using Agar well diffusion assay and diameter of zone of inhibition was measured.

Keywords : *antibacterial activity, phytochemical analysis, raphanus sativus, zone of inhibition.*

GJMR-L Classification : *NLMC Code : QU 34, FOR Code: 860803*

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Antibacterial Activity of *Raphanus Sativus Linn.* Seed Extract

Faiyaz Ahmad [α], Izharul Hasan [σ], Danish Kamal Chishti [ρ] & Haqeeq Ahmad [ω]

Abstract - ***Raphanus sativus Linn.*** (Radish) is an annual herb of family Cruciferae or Brassicaceae and grown as an edible root.

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Methods : The antibacterial activity was performed in vitro using Agar well diffusion assay and diameter of zone of inhibition was measured.

Results : Among all the extracts Ethanolic and Methanolic extracts showed maximum antibacterial activity against all the bacterial strain used with a zone of inhibition ranges from 12-21mm and the least activity was observed in Aqueous cold extract with zone of inhibition ranges from 7-9mm. The test results were compared with standard antibiotics chloramphenicol and Ciprofloxacine.

Conclusions : The qualitative analysis of different extracts of Raphanus sativus seed reveals the presence of Alkaloids, Flavonoids, Glycosides, Phenols, Tannins, Saponin, Sterols and Protien which may be responsible for the observed antibacterial activity. The results suggest that ethnolic and methnolic extracts can be used in the treatment of infection caused by these bacterial strains used in this study.

Keywords : antibacterial activity, phytochemical analysis, raphanus sativus, zone of inhibition.

I. Introduction

According to World Health Organization (WHO), the increase of resistance to antibiotics by bacterial pathogens is a growing problem in both developed and developing countries (1). The problem of microbial resistance is growing and the outlook of the use of antimicrobial drugs in future is uncertain. Therefore action must be taken to reduce this problem, for example, to control the use of antibiotics, to develop research to better understanding of the genetic mechanism of resistance and to continue study to develop new drugs either synthetic or natural (2).

For along period of time, plants have been a valuable source of natural products for maintaining human health (3). Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Medicinal plants represent a rich source of antimicrobial agent (4). Different parts of plants, herbs and spices have been used for many years for the prevention of infection. The use of plants with known antimicrobial properties can be of great significance in treatment of infections (5).

A renewed interest in plant based antimicrobials has arisen during the last twenty years, but still plant based antimicrobials are poorly explored. Screening of plants extracts for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective compounds (6). The antimicrobial compounds from plants may inhibit bacteria through different mechanism than the conventional antibiotics, and could therefore be of clinical value in the treatment of microbial infection (7).

Radish, ***Raphanus sativus Linn.*** (Brassicaceae family) is an annual herb, consumed as vegetable. Commonly known as Mooli. It is coarse, rough or glabrous. Leaves are lyrate, pinnate or pinnatifid. Flowers are large yellow, white or pale lilac, veined with purple, in long ebracteate racemes. Seeds are pendulous, globose; cotyledons conduplicate. Cultivated all over sub-continent up to 16,000 ft in temperate and warm countries (8). It is well reputed in Unani System of Medicine, useful for urinary complaints and piles. Almost all parts of the plant including leaves seeds and roots are utilized in medicine. The fresh juices obtained from leaves are diuretic, laxative. Roots are used for urinary complaints and syphilitic disease; they are a reputed medicine for piles and gastrodynic pains. The seeds are expectorant, diuretic, laxative, carminative, antitussive and stomach tonic (8, 9, 10, and 11). The present study aims at assessing the antibacterial property of R. *sativus* seed extract, to substantiate the use of radish in Unani System of medicine in infectious diseases.

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II. Materials and Methods

a) Plant Materials

The sample of seeds of *Raphanus sativus* [Tukhm-e-Mooli] were collected from local market of Hyderabad, Andhra Pradesh, and was properly identified authenticated on the basis of literary description available in the Unani classic as well as modern literature by Botanist Dr. V.C. Gupta, Deputy director, Central Research Institute Of Unani Medicine, Hyderabad (C.R.I.U.M.) and Dr. Hakeem. Mohd Yadullah Ex. C.M.O. Govt. Nizamia General Hospital Hyderabad and renowned Unani practitioner. Voucher sample was prepared and preserved in the Herbarium of C.R.I.U.M., Hyderabad for further reference.

b) Preparation of plant Extract

Different extracts of *Raphanus sativus* seeds were prepared for analysis in the present study (a) Ethanol (b) Methanol (c) Ethyl Acetate (d) Chloroform (e) benzene (f) Aqueous Hot (g) Aqueous Cold. Ten (10) grams powdered drug soaked in 100 ml of different solvents for 24 hrs & filtered through whattman's filter paper No.1. The filtrate was concentrated by evaporation of solvent on hot plate and water bath at room temperature. All extracts were stored at 4^0 C until further use.

c) Preparation of Test Sample

A stock solution of the extracts was prepared at the concentration of 100mg/ml and store at 2^0C till further use.

d) Source and Maintenance of Organisms

A total 8 strains including gram positive (Staphylococcus aureus, ATCC25923) and gram negative (E.coli-ATCC25922, Pseudomonas aeruginosa-ATCC 27853, Shigella sonnei- ATCC 25931, Salmonella typhi-ATCC 25241, Proteus vulgaris- ATCC 6380, Klebsiella pneumonie-ATCC27736, Salmonella paratyphi -ATCC 9150) bacteria were selected to assess the susceptibility test against the drug extract. The strains were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. They were sub cultured on nutrient agar for every 15 days and maintained on nutrient agar slants at 4^0C. Fresh inoculums were taken for the test.

e) Culture Media

Muellar Hinton Agar (Himedia, India) was prepared according to the manufacturer's instructions, autoclaved at 15 lbs pressure and 121^0C for required time and dispensed into petridishes more than half. Set plates were incubated overnight at 37^0C to ensure sterility before use.

f) Preparation of inoculums

Select & label test cultures that are to be used for (plant extract) Sensitivity Assay. Prepare nutrient agar plates. 3-4 colonies should be selected from the agar plate culture. The top of the each colony is touched with loop & transferred in to into a test tube containing4-5 ml nutrient broth. The test tubes which containing broth cultured are incubated at 37°C until it achieves the turbidity.

g) Evaluation of Antibacterial Activity

The *in-vitro* antibacterial activity of the extracts was determined by agar well diffusion assay (Reeves, 1989). All strains were first grown in Mueller Hinton broth (MHB) under shaking condition for 4 h 37^0C and after the incubation period 0.1ml of the test inoculums was spread evenly with a sterile glass spreader on Mueller Hinton Agar (MHA) plates. The seeded plates were allowed to dry in the incubator at 37^0C. Wells were made using sterile 6 mm cork borer in the inoculated MHA plates. The wells were filled with 200µl of the extracts (re-suspended in respective solvents) and negative controls 1:1 (solvent: water). The concentration of stock extracts was 200 mg /ml. The inoculated plates were incubated at 37^0C for 24 h. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the well. The size of zone of inhibition was measured and the bacterial activity was expressed in term of average diameter of the zone of inhibition in millimeters. The results were compared with the standard antibiotics, Chloramphenicol (25mcg) and Ciprofloxacin (25mcg). The photographs were taken in U.V-visible documentation system.

h) Statistical Analysis

Calculations of antibacterial activity were determined by Standard Deviation and Mean of replicates.

i) Screening for Secondary Metabolites

Secondary metabolites are identified in the extracts of R. *sativus* by using standard methods.1 mg of each extract was dissolved in 100 ml of the respective solvent and filtered through Whattman filter paper No.1. Thus, the filtrates obtained were used as test solutions for the screening. The details for the qualitative analysis (14, 15, 16) were described. Table**1**.

III. Results

The results are listed in Table**2**. Results obtained in the present study relieved that tested medicinal plant extracts posses potential antibacterial activity against all selected bacteria (agar well diffusion method). Among all the extracts Ethanolic and Methanolic extracts showed maximum antibacterial activity against all the bacterial strain used with a zone of inhibition ranges from 12-21 mm and the least activity was observed in Aqueous cold extract with zone of inhibition ranges from 7-9 mm. The test results were

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compared with standard antibiotics Chloramphenicol (25μg) and Ciprofloxacin (25μg).

The plant extracts were also screened for qualitative analysis to know the relative distribution of the secondary metabolites which may be responsible for the potent antibacterial activity. Flavonoids are extracted into Ethanol, Aqueous hot and Aqueous cold. Alkaloids are extracted into Ethanol, Methanol, Chloroform, Aqueous hot and Aqueous cold. Glycosides are present in all solvents. Carbohydrates are extracted only in Methanol, Aqueous hot and Aqueous cold. Phenols are extracted into Ethanol, Chloroform and Aqueous hot. Saponins are extracted into Methanol, Chloroform, Aqueous hot and Aqueous cold. Sterols are found in Ethanol, Methanol, Chloroform and Benzene. Tannins are extracted into Ethanol, Chloroform and Aqueous hot. While Protiens are present only into Benzene. Table**3**.

IV. Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antibacterial activity assay (17). Crude plant extracts are generally a mixture of active and non-active compounds. A number of medicinal plants described in Unani System of Medicine still need to be testify according to the modern parameters to ensure their activity and efficacy. Many reports are available on the antibacterial, antifungal and anti-inflammatory properties of plants (18, 19, 20, 21). Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

In India, mortality rate due to infections is largely due to *S. aureus, Ps. aeruginosa, K. pneumonia, E. coli, P.vulgaris, S.sonnie, S.typhi, S. paratyphi.* (22). The treatment and management of infections caused by these strains has become very difficult, therefore, the challenge to discover newer and potent drugs is ever increasing. Therefore, studies were undertaken to test the extracts of *R. sativus* against these pathogens. The highest activity was observed in Ethanol and Methanol extracts followed by Ethyl acetate, chloroform, Benzene, aqueous hot and aqueous cold.

The highest antibacterial effect of Methanol and Ethanol extract against these organism may be due to the ability of the Ethanol and Methanol to extract some of the active properties of these plants like Flavonoids, phenolic compounds, Saponins and other secondary metabolites which are reported to antibacterial (5). Flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to complex with extra cellular and soluble proteins and to complex with bacterial cell wall; more lipophilic Flavonoids may also disrupt microbial membrane (23). Phenol and polyphenols present in the plants are known to be toxic to micro-organism (24). Antibacterial activity of tannins may be related to their ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins, they also complex with polysaccharides (25) .The broad spectrum antibacterial activity exhibited by R. *sativus* may be attributed to the various active constituents presents in it which either due to their individual or combined action. Thus, the study ascertains the value of R. *sativus* used in Unani System of Medicine. This could be of considerable interest to the development of new drugs.

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Table : 1

S.No.	Secondary metabolites	Experiment	Observation	Inference
1.	**Alkaloids**			
	Dragendroff 's Test	Few mg of alc. Or aq. Ext. of drug in 5 ml of dist. Water and add 2M HCL, then add few drops of Dragendroff's reagent	An orange or orange -red precipitate is formed	Present
2.	**Flavanoids**			
	(a) Shinoda test	To 0.5 ml of alc. ext. of the drug add 5 -10 drops of dil. HCL followed by addition of small piece of Magnesium. Boil the solution for	A pink or reddish pink or brown colour is produced.	Present
	(b) NaOH test	few minutes. 1 ml of 1N NaOH solution w as added to the 1ml of test solution.	Formation of yellow colour	Present

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3.	**Glycosides**			
	(a) Conc.H_2SO_4 test	1ml of conc.H_2SO_4 was added to 1ml of test solution and is allowed to stand for 2 minutes.	Formation of reddish colour	Present
	(b) Aq NaOH test	To alc. Ext. of the drug add 1ml of water and adds aq.NaOH solution.	Formation of yellow colour	Present
4.	**Carbohydrates**			
	(a) Benedict's test	To o.5 ml of aq. Ext. of the drug add 5 ml of Benedict's solution and boil for 5 min.	Formation of colour ppt.	Present
	(b)Molisch's test	To 2 ml of aq. Ext. of the drug add 2 drops of freshly prepared 20% alc. α-naphthol and mix, pour 2 ml of conc. H_2SO_4 through the wall of the test tube.	A red-violet ring is formed at the junction of the two liquids, which disappears on addition of excess of alkali	Present
5.	**Phenol**			
	(a) Ferric chloride test	To alc. Or aq. ext. of the drugs add 2 ml of dist. Water and add few drops of 10% aq. $FeCl_3$ solution.	A blue or green colour is produced.	Present
	(b) Aq. Lead acetate test	To alc. Or aq. ext. of the drugs add 5 ml of dist. water and add few drops of 1% aq. lead acetate solution.	A yellow ppt. is formed.	Present
6.	**Saponins**			
	Foam test	To 5 ml of aq. ext. of the drug add drops of Sodium bicarbonate solution shake the mixture vigorously and leave for 3 min.	Honey comb like froth is formed.	Present
7.	**Sterols/Steroids**			
	Salkowski test	Add 1 ml conc. Sulphuric acid to 2 ml of chloroform ext. of the drug care fully through the wall of the test tube.	A red colour is produced in the chloroform layer or at the junction of the two liquids.	Present

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8.	**Tannins**			
	Ferric chloride test	To 1-2 ml of aq. ext. of the drug add few drops of 5% aq. ferric chloride solution.	A bluish black colour is produced which disappear on addition of a few ml of a dil. Sulphuric acid solu tion followed by the formation of a yellow-brown ppt.	Present
9.	**Proteins**			
	Millon's test	To aq. ext. of the drug add 1 ml of dist. water and add 5-6 drops of Millon's reagent.	A white ppt. is formed which turns red on heating.	Present

Table : 2 Antibacterial Activity.

EXTRACTS	**Diameter of Zone of inhibition(mm)**							
	E.coli	***K.pneumoniea***	***P.valgaris***	***Ps.aeruginosa***	***S.aureus***	***S.sonnie***	***S.paratyphi***	***S.typhi***
ETHANOL	14.5±0.7	17±0.5	18±4.2	21.3±6.6	19±7.0	13.6±2.0	13.3±1.5	16.6±1.5
METHANOL	12.5±0.7	14.6±2.5	19.5±0.7	14.6±2.3	13.5±0.7	15.3±2.0	14.6±1.5	15.6±0.5
ETH. ACETATE	9±1.4	NA	22.5±4.9	18±0.5	18±0.5	NA	NA	19.6±0.7
CHLOROFORM	10±0.5	NA	19±0.5	18.3±3.5	10±0.5	NA	NA	14±2.0
BENZENE	12.5±0.7	NA	18±5.6	NA	9±0.5	NA	NA	16.3±1.5
AQ. HOT	12±0.5	11.6±0.5	9±0.5	13.3±2.0	12±0.5	12±0.5	NA	NA
AQ. COLD	NA	9.3±0.5	9±0.5	9.3±0.5	9±0.5	9.6±0.5	7±0.5	7±0.5
CHLORAMPHENICOL(25µG)	29±0.5	28±0.4	20±0.5	9±0.5	NA	16±0.3	14±0.4	21±0.5
CIPROFLOXACIN (25µG)	27±0.4	26±0.3	30±0.4	30±0.4	25±0.5	27±0.4	30±0.5	35±0.5

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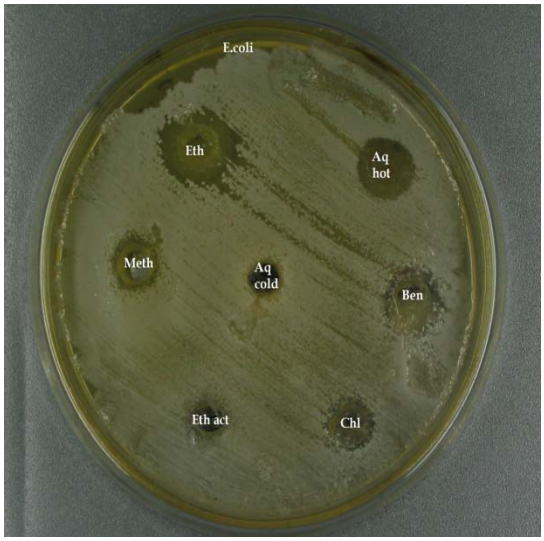
Table : 3

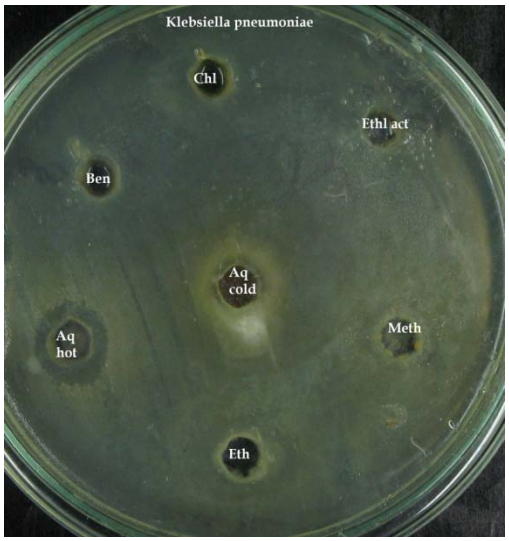
Secondary Metabolites	Name of Test	Results (+/-)						
		Et	Mt	Ea	Ch	Ben	Aq H	Aq C
Alkaloids	Dragendroff's	++	++	--	++	--	++	++
Flavonoids	Shinoda	++	--	--	--	--	++	++
	NaOH	++	--	--	--	--	++	++
Glycosides	Conc.H_2SO_4	++	++	++	++	++	++	++
	Aq NaOH	++	++	++	++	++	++	++
Carbohydrates	Benedict's	--	++	--	--	--	++	++
	Molisch's	--	++	--	--	--	++	++
Phenol	Ferric chloride	++	--	--	++	--	++	--
	Aq. lead acetate	++	--	--	++	--	++	--
Saponins	Foam	--	++	--	++	--	++	++
Sterols	Salkowski	++	++	--	++	++	--	--
Tannins	Ferric chloride	++	--	--	++	--	++	--
Proteins	Millon's	--	--	--	--	++	--	--

Et= Ethanol, **Mt**= Methanol, **Ea**= Ethyl acetate, **Ch**= Chloroform, **Ben**= Benzene, **Aq**=Aqueous, **H**=Hot, **C**=Cold

Figures



E.coli



Klebsiella pneumonie

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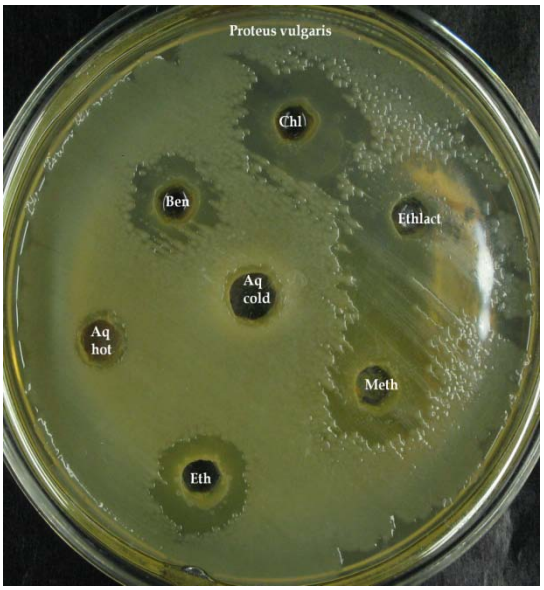
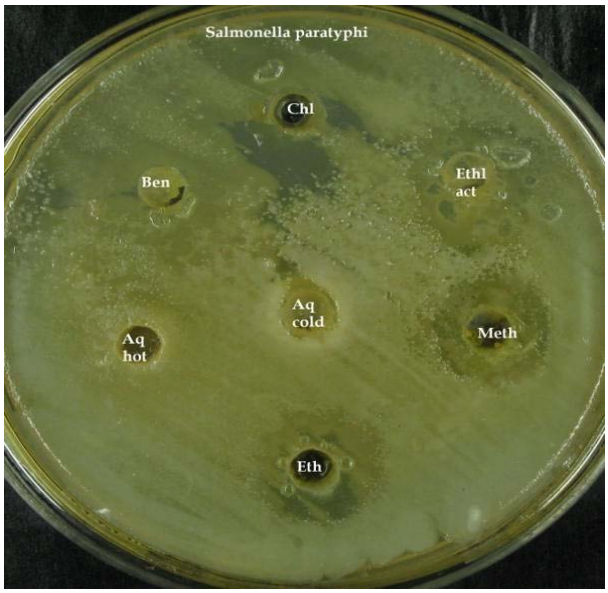
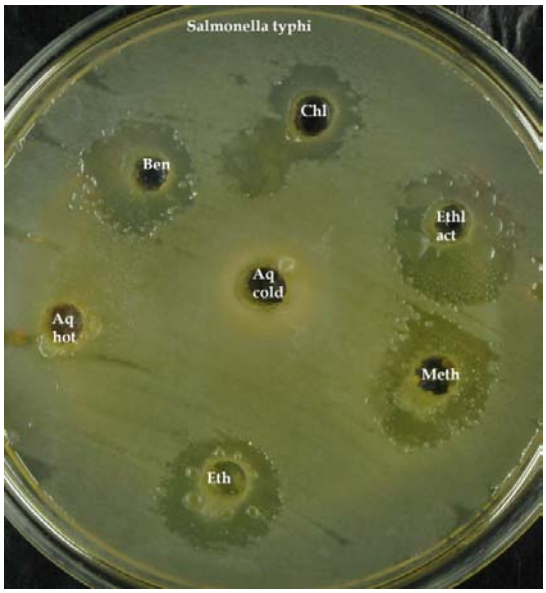


Proteus vulgaris

Pseudomonas aeruginosa



Salmonella paratyphi



Salmonella typhi

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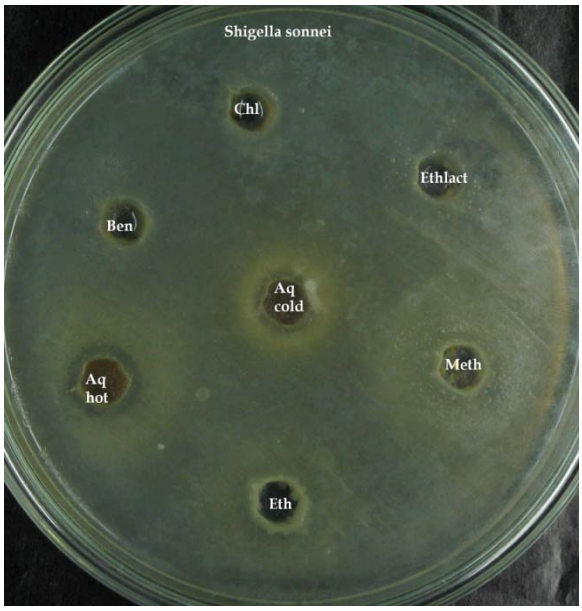
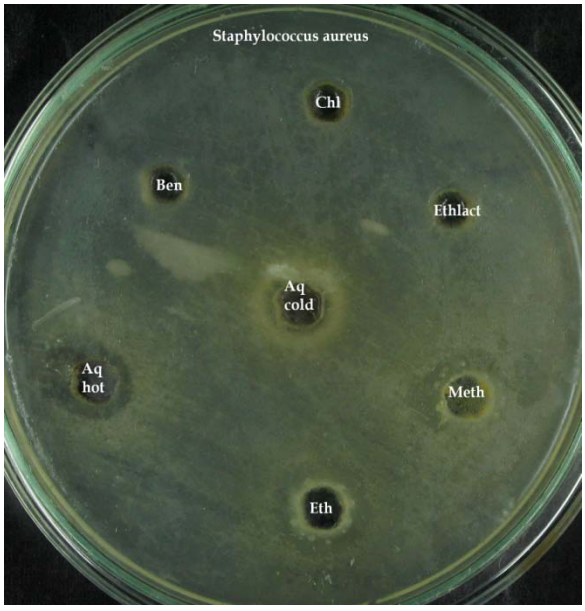


Shigella sonnei



Staphylococcus aureus

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Early Virological Response of the First Line Combination Therapy (Pegylated Interferon α - 2a and Ribavirin) in Iraqi Chronic Hepatitis C Patients and Their Psychological Adverse Effects

By Dr. Vian Ahmed Wasta Ismael, Dr. Kassim Al-Shamma & Dr. Hiwa Abubakir Hussein

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GJMR-L Classification : NLMC Code : MSC 2010: QV 38, QV 77.2

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I. Introduction

The hepatitis C virus (HCV) is a major public health problem and a leading cause of chronic liver disease (Williams R, 2006). Hepatitis C is the principal cause of death from liver disease and the leading indication for liver transplantation in the United States (Kim W, 2002).

Approximately 20-30% of patients with chronic HCV infection progress to end stage liver disease within 20 years and a small percentage develop hepato-cellular carcinoma (Walker R and Edwards O, 2007). HCV infection is now the leading worldwide indication for liver transplantation (Ryan K and Ray C, 2004, Walker R and Edwards O, 2007).

First-line treatment for HCV includes pegylated interferon plus ribavirin. The dosing regimen varies with the specific product and the duration of therapy varies with the product and HCV genotypes (Wells B *et al*, 2006).

Interferon (IFN) was first described in 1957 as an antiviral compound in chick embryo cells (Greenwood D *et al*, 2007). Its efficacy for treatment of HCV first was recognized when Hoofnagle *et al* (1986) published a preliminary findings when HCV was known as non-A, non-B hepatitis (Sangik O and Afdhal N, 2006). The United States Food and Drug Administration (FDA) approved alpha interferon monotherapy for the treatment of chronic HCV infection in 1992. Ribavirin was approved for use as an adjunct to interferon therapy of hepatitis C in 1998. Pegylated forms of interferon in combination with ribavirin were approved in the United States in 2001 (Hoofnagle J, 2009). There are two licensed pegylated interferons, peg interferon α-2b (Peg-Intron, Schering Plough Corp.), with a 12-kd linear polyethylene glycol (PEG) covalently linked to the standard interferon α-2b molecule, and peg interferon α-2a (Pegasys, Hoffmann-La Roche) with a 40-kd branched PEG covalently linked to the standard interferon α-2a molecule (Zeuzem S *et al*, 2003). The doses of these two forms of pegylated interferons (Peg IFNs) differ (Ghany M *et al*, 2009). Peg IFN α-2b is dosed according to body weight (1.5 μg/kg once weekly), while the larger Peg IFN α-2a is given in a fixed dose of 180 μg once weekly (Cornberg M *et al*, 2002). Peg IFN α-2b may also be dosed at 1.0 μg/kg once patients become negative for HCV-RNA without major declines in sustained virological response (SVR) rates (McHutchison J *et al*, 2009, Manns M *et al*, 2011).

Pegylation of interferons increases the persistence of the interferon in the blood, extend half life, better toleration and much importantly produces much superior virological response (Greenwood D *et al*, 2007).

The two licensed peginterferons have been shown in head-to-head comparison to be equivalent in efficacy and to have similar safety profiles (McHutchison J *et al*, 2009). Although smaller trials from southern Europe have suggested slightly higher SVR rates in patients treated with Peg IFN α-2a (Ascione A *et al*,

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2010, Rumi M *et al*, 2010), a large US multicentre study did not detect any significant difference between the two Peg IFNs in combination with ribavirin (RBV) regarding SVR (McHutchison J *et al*, 2009).

Ribavirin is a synthetic nucleoside in which ribose is linked to a triazole derivative. Like other nucleoside analogues it has to be activated intracellularly by phosphorylation (Greenwood D *et al*, 2007). The precise mode of action has proved elusive, though there are several theories including the possibility that it causes lethal mutations in viral nucleotides (Greenwood D *et al*, 2007). Ribavirin has limited utility as monotherapy and should be administered twice daily with food when used in combination with α-interferons (DiPiro J *et al*, 2005).

Meta-analyses and systematic reviews confirm that a combination of PegIFN with RBV is effective in treating patients with chronic hepatitis C (CHC), leading to high levels of SVR (Strader D *et al*, 2004). In general, the combination of RBV with α-interferons is associated with numerous adverse events to multiple organ systems, and these should be discussed with patients prior to initiation of therapy (DiPiro J *et al*, 2005).

This study was designed to measure early virological response (ERV) in HCV infected patients receiving first line combination therapy (Peg interferon α-2a and ribavirin) and to evaluate response according to different doses of ribavirin but fixed dose of peg interferon α-2a. Also this study was conducted to monitor appearance of psychological adverse effects to guide the patients and provide necessary instructions.

II. Subjects and Methods

a) Patients

This study was conducted during the period from the 15th March 2012 till 1st October 2012, which was carried out in Gastro-enterology center at General teaching hospital in Sulaimania city. Fifty six patients (30 males and 26 females) with an age of 18-70 years were divided into three groups according to viral genotypes (36 patients infected with genotype 1 and 20 patients infected with genotype 4), dose of the ribavirin and body weight of the patients. Throughout the study period, six patients were lost (4 males and 2 females) and only fifty patients (26 males and 24 females, in whom 32 patients were infected with genotype 1 and 18 patients with genotype 4) followed up. Four of the six patients stopped taking the drug (poor adherence), one female died (car accident), and the other female withdraw the drug after one month of treatment because of severe dehydration, arthralgia, myalgia, head ache, nausea and vomiting. All patients were recieving combination of 180 μg/week of Peg IFN α-2a s.c. injection (Pegasys® by Roche pharmaceutical company, Switzerland) and different doses of ribavirin capsules (Rebetol® by Schering pharmaceutical company, USA).

For monitoring of hematological and other common adverse effects from combination therapy that may necessitate dose adjustment or even withdrawal of the drugs, the patients were examined weekly for the first month then monthly for the other 2 month.

Ethical authorization and permission were submitted from each of college of pharmacy, directory of health and gastroenterology center in Sulaimania city. Informed concern had been taken from patients studied.

The previously diagnosed patients were recruited into the following prospective groups:

Group A : This group included fourteen patients infected with HCV genotype one, 10 males and 4 females ranging 22-65 years (mean ± SD, 45.4 ± 12.15), with body weights more than 75 kg, taking combination of PegIFN α-2a 180 μg once weekly as subcutaneous injection and RBV capsule 1200 mg per day (three 200 mg capsules after breakfast and three 200 mg capsules after dinner).

Group B : Included eighteen patients infected with HCV genotype one, 9 males and 9 females ranging 21-67 years (44.94 ± 14.7), with body weights equal or less than 75 kg, taking combination of Peg IFN α-2a 180 μg once weekly as subcutaneous injection and RBV capsule 1000 mg per day (three 200 mg capsules after breakfast and two 200 mg capsules after dinner).

Group C : Included eighteen patients infected with HCV genotype four, 7 males and 11 females ranging 18-65 years (43.78 ± 13.28), taking combination of Peg IFN α-2a 180 μg once weekly as subcutaneous injection and RBV capsule 800 mg per day (two 200 mg capsules after breakfast and two 200 mg capsules after dinner).

b) Healthy Subjects

Twenty healthy individuals were involved as a control group, including 8 males and 12 females ranging 19-69 years (39.1 ± 13.4).

c) Inclusion criteria

- Patients confirmed to have HCV infection, genotypes 1 and 4.
- Patients between 18-70 years old of both genders.
- Treatment naïve patients.
- Patients willing to be treated and to adhere to treatment requirement.

d) Exclusion criteria

- Patients with HIV or HBV co-infection.
- Patients with solid organ transplantation (heart, lung, liver, and kidney)
- Patients with decompensated liver disease.
- Patients allergic to any one of the components of combination therapy.
- Difficult to follow up patients (alcoholics, patients who travel frequently).
- Breast feeding and pregnancy or patients unwilling to comply with adequate contraception.

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- Patients with thalasemia, cytopenia, or severe anemia.
- Patients with renal failure.
- Patients with severe psychiatric disorder.
- Patients with severe immunosuppresion.
- Patients with heart failure or significant coronary or CVD.
- Patients with untreated thyroid disease.
- Patients with unknown HCV genotype (refused to do viral genotyping).

e) Sample collection and preparation

4 ml of venous blood was collected from each patient. The blood was drawn by venipuncture under basal condition using tourniquet with vacationer system, then centrifuged at 3000 rpm to seperate plasma and stored in ACD or EDTA tube and freezed at -20 ℃ (within 4 hrs of collection) and analyzed within 2 week. The assessments were done twice for each patient, once before starting treatment and second time three months after starting treatment with combination therapy of peg IFN α-2a and RBV.

f) Sample processing and extraction

Purification of viral nucleic acid from cell was carried out using genomic DNA extraction method in which nucleic acids of the virus are lysed quickly and efficiently using lyses buffer which is a highly concentrated solution of chaotropic salt. When combined with ethanol, the buffer creates optimum conditions for nucleic acid binding to the glass fiber matrix of the column tube. Contaminants such as salts, metabolites and soluble macromolecular cellular component are removed in the washing step. Nucleic acid is eluted in RNAase-free water and is then ready for use in subsequent reactions including real time RT-PCR and other enzymatic reactions (Bioneer Inc., 2009).

g) Amplification of viral nucleic acid

Amplification of viral nucleic acid (RNA for HCV) was carried out by real time RT-PCR procedure using EXICYCLER® (BIONEER/ South Korea). RNA templates are first reverse-transcribed to generate complementary cDNA strands followed by a DNA polymerase-mediated cDNA amplification. DNA detection simultaneous to amplification is preferentially achieved by the use of target sequence-specific oligonucleotides linked to two different molecules, a fluorescent reporter molecule and a quenching molecule. These probes bind the target cDNA between the two PCR primers and are degraded or released by the DNA polymerase during DNA synthesis. In case of degradation the reporter and quencher molecules are released and separated, which results in the emission of an increased fluorescence signal from the reporter. The fluorescence signal, intensified during each round of amplification, is proportional to the amount of RNA in the starting sample (Mauss S *et al*, 2012).

h) Psychological evaluations

For evaluation of psychological conditions of HCV infected patients before treatment and three months after starting treatment with 180 μg/week of s.c. peg IFN α-2a and different doses of RBV, each patient were interviewed and filled a used questionnaire. The questionnaire was prepared by a psychologist Dr. Rebwar H. Gharib at 2008 for his research (Gharib R, 2008) using DSM-IV scoring system (American Psychiatric Association, 1994). According to the questionnaire, patients who presented with at least 5 of depressive symptoms during the same two-week period or more, at least one of which is either depressed mood or loss of interest, is considered to have major depression. Patients who presented with at least 3 of depressive symptoms during the same two-week period or more, at least one of which is either depressed mood or loss of interest, is considered to have minor depression. Insomnia, suicidal idea and suicidal attempt were also considered separately.

i) Statistical analyses

All data are represented as mean ± standard error of means (SEM). Statistical analysis were carried out using paired sample T-test to compare treatment groups, focusing on changes from pre-treatment values and after three months of starting treatment of each group. Statistical analyses were carried out using SPSS 16.

III. Results

a) Effects of combination therapy on viral load

Table (1) and figure (1) show a significant reduction in viral load (amount of HCV-RNA in serum) three months after starting treatment compared to viral load before starting treatment. Nearly in all patients (47 patients), the viral load became no detectable in serum, but in only two patients (one 38 years old female, and one 36 years old male, both in group C) the virus was still detectable but comparing to pre-treatment amount there was more than two log reduction. Only one 63 years old female in group B was resistant to treatment and viral load increased by 2 times the pre-treatment value three months after starting treatment. In those patients whose serum viral RNA became no detectable, viral load reduced to 0.0 ± 0.0 IU/ml compared to pre-treatment values of 19427000 ± 527847 IU/ml (very high), 2374000 ± 331629 IU/ml (high), 914420 ± 31540 IU/ml (moderate) and 258500 ± 20169 IU/ml (low) with percent reduction of 100% .

b) Other parameters

In all three groups (as showed in tables 2 and 3), there were significant reductions ($p < 0.05$) in the levels of each of hemoglobin (Hb), white blood cell count (WBC), absolute neutrophil count (ANC), platelet count (PLT), random plasma glucose level, Alanine

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aminotransferase (ALT), Alkaline phosphatase (ALP), serum albumin and body weight three months after starting treatment compared to pre-treatment values. Neither dose reduction nor pharmacological interventions were required, since hematological reductions were not severe.

c) Psychological condition

In figure 2 for group A patients (n=14); before starting treatment, two patients (one male and one female) were complaining from isolated insomnia (insomnia alone, without any other psychological symptoms), which represents 14.3% of all group A patients, who both became suffering from major depression later three months after starting treatment. And one female patient was complaining from minor depression before treatment representing 7.1% of all the group's patients who also became suffering from major depression three months after starting treatment. The remaining 78.6% were psychologically normal patients. After 3 months of starting treatment with s.c. Peg IFN α-2a 180 μg/week and RBV 1200 mg/day, three cases (21.4%) of minor depression, four cases (28.6%) of major depression one of whom also had suicidal idea, three cases (21.4%) of isolated insomnia were reported. Among those who reported major depression, one patient was male (who also had a suicidal idea) and the other three were females, all three patients with minor depression were male, and those with isolated insomnia were two males and one female. Overall, three months after starting treatment, only four patients (28.6%) were not complaining from psychiatric symptoms (three males and one female), while the remaining ten patients (71.4%) were complaining from psychiatric symptoms as individualized above.

In figure 3 for group B patients (n=18); before starting treatment, six patients (one male and five females) were complaining from minor depression which represents 33.3% of all group B patients, one female of whom had suicidal idea. Later, three months after starting treatment, all of these six patients became suffering from major depression, two females of these became having suicidal idea without suicidal attempt. And only one female patient representing 5.6% was complaining from isolated insomnia, who became majorly depressed and died later after three months of starting treatment as a result of suicidal attempt. The remaining 61.1% were psychologically normal patients. After 3 months of starting treatment with s.c. Peg IFN α-2a 180 μg/week and RBV 1000 mg/day, seven cases (38.8%) of minor depression, five cases (27.8%) of major depression three of whom had suicidal idea, three cases (16.7%) of isolated insomnia were reported. Unfortunately, 5 days after my interview with patients, one 43 years old female who had isolated insomnia alone before starting treatment, committed suicide by jumping out of a building, after three days of staying at hospital, she passed away. One point of note is that, this female had experienced major depression and frequent suicidal idea compared to the pre-treatment state, before committing suicide. Among those who reported major depression, two patients were male and the other three were females, two males and five females were with minor depression, and those with isolated insomnia were two males and one female. Overall, three months after starting treatment, only three patients (16.7%) were not complaining from psychiatric symptoms, while the remaining fifteen patients (83.3%) were complaining from psychiatric symptoms as individualized above.

In figure 4 for group C patients (n=18); before starting treatment, two female patients were complaining from minor depression which represented 11.1% of all group C patients, one of them was also complaining from suicidal idea. One female (5.6%) was complaining from major depression before treatment who interestingly became minor depressed three months after starting treatment. No one was complaining from isolated insomnia before treatment. The remaining 83.3% were psychologically normal patients. After 3 months of starting treatment with s.c. Peg IFN α-2a 180 μg/week and RBV 800 mg/day, four cases (22.2%) of minor depression, five cases (27.8%) of major depression one of whom had suicidal idea and tried to commit suicide, four cases (22.2%) of isolated insomnia were reported. Unfortunately, three days before my interview with patients, one 54 years old female who already had minor depression and suicidal idea even before starting treatment, tried to commit suicide by burning herself, but she was lucky and rescued by her son who prevented her from doing such a thing. One point of note is that, at the time of interview, three days before trying to commit suicide, this female had experienced major depression and more frequent suicidal idea compared to the pre-treatment state. Among those who reported major depression, one patient was male and the remaining four were females, two males and two females were with minor depression, and those with isolated insomnia were two males and two females. Overall, three months after starting treatment, only five patients (27.8%), two males and three females, were not complaining from psychiatric symptoms, while the remaining thirteen patients (72.2%) were complaining from psychiatric symptoms as individualized above.

In figure 5 for control group (n=20); Two patients (one male and one female) were complaining from minor depression which represents 10% of all control group individuals, one female with major depression and suicidal idea which represents (5%), and seven patients (two males and five females) representing 35% were complaining from isolated insomnia, the remaining 50% were psychologically normal individuals.

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Finally in figure 6 we may say that, among all three groups of patients (A, B, and C), percent of patients experienced major depression and percent of those who were psychologically normal three months after starting treatment, were greater in group A (28.6% for each) compared to group's B and C patients. For minor depression, the percent of patients experiencing it, was greater in group B patients (38.8%) compared to other groups, and isolated insomnia was more frequent among group C patients than group A and B patients, and percent of patients experienced major depression were the same in both groups B and C (27.8%). One point of note, percent of individuals experiencing isolated insomnia was greater (35%) among control group than other three patient groups after three months of treatment.

IV. Discussion

Hepatitis C virus infection is still a global and the possible new approaches for conquering the health care challenges. The standard of care (SOC) therapy for patients with chronic hepatitis C virus infection has been the use of both peg interferon and ribavirin (Ghany M *et al*, 2011). These drugs are administered for either 48 weeks (HCV genotypes 1, 4, 5, and 6) or for 24 weeks (HCV genotypes 2 and 3), inducing sustained virological response rates of 40%-50% in those with genotype 1 and of 80% or more in those with genotypes 2 and 3 infections (Manns M *et al*, 2001, Fried M *et al*, 2002a, Hadziyannis S *et al*, 2004). Although PegIFN and RBV remain vital components of therapy, the emergence of direct acting antivirals has led to the concept of triple therapy in many patients with genotype 1 chronic HCV infection (Ghany M *et al*, 2011).

The therapy of hepatitis C began almost 26 years ago with a small trial of recombinant human interferon alfa (Hoofnagle J *et al*, 1986). The rationale for using interferon was its broad antiviral effects and the suspicion that it might be active against the still-undiscovered agent of non-A non-B hepatitis. Not until the discovery of the HCV, at 1989 (Feitelson M, 2003), were the effects of interferon understood. Nevertheless, interferon was approved for use for hepatitis C treatment in the United States in 1992 (Hoofnagle J, 2009).

The second important advance in hepatitis C therapy came with the use of ribavirin. Ribavirin was approved for use as an adjunct to interferon therapy of hepatitis C in 1998. A third advance in therapy of hepatitis C came soon thereafter, with the introduction of pegylated forms of interferon that allowed for once-weekly (rather than thrice-weekly) injections. Peg nterferon was approved in the United States in 2001 (Hoofnagle J, 2009). The treatment paradigm for HCV has changed with the recent FDA approval of two first generation protease inhibitors, telaprevir, and boceprevir for genotype 1 infected individuals. Nonetheless, ribavirin and pegylated interferon remain integral components of treatment (Ghany M *et al*, 2011).

In our community, combination of peginterferon and ribavirin is still the first choice because of high cost of triple therapy and difficulties in providing these drugs on continuous bases. Despite that, majority of our patients cannot afford such a large amount of money for providing the drugs themselves continuously. So in order to determine advantage and effectiveness of this first line combination therapy (by measuring early virological response which is a main predictor for sustained virological response in majority of patients) in HCV infected patients in our communuity, its psychological effects after three months of treatment, present study has been conducted.

The results of present study are somewhat conflicting and out of line with the results of other studies because patients treated in clinical trials, represent a highly selected population not necessarily representing general HCV infected population (Ferenci P *et al*, 2005). Therefore, it is not clear if the reported efficacy and safety of peg interferon α and ribavirin regimen would be validated in routine clinical practice.

Response to standard treatment with peg interferon α-2a and ribavirin in patients with chronic hepatitis caused by HCV, including genotypes 1 and 4, has been widely studied and documented in numerous populations (Fried M *et al*, 2002a, Hadziyannis S *et al*, 2004). Approximately 80% of patients who have genotype 1 and virtually all patients who have genotypes 2 and 3 achieve an early virological response (Davis G, 2002, Fried M *et al*, 2002a, Lindsay K, 2002, Shiffman M *et al*, 2007a).

Early virologic response (EVR) was defined as the > 2 $\log_{10}$ reduction in HCV RNA in serum 12 weeks after starting treatment. In case of total absence of HCV RNA in serum 12 weeks after starting treatment, a complete early virologic response (cEVR), which is a more promising predictor of sustained virological response (SVR) than EVR, is obtained (Mauss S *et al*, 2012). Over all, in this study among all participants, 94% achieved cEVR (100%, 94.4% and 88.9% for groups A, B and C respectively) 4% achieved > 2 $\log_{10}$ reduction in HCV RNA, i.e., EVR (11.1% of group C patients) and 2% null responder (5.6% of group B patient) who discontinued drugs after 12 weeks of starting treatment after confirming increased viral load, patient's instruction and acceptance.

In a retrospective analysis done by Gheorghe L *et al* (2005) in Romania that consisted of 174 HCV infected patients, therapy was stopped in patients who do not achieve 2 log reductions in viral load 12 weeks after starting treatment, the same strategy that followed in present study.

Early virological response (EVR) in a clinical trial at Beth Israel Medical Center NY (Johnson T *et al*, 2004) was 63% in genotype1 patients treated with peg interferon α-2a and ribavirin. This difference in present study results from that in Beth's, may be explained by

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more advanced illness and a high proportion of black patients in Beth's study.

In one of a phase III trials of peg interferon α-2a and ribavirin, by week 12 of therapy, EVR was achieved by 86% of patients (Fried M *et al*, 2002a) which is near to present study results. These results suggest that patients who have EVR who remain PCR positive at 12 weeks (not complete absence of HCV RNA) should have PCR testing repeated after 24 weeks before making any decision about discontinuing therapy. Achievement of EVR can provide a goal to motivate patient adherence during the first months of therapy, and early testing provides the opportunity to reassess the need for continued treatment. Consequently, when an EVR is absent, discontinuation of therapy should be considered because the likelihood of sustained response is negligible, but the decision must be made on an individual patient basis. If uncertainty exists, retesting should be considered before stopping therapy (McHutchison J and Fried M, 2003, Manns M, 2004), which was the case with the female patient in group B in this study, when repeated viral load testing after one week of last PCR showed the same increase in HCV RNA compared to baseline level.

In a study done by Ascione A *et al* (2010) in Italy including both genotypes 1 and 4, EVR was obtained in 85% of all patients. The majority of patients obtained a complete EVR, while the number of those who obtained a partial EVR was only 8.8%. The results of Ascione A *et al*'s study are nearly the same as that in present study.

In the registration trials of peg interferon α-2a plus ribavirin, 10% to 14% of patients had to discontinue therapy due to an adverse event (Manns M *et al*, 2001, Fried M, 2002b), compared to 2% discontinuation in present study. Laboratory abnormalities are the most common reasons for dose reduction. Among these, neutropenia (absolute neutrophil count [ANC] of 1500 mm^3) was a frequent laboratory abnormality, occurring in 18% to 20% in the two large phase III clinical trials where the dose was reduced 50% for an ANC of 750 mm^3 and permanently discontinued for an ANC of < 500mm^3 (Manns M *et al*, 2001, Fried M *et al*, 2002a). Severe neutropenia, ANC <500 mm^3, occurred in 4% of subjects. None of these lab abnormalities were reported in present study participants. Actually these abnormalities happened in participants but not so severe to necessitate dose reduction but if study participants were followed up further (i.e., more than three months) these severe effects that call for dose modification may appear. Interferon causes anemia via bone marrow suppression and ribavirin causes anemia via hemolysis (De Franceschi L *et al*, 2000).

For psychological presentation, among all 50 patients 28% presented with major depression 10% of whom had suicidal idea and 4% committed suicide. 28% presented with minor depression, 20% with isolated insomnia and the remaining 24% were psychologically normal HCV infected patients.

Major depression was more common in group a patients (28.6%) than group's B and C patients (27.8% for each group), minor depression was more common in group B patients (38.8%) than group A patients (21.4%) and group C patients (22.2%). Isolated insomnia was more common among group C patients (22.20%) as well as control group (35%) compared to group a patients (21.4%) and group B patients (16.7%). These results demonstrate that appearance of any type of depression or psychological symptoms is more related to individual's susceptibility for developing symptoms and surrounding environment, because as it obvious, all three groups of patients treated with equal doses of peg interferon α-2a but the percent of patients showed psychological presentations differ, and its known that it is interferon that induce psychological abnormalities not ribavirin, that's why psychological presentations in any one of the groups is not related to the variations in ribavirin dosing regimen. Only one interesting female patient in group C, who experienced major depression before starting treatment, became minorly depressed at 12th week of treatment. The pre-treatment depression in this patient was because of knowing that she is infected with HCV and misunderstood by some specialists that it is not curable and should be isolated from family and friends so as not to infect others. But later when we explained the disease course, routes of transmission and precautions that needed to be made, she felt better but still minor depressed after three months this one may be the effect of interferon on neurotransmitters.

Manns M *et al* (2006) demonstrated that depression in HCV-infected individuals occurs in up to 60**%** (Hilsabeck R and Malek A, 2004, Zacks S *et al*, 2006). During HCV treatment with interferon based regimens the prevalence of depression has been reported to be between 10%–40% depending on the screening method used (Zacks S *et al*, 2006). Recently, data from the Virahep-C study, a prospective analysis of depression during HCV genotype 1 treatment with peg interferon and ribavirin, demonstrated that low social support was independently associated with pre-treatment and on-treatment development of depression (Evon D *et al*, 2009). At any time during the Virahep-C study 20% had depressive symptoms whereas 18% of non-responders compared to 10% of responders had depression 6 months after the end of treatment, a difference that may be explained by the failure of a challenging 48-week treatment course. This explains that occurrence of depression is more common during first three months of treatment, the time when the patients are still unaware of their response rate to treatment. In Virahep-C study 21% of patients developed depression during the first 12 weeks, a result which is nearly the same as present study findings.

A recent study of 1010 HCV infected patients demonstrated that suicide risk was higher in males and in patients under the age of 45 (Kristiansen M *et al*,

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2011), this finding is in contrary with that in present study, because the two patients who had suicidal idea and committed suicide (one died and the other one prohibited) were both females and the other three patients who had suicidal idea but did not try to commit it were 2 females and 1 male and all were above 40 years old. Different conclusions were reported in a study of almost 400 HCV-infected patients with genotype 1 on peg interferon and ribavirin, where just 3.5% reported suicidal ideation and none attempted suicide (Evon D *et al*, 2009), this may be due to extensive supervision and guidance of patients by psychiatrists and family involvement strategy but neither is done during present study period.

Elsewhere in the literatures, many HCV treatment studies have reported various depression and suicidal ideation rates, making the application of these results to clinical practice impractical. This inconsistency in reporting may reflect the utilization of different depression screening methods, physician and patient biases in diagnosing and reporting symptoms, and variable treatment protocols followed. It is important to acknowledge the effect of interferon on the thyroid and the potential development of depressive-like symptoms related to thyroid dysfunction, mimicking, or even masking depressive symptoms related to interferon use (Papafragkakis H *et al*, 2012).

In a cross-sectional study of 43 patients who had chronic hepatitis C and not receiving interferon α, Kraus M *et al* (2001) found several factors that correlated significantly with depression. Higher rates of depression were observed in older patients (>50 years; P = 0.024), in patients who were aware of their hepatitis diagnosis for more than 5 years (P = 0.003), and in patients who were informed that they were not eligible for interferon α therapy (P = 0.001). Furthermore, a lower incidence of depression was noted in patients who had been diagnosed with HCV recently (1–6 months; P = 0.003). If such evaluations were done in present study in HCV infected patients before starting treatment, at least some if not all explanations made by Kraus *et al* may be applicable especially age more than 50 years and newly diagnosed ones.

Finally we should not exclude the major role of patient care and need of clinical pharmacist interventions in improving patient's adherence and response to standard therapy. Interim results of a prospective, randomized, controlled multicenter study indicate that active intervention with patient education, aggressive side effect management, and expanded supportive nursing intervention with cognitive behavioral therapy by way of telephone calls for patients infected with HCV who are treated with Peg-IFN and RBV therapy is feasible, can decrease the dropout rate in the first 12 weeks of therapy, and is associated with significant improvements in patient quality of life at early time points in treatment (Sarrazin C et al, 2010a). In present study some if not all of mentioned above strategies followed, so this may be the main cause of high rate of EVR especially cEVR and low rate of unwanted effects of treatment on organ functions.

V. Conclusions

- First line combination therapy with Pegylated interferon α-2a and ribavirin is highly effective in early eradication of hepatitis C virus (98% EVR) in Iraqi chronic hepatitis C patients and can be used relatively safely .
- Lower doses of ribavirin lowers percent of patients who achieve complete EVR.
- Development of major depressive symptoms occurred frequently during Pegylated interferon α-2a and ribavirin treatment and was predicted by baseline depression scores and higher doses of ribavirin.
- Patient care, patient instruction by clinical pharmacist and family involvement strategies have a great role in improving adherence, minimizing early drug withdrawal, managing mild unwanted disturbing effects and help minimizing unwanted psychological effects.

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Table 1 : Viral load (amount of HCV RNA in serum in IU/ml) of HCV infected patients, genotypes one and four, before treatment and 3 months after starting treatment with PegIFN α-2a 180 μg/week and RBV 1200, 1000 or 800 mg/day. Each value represents the mean ± standard error. Number of patients = 47 (Very high viral load=13, High viral load=15, Moderate viral load=5, Low viral load=14).

Time of treatment	Viral load (IU/ml)			
	Very high	High	Moderate	Low
Pre-treatment	19427000 ± 527847	2374000 ± 331629	914420 ± 31540	258500 ± 20169
After 3months	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*

*$p < 0.05$ significant difference between treated and pre-treated values.

Very high; viral load > 10,000,000 IU/ml
High; viral load > 1,000,000 IU/ml
Moderate; viral load > 500,000 IU/ml
Low; viral load < 500,000 IU/ml

Table 2 : Pre-treatment values.

Variables	Group A	Group B	Group C
Hb (g/dl)	14.8 ± 0.4	13.82 ± 0.39	13.1 ± 0.36
WBC (/mm³)	7157.1 ± 583.4	5982.8 ± 403.9	5422.2 ± 325.8
ANC (/mm³)	4198.7 ± 434.2	3370 ± 245.6	3168.7 ± 261.3
PLT (/mm³)	209210 ± 14157	235280± 12978	202060 ± 12483
Plasma glucose level (mg/dl)	103.36 ± 2.5	101.9 ± 1.6	99.17 ± 1.8
ALT (IU/L)	55.1 ± 4.4	59.2 ± 2.86	55.3 ± 3.6
ALP (IU/L)	273.5 ± 13.5	300.5 ± 12.3	353.4 ± 11.2
S.Albumin (g/dl)	4.04 ± 0.1	4.2 ± 0.10	4.01±0.12
Body weight (Kg)	88.07 ± 2.1	66.56 ± 1.83	80.06 ± 3.25

Table 3 : Values three months after starting treatment.

Variables	Group A	Group B	Group C
Hb (g/dl)	13.5 ± 0.3	11.5 ± 0.35	11.5 ± 0.35
WBC (/mm³)	4892.9± 475.9	3800± 305.6	3800± 305.6
ANC (/mm³)	2679.8± 329.9	2015 ± 187	2015.2 ± 187.1
PLT (/mm³)	178930± 10401	158110 ± 12905	158110 ± 12905
Plasma glucose level (mg/dl)	88.57 ± 2.16	90.4 ± 1.5	88.44 ± 2.6
ALT (IU/L)	47.9 ± 3.58	41.3 ± 1.98	45.7 ± 1.9
ALP (IU/L)	192.7 ± 11.6	227 ± 11.09	276.7 ± 9.1
S.Albumin (g/dl)	3.58 ± 0.1	3.8 ± 0.09	3.57±0.096
Body weight (Kg)	80.36 ± 2.4	62 ± 2.39	74.2 ± 3.02

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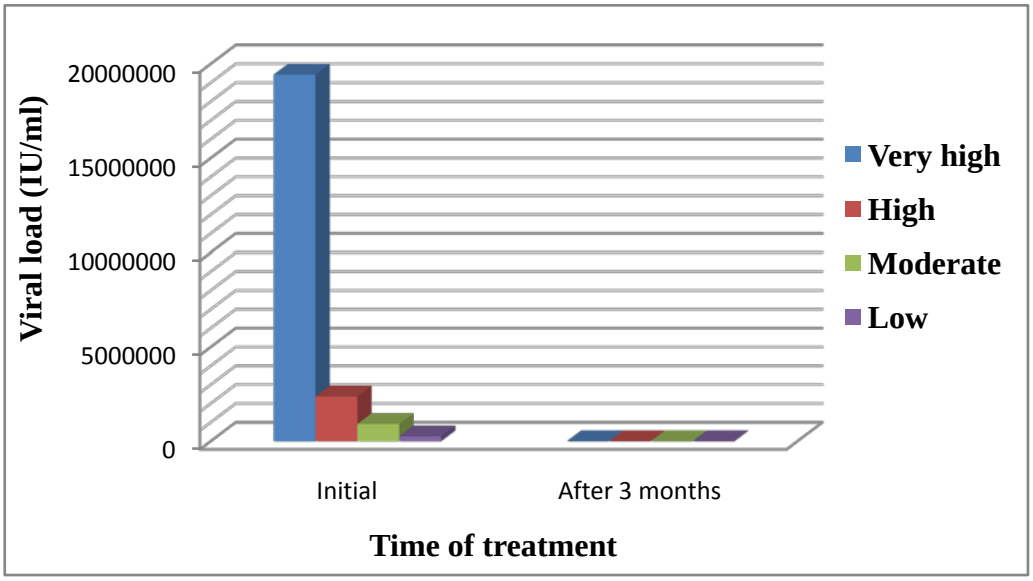
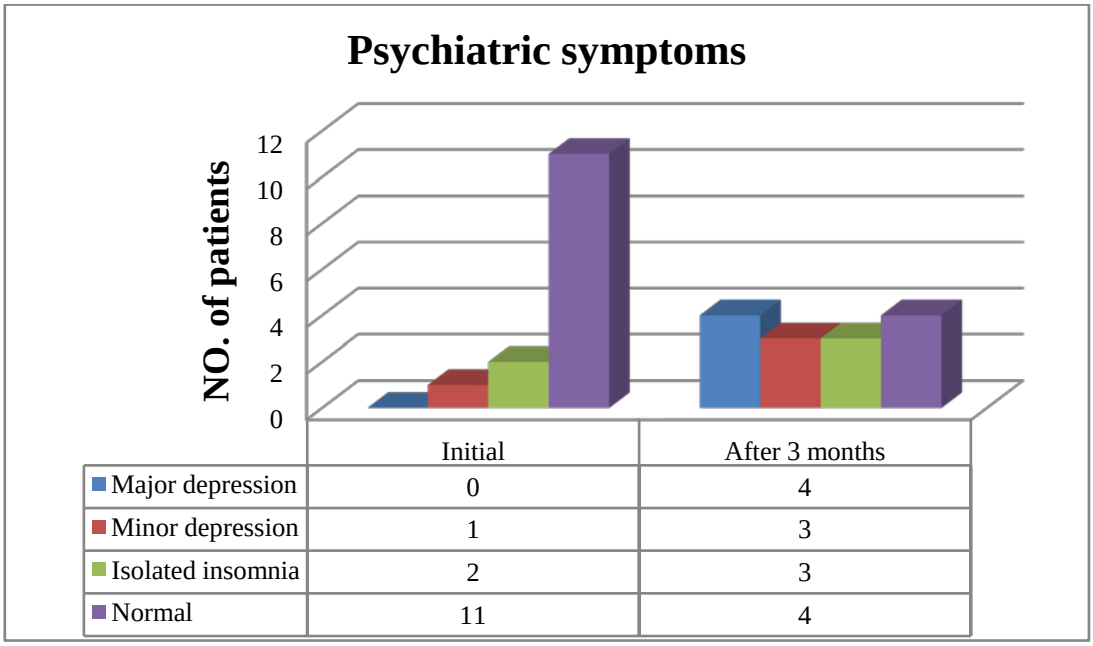


Figure 1 : Histogram showing viral load (amount of virus RNA in serum) in newly diagnosed HCV infected patients, genotypes one and four, treated with s.c. PegIFN α-2a 180 μg/week and RBV 1200, 1000 or 800 mg/day for 3 months (n=47).



	Initial	After 3 months
Major depression	0	4
Minor depression	1	3
Isolated insomnia	2	3
Normal	11	4

Figure 2 : Data chart showing psychological presentation in newly diagnosed HCV infected patients in group A, before treatment and three months after starting treatment with s.c. PegIFN α-2a 180 μg/week and RBV 1200 mg/day (n=14).

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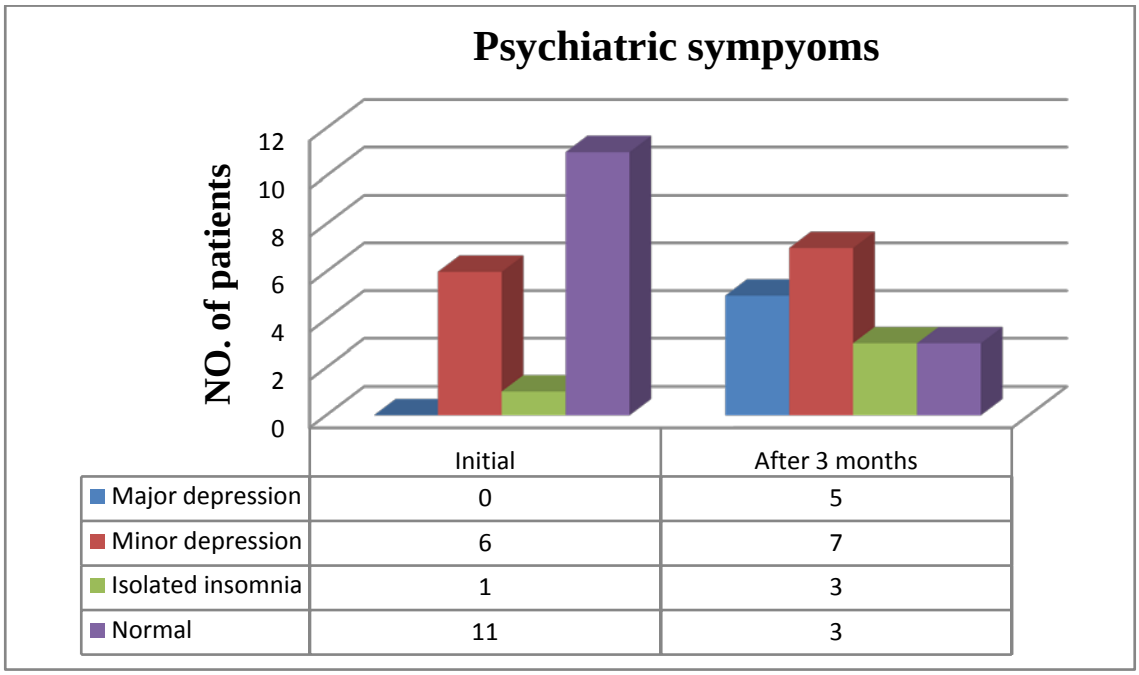

Figure 3 : Data chart showing psychological presentation in newly diagnosed HCV infected patients in group B, before treatment and three months after starting treatment with s.c. PegIFN α-2a 180 μg/week and RBV 1000 mg/day (n=18).

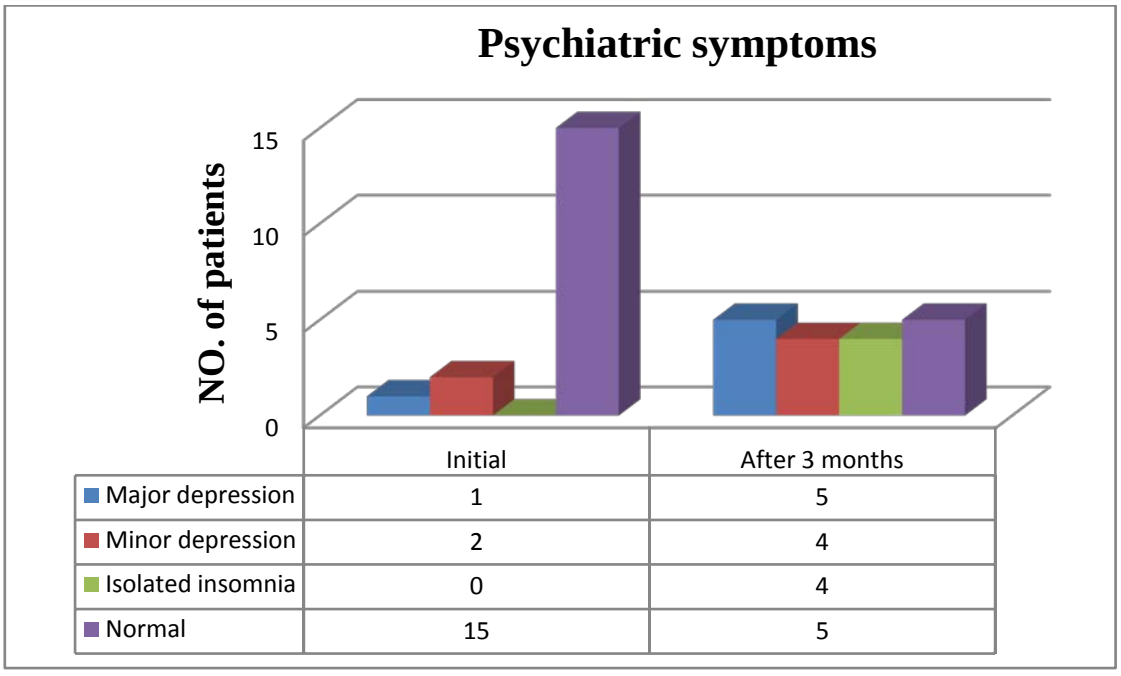

Figure 4 : Data chart showing psychological presentation in newly diagnosed HCV infected patients in group C, before treatment and three months after starting treatment with s.c. PegIFN α-2a 180 μg/week and RBV 800 mg/day (n=18).

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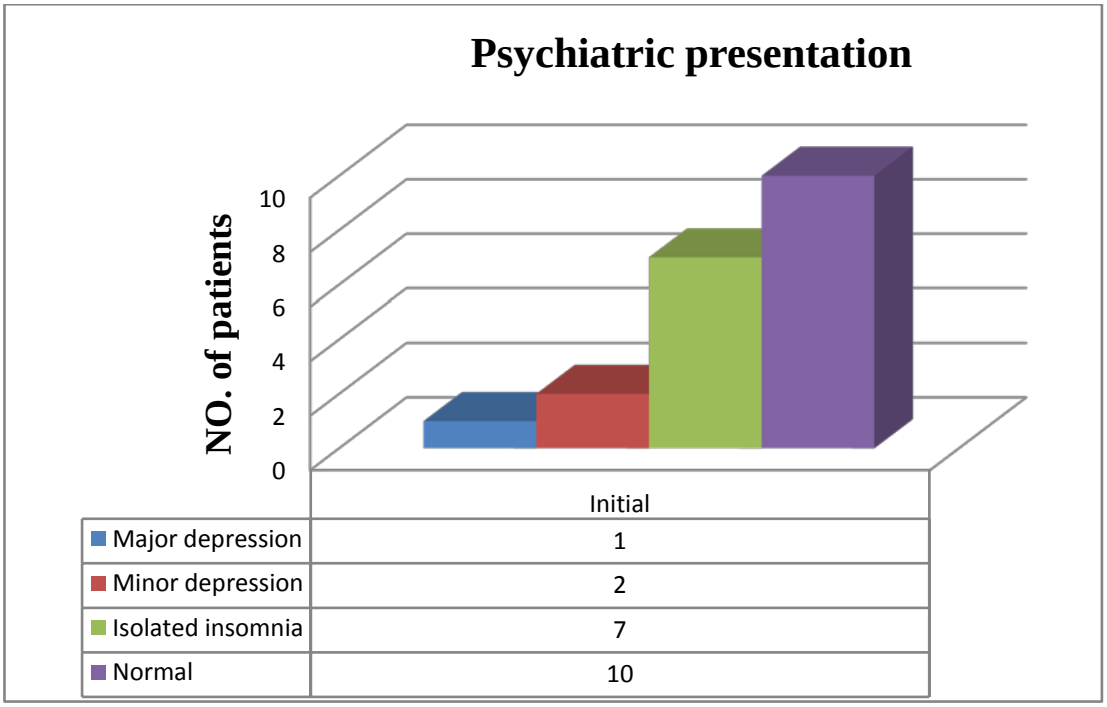

Figure 5 : Data chart showing psychological presentation in control group (n=20).

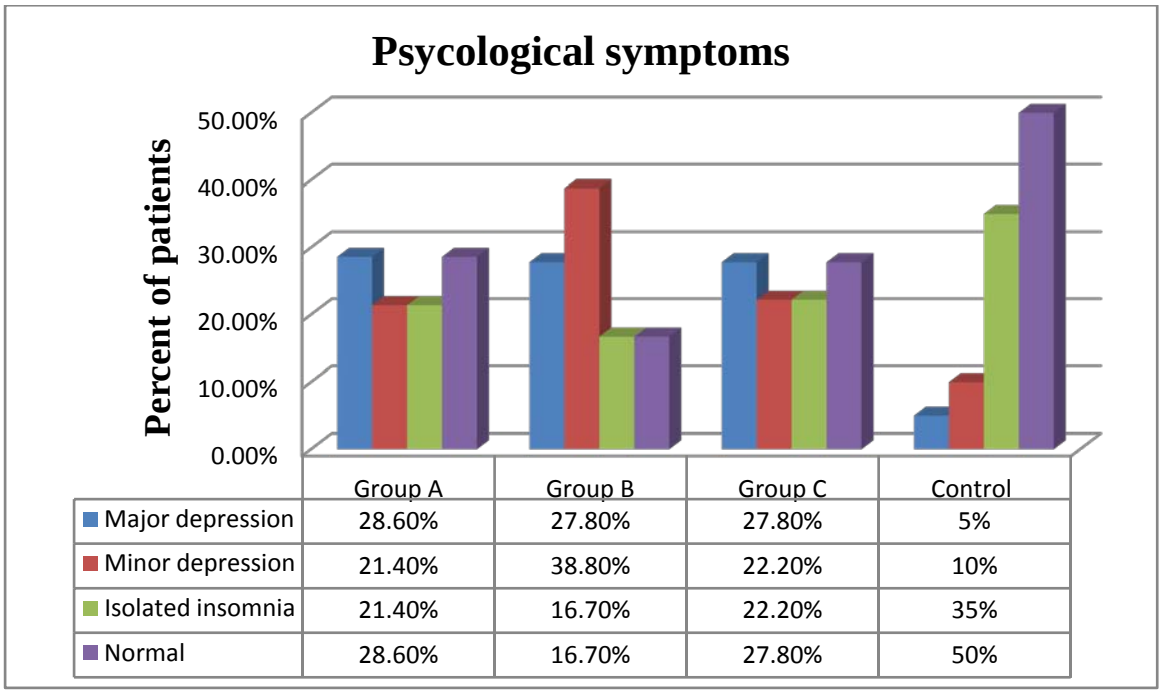

Figure 6 : Data chart showing percent comparison in psychological presentation of A, B, C and control groups (n=70).

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Roles of Cyclin Dependent Kinase and Cdk- Activating Kinase in Cell Cycle Regulation: Contemplation of Intracellular Interactions and Functional Characterization

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Abstract - Cyclin dependent protein kinases (CDKs) play vital role in gene expression and cell cycle regulation. CDKs require cyclin binding activity, phosphorylation through CDK activating kinase (CAK), Cdc25, Wee 1 kinase. Non-cyclin CDK activators include CDK5 activators, Viral Cyclins and RINGO/Speedy. Among all CDK activators, CAK carries prime importance. The time frame of activating phosphorylation varies across different model organisms. A literature search was performed via using Keywords: Cyclin-dependent kinases, CDK activating kinases, Interactions of CDK activating kinase, Association of CDK activating enzymes with cellular proteins, Cell cycle regulation via CDKs, Structure and Function of CDK activating kinases in Pubmed and Google scholar. The key findings on the basis of previous studies illustrated that the CDK3, CDK4 and CDK6 are associated with regulation of G1-S phase transition; CDK2 is involved in entrance to S phase and DNA replication; while CDK1 is vital for mitosis. The CDK activity is regulated via cyclin binding, cyclin-dependent kinase inhibitors CKIs, CDK phosphorylation at ATP-binding pocket for inhibition while for activation CDK phosphorylation occurs at T-loop conserved residue.

Keywords : *cyclin dependent kinases, CDK, CDK activating kinase, CAK, CKI, cell cycle regulation, structural characterization of CAK, functional characterization of CAK.*

GJMR-L Classification : *NLMC Code : QU 55.2, QU 21*

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Roles of Cyclin Dependent Kinase and Cdk-Activating Kinase in Cell Cycle Regulation: Contemplation of Intracellular Interactions and Functional Characterization

Umar Saeed [α], Nasir Jalal [σ] & Muhammad Ashraf [ρ]

Abstract - Cyclin dependent protein kinases (CDKs) play vital role in gene expression and cell cycle regulation. CDKs require cyclin binding activity, phosphorylation through CDK activating kinase (CAK), Cdc25, Wee 1 kinase. Non-cyclin CDK activators include CDK5 activators, Viral Cyclins and RINGO/Speedy. Among all CDK activators, CAK carries prime importance. The time frame of activating phosphorylation varies across different model organisms. A literature search was performed via using Keywords: Cyclin-dependent kinases, CDK activating kinases, Interactions of CDK activating kinase, Association of CDK activating enzymes with cellular proteins, Cell cycle regulation via CDKs, Structure and Function of CDK activating kinases in Pubmed and Google scholar. The key findings on the basis of previous studies illustrated that the CDK3, CDK4 and CDK6 are associated with regulation of G1-S phase transition; CDK2 is involved in entrance to S phase and DNA replication; while CDK1 is vital for mitosis. The CDK activity is regulated via cyclin binding, cyclin-dependent kinase inhibitors CKIs, CDK phosphorylation at ATP-binding pocket for inhibition while for activation CDK phosphorylation occurs at T-loop conserved residue. Structural and functional characterization of CDK activating kinases and interactions with other cellular proteins were also discussed in detail. Loss of CAK activity usually leads toward transcriptional defects and cell cycle arrest. Identification of CDK and CDK activating kinases inhibitors could provide potential therapeutic options against human neoplasias.

Keywords : cyclin dependent kinases, CDK, CDK activating kinase, CAK, CKI, cell cycle regulation, structural characterization of CAK, functional characterization of CAK.

I. Introduction

Cyclin-dependent kinases (CDKs) are group of protein kinases (serine/threonine kinases), activated via formation of a complex with cyclin molecules, involved in cell cycle regulation. CDKs are considered as potential target molecules for anti-cancer medication. The level of CDK remains constant in a cell, while cyclin level fluctuates depending upon cell cycle stage. It has been reported that each cyclin is associated with one or two CDKs and most of the CDKs get associated with one or two cyclin molecules. Cyclin-CDK complex formation results into activation of CDK active site. Formation of this complex is regulated via various phosphate and kinase molecules including CDK-activating kinase (CAK), Cdc25 and Wee 1 kinase (1). CDK also get activated via non-cyclin CDK activators such as CDK5 Activators, Viral Cyclins and RINGO/Speedy (2,3). Some of the alternative names for CDK include cell division protein kinase 1, Cell division control protein 2 homolog and p34 protein kinase (4). CDKs have been categorized into CDK1 / CDC2, CDK2, CDK3, CDK4, CDK5, CDK5R1, CDK7, CDK8, CDK9 / CDC2L4, CDK16 / PCTAIRE1, CDKL2, CDKL3, CDKL4, CDKL5 (1). The phosphorylation at threonine-14 or tyrosine-15 causes inactivation or deregulation of its enzymatic potential while phosphorylation at threonine-161 around the T-loop activates it (5). The CDK 7 member acts indirectly, by acting as CDK-activating kinase (CAK) that cause phosphorylation of other CDKs (especially CDK1, CDK2, CDK4, and CDK6 molecules)(6).

Cyclin-dependent kinase activity requires phosphorylation at active site of threonine residue. The phosphorylation time frame varies across model organisms. It has been reported in mammalian cells that the activating phosphorylation take place after cyclin binding; while in yeast cells, the activating phosphorylation usually occurs before cyclin binding. The activity of CDK kinase is not regulated via known cell-cycle pathways. It has been reported that cyclin binding is actually a limiting step for CDK activation (6). The CDK activating kinase is usually composed of CDK7, cyclin H and Mat1assembly protein. Phosphorylation of activation segment is not prerequisite for CDK7/cyclin H complex activation in presence of Mat1; while in absence of Mat1, phosphorylation at Ser170 and Thr176 in the activation segment of CDK7 is required for its activity. It has been reported that CDK7/cyclin H and CDK2/cyclin A do not

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self phosphorylates, but have ability to tendency to phosphorylate each other (7).

Morgan (2007) reported various CAKs from S. *cerevisiae*, S. *pombe*, D. *melanogaster*, X. *laevis* and H. *sapiens*. S. *cerevisiae* possesses CAKs including CAK1 (also known as Civ1) and Kin. The CAK 1 are monomer with non cyclin partner, while Kin 28 are CDK7 related with no CAK activity. S. pombe possesses CAKs including Csk1 and Mcs 6. The Csk1 is monomer and related to Cak1 while Mcs6 is related to CDK7 and usually binds to cyclin Mcs2. D. *melanogaster*, X. *laevis* and H. *sapiens* possesses CDK7 as CAK that forms trimer with cyclin H and Mat1. The CAK (CDK1) of X. *laevis* is also recognized as M015. It has been reported that CAK activity remains high during cell cycle via unknown control mechanism. In G0 quiescent state CAK activity is comparatively low, compared to tumor cells (6). It is a matter of fact that CAK is localized to nucleus in many vertebrates. This phenomenon suggests that CAK is involved in transcription along with cell regulation. It has been reported that CDK7 (a type of CAK) is involved in phosphorylation of cellular transcriptional machinery (8,9). Serizawa et al, reported strong association of CDK-activating kinase subunits with transcription factor Transcription Factor IIH (TFIIH) which suggested their role in transcriptional regulation as well as in cell-cycle control (10). Shiekhattar et al, reported CAK complex as an important component of human transcription factor TFIIH, their findings suggested that phosphorylation of both Cdc2 and CDK2 creates link between cell cycle regulation and transcription (11).

II. Literature Search

A review of literature was conducted via accessing latest research articles from Pubmed, Google Scholar by using the key words: Cyclin-dependent kinases, CDK activating kinases, Cell cycle regulation via CDKs, Interactions of CDK activating kinase, Association of CDK activating enzymes with cellular proteins, Structure and Function of CDK activating kinases. Most relevant research articles of previous two decades were considered for review. The anatomical and biological context of Cak1 was kept into consideration and CAK1 related enzymatic, physical and regulatory interactions were contemplated. High impact information was pooled into three categories of "Association of CDK activating kinases (CAKs) with Cyclin-dependent kinase", "Structural characterization of CDK activating kinases (CAKs) activation" and "Functional characterization of CDK activating kinases: interactions with other cellular proteins".

a) Association of CDK-activating kinases (CAKs) with Cyclin-dependent kinase

TFIIH was identified initially as basal transcription factor associated with transcription of protein-coding genes. The cloning of nine vital TFIIH subunits revealed its importance in repair of damaged DNA and cell cycle regulation (both of which are fundamental processes in cell). It is quite obvious that TFIIH is involved in various other cellular metabolic process, thus mutation in some of its subunits may cause serious human disorders leading towards complex pleiotropic symptoms such as susceptibility towards cancer, developmental abnormalities and UV-light sensitivity. The study conducted by Keriel et al discussed ternary subcomplex of TFIIH and its importance as CDK-activating kinase due to its tendency towards activating CDKs via phosphorylation along with its vital enzymatic activities of RNA synthesis and DNA repair (12). Nasmyth et al and Beach et al, reported a single CDK (Cdc28p or its ortholog Cdc2) was found responsible for all important cell cycle transitions (13,14). The CDK3, CDK4 and CDK6 are involved regulation of G1-S phase transition, whereas CDK2 is associated with entrance into S phase and replication of DNA; while CDK1 is vital for mitosis (15-19). The CDK activity is regulated in cells via four basic mechanisms; which includes, binding of cyclin proteins to get activated, inhibition of CDK activity via cyclin-dependent kinase inhibitors, conserved residues phosphorylation at ATP-binding pocket of CDK (for inhibition of its activity) and phosphorylation at a conserved residue of CDKs T-loop (for its activation) (20). Loss of CAK activity usually lead towards cell cycle arrest and transcriptional defects (21-23). Phosphorylation at conserved threonine residue of T-loop do not play a direct role during catalysis, instead it tends to stabilize CDK-cyclin complex (24-25). Various model systems indicated that the phosphorylation may proceed independent to complex assembly, contrarily the assembly of complex may also occur before or after phosphorylation as shown in figure 1 (26).

In 1996, while working on S. *cerevisiae*, studies conducted by Espinoza et al, Kaldis et al and Thuret et al elaborated identification of novel CAK protein. The CAK (CAK1/Civ1) enzyme of yeast was isolated and purified via assistance of biochemical fractionation. There exists a strong correlation between Cak1 and Cdc28 (of budding yeast), as compared to rest of kinases. The Cdc28 usually lack consensus sequence of Gly–x–Gly–x–x–Gly in the ATP-binding loop (where X represents aminoacid). In CAK1 the aforementioned sequence is replaced by Asp–Ile–Thr–His–Cys–Gln. A 45 kDa purified bacterial CAK1 has tendency to phosphorylate both cyclin bound form of CDK2 and monomeric Cdc28 at invitro conditions. This ability indicates the ability of CAK1 to function in absence of regulatory subunit protein or post-translational modifications. The yeast cell extract may be used to purify both CAK1 along with Cdc28. Studies suggested that depletion of CAK1 after binding of specific

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antibodies, reduces CAK activity which clearly indicated vital role of CAK1. On the contrary, over expression of CAK1 in purified yeast extract yielded increased CAK activity (27,28,29).

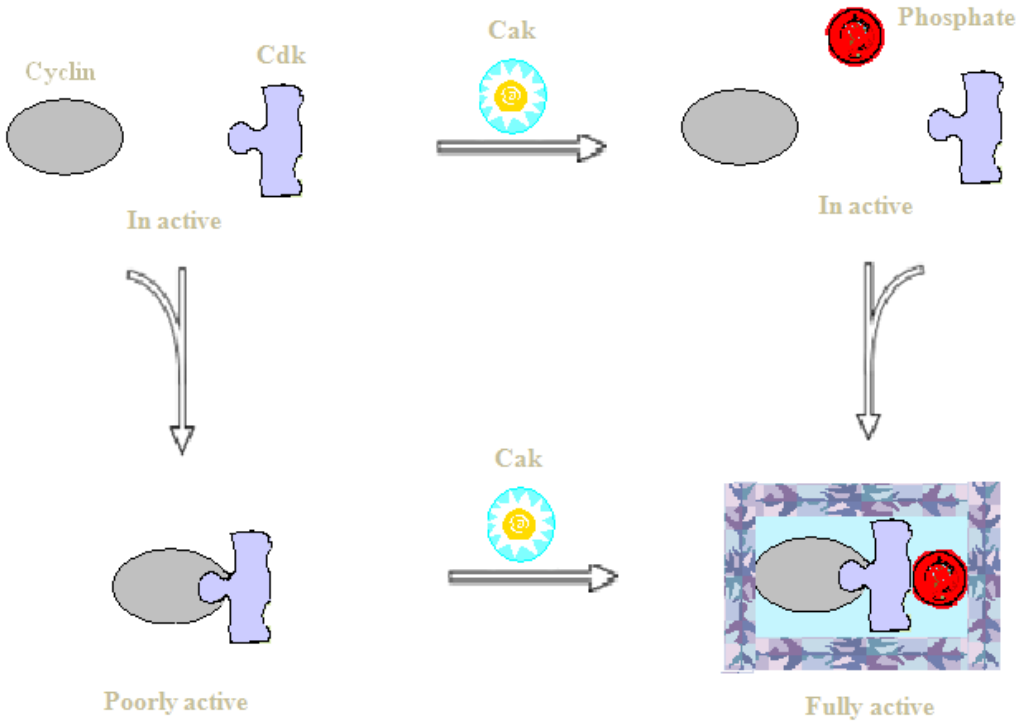

Figure 1 : The figure represents that CDK requires association with a cyclin subunit, phosphorylation via CAK. It suggests that complex assembly may occur either before of after phosphorylation.

Although CAK1 is structurally more related to CDKs, yet there exists some dissimilarity. The CAK is unique in the sense that it exists as monomer during its functionally active form, and it also lacks the glycine-rich loop in its structure. It can phosphorylate Cdc28 monomer. The phosphorylated Cdc28 could get activated via addition of cyclin molecule which supported the indication that cyclin binding prior to CDK phosphorylation is not a necessary step. It can be inferred from the literature that; for catalysis, the phosphorylation and cyclin binding only tends to provide structural stability, which further illustrates that aforementioned events are not necessary steps for catalysis (30,31). A true homologue of CAK1 (of S.cerevisiae) exists in higher eukaryotes which are regulates CAK activity (26).

b) Structural Characterization of CDK activating kinases (CAKs) activation

The binding of cyclin molecule and CDK activating kinase to CDK2 leads towards important conformational changes at active site. Insights into ATP binding at active site revealed orientation of phosphate outwards, while substrate binding at active site cleft. During inactive state, CDK2 is unable to bind substrate molecule and gets disoriented ATP positioning. Inactive conformation causes PSTAIRE helix to move outwards via a L12 helix push as shown in figure 2. The disoriented ATP positioning is due to PSTAIRE helix disposition which carries glutamate 51 residue (vital for positioning ATP phosphates) (6,9). During activation state, conformational changes appear after cyclin A binding to the molecule. At this state, the T-loop displace from entrance point of active site thereby reducing blockage of substrate binding site. Active conformation causes PSTAIRE helix to move inside along with L12 helix rearrangement as beta strand, which results into glutamate 51 interaction with lysine 33 residue. During this state, there occurs to be repositioning of Aspartate 145. Aforementioned structural modifications and rearrangements results into most appropriate binding of ATP phosphates. After phosphorylation of threonine 160 of CDK via CAK, the interactions between T-loop and cyclin A gets increased.

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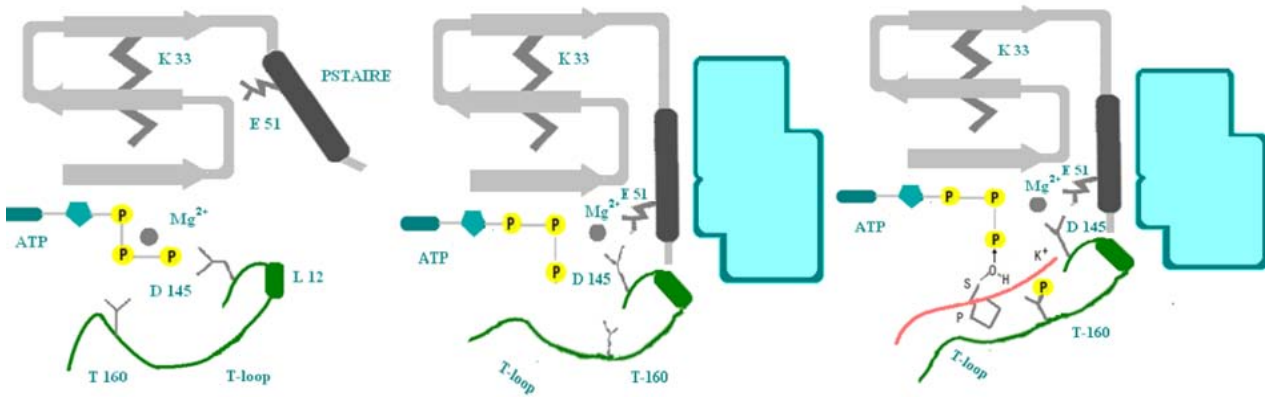
The event of phosphorylation increases stability and activity of cyclinA-CDK2 complex. It has been reported that different conformational changes appear in CDKs depending upon types of cyclin molecules.



(a) (b) (c)

Figure 2 : The figure shows activation of CDK2: part (a) describes CDK 2 monomer, part (b) shows CDK2+ Cyclin A, while part (c) shows CDK2+ Cyclin A+ Thr 160 Phosphorylation+ substrate peptide association.

c) Functional characterization of CDK activating kinases: interactions with other cellular proteins:

CAK exist as trimeric enzyme containing CDK7, Cyclin H and MAT1. CAK was unusually identified as 44 kDa CAK1 protein which resembled CDKs. The activity of CAK1 remained constant throughout cell cycle. The responsible gene (CAK1) was found essential for cell viability. The information revealed that there exist a difference among CAK of vertebrates and non-vertebrates which suggest distinct mechanisms of CDK activation among vertebrates and non-vertebrates (32). It has been reported that CDK7 is vital for mitosis and CDK activating kinase at invivo conditions. It was found that CDK7 is essential for Cdc2/cyclin A and Cdc2/Cyclin B complexes and cell division (33). Schindler et al, reported that CDK activating kinase, CAK1p is involved in activation of meiotic S phase via Ime2p. There are many Cdc28 independent functions of CAK1 which are unique with respect to meiosis. An example of such functions is to induce S phase, whose regulation is different in both mitosis and meiosis. During mitosis, Cdc28 protein usually controls its S-phase promoting ability via destroying its inhibitor through signaling event. During meiosis, the Ime2p protein kinase induces signaling which causes Sic1 destruction. It was found that it is CAK1 which is involved in Ime2p activation, which suggests Ime2p as potent target for CAK1p regulation (34).

It has been reported that CAK1p nucleotide binding pocket is significantly different from other protein kinase molecules which suggest importance of specific target molecule as inhibitory drug. The 5`-fluorosulfonylbenzoyladenosine (which as an ATP analog) usually inhibit protein kinases, but its activity has been found insensitive towards CAK1p (35). Yao et al reported CAK1 as physiological regulator of Bur1 kninase. This indicates that activation of Bur1-Bur2 cyclin dependent kinase complex is dependent upon CAK1 (36). CAK1 is involved in Ctk1 C-terminal domain phosphorylation at Thr-338. Invitro study revealed that CAK1 directly phosphorylates Ctk1 in S. *cerevisiae* (37). Espinoza et al reported that CAK1 is required for Kin28 phosphorlyation and invivo activation of Cdc28 (38). Immunofluorescence and biochemical subcellular fractionation techniques have confirmed that CAK1p is completely dispersed in cell. It has been reported that CAK1p level is usually stable during growth phase or stationary phase, while its level fluctuates during meiosis. This phenomenon depicts CAK1p regulation at both transcriptional and post transcriptional level (39).

The CAK usually exist as “free CAK” and “associated CAK”. Quantitatively, free CAK is predominant as compared to associated CAK. The “free CAKs” are involved in phosphorylating CDKs, which controls cell cycle regulation. The “associated CAKs” are associated with transcription factor TFIIH. These CAKs are involved in phosphorylating transcriptional proteins (such as RNA polymerase II). The CAK molecule is also involved in promoter clearance and transcription (from pre-initiation to the initiation stage). CAK are also involved in enhancing transcription rate by phosphorylating estrogen receptors and retinoic acid which leads towards increased expression of target genes. CAK plays a vital role in DNA damage response and CAK inhibition usually prevents cell cycle progression (9).

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III. Conclusion

Studies depicted that increased activation of certain cellular proteins may causes pathogenesis of tumor formation and cancer propagation while elevated activation of such proteins can be inhibited via ATP and other potential inhibitors to cure associated cancers (40, 41). The CDK activating kinase is an important cell cycle regulating molecule. Cancer associated cell cycle defects are frequently mediated through alterations in CDK activity. Research suggests that the tumor cells require specific interphase CDKs for abnormal proliferation, therefore inhibition of CDK and CDK activating kinases could provide potential therapeutic target against human neoplasias.

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To Evaluate the Clinical Efficacy of Tila E Hadaf in the Management of Erectile Dysfunction

By Izharul Hasan, Danish Kamal Chishti, Fakhra Talat & Yusuf Jamal
AU Tibbia College and Hospital

Abstract - The effect of erectile dysfunction potentially interferes with men's self esteem, confidence, relationship, and overall sense of well being. The problem is increasing in all segments of the sexually active male population and affects both men and his partner. In younger man increase is attributed to substance abuse, such as recreation drugs and alcohol. Middle aged men are affected by medical conditions such as diabetes, hypertension, sexual diseases, organ transplant, coronary artery bypass surgeries and cancer, or the therapy of these problems. The older population is living longer, fuller lives and expects to remain sexually active, regardless of any existing medical conditions. Stress factors associated with modern life styles are affecting men of all ages and contribute greatly to the overall causes of erectile failure. Early identification, behavior modification and increased therapeutic options may improve patient's outcome. By improving the knowledge and therapeutic options, it may be possible to identify patients at risk of erectile dysfunction and thus to lead a normal healthy life.

Keywords : *erectile dysfunction, Tila e Hadaf.*

GJMR-L Classification : *NLMC Code : WM 611*

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To Evaluate the Clinical Efficacy of Tila E Hadaf in the Management of Erectile Dysfunction

Izharul Hasan [α], Danish Kamal Chishti [σ], Fakhra Talat [ρ] & Yusuf Jamal [ω]

Abstract - The effect of erectile dysfunction potentially interferes with men's self esteem, confidence, relationship, and overall sense of well being. The problem is increasing in all segments of the sexually active male population and affects both men and his partner. In younger man increase is attributed to substance abuse, such as recreation drugs and alcohol. Middle aged men are affected by medical conditions such as diabetes, hypertension, sexual diseases, organ transplant, coronary artery bypass surgeries and cancer, or the therapy of these problems. The older population is living longer, fuller lives and expects to remain sexually active, regardless of any existing medical conditions. Stress factors associated with modern life styles are affecting men of all ages and contribute greatly to the overall causes of erectile failure. Early identification, behavior modification and increased therapeutic options may improve patient's outcome. By improving the knowledge and therapeutic options, it may be possible to identify patients at risk of erectile dysfunction and thus to lead a normal healthy life. The present study reveals that overall effect of Tila e Hadaf was found quite encouraging in the treatment of erectile dysfunction and significant improvement was observed in subjective parameters.

Keywords : *erectile dysfunction, tila e hadaf.*

I. Introduction

Erectile dysfunction (ED) is defined as the inability to achieve or maintain an erection sufficient for satisfactory sexual performance (Impotence NIH Consensus Statement 1992). The researchers assessed the sexual function of 31,742 men between the ages of 53 and 90, who were enrolled in the Health Professionals Follow-up Study and had responded to a questionnaire mailed in 2000 that, among other questions related to health, asked about sexual function, physical activity, body weight, smoking and marital status. Men who had been diagnosed with prostate cancer were excluded from the findings. Thirty-three percent of the participants reported experiencing erectile dysfunction in the previous three months. ED was defined as the inability, without treatment, to have and maintain an erection adequate for intercourse (Feldman HA, Goldstein I, Hatzichristou DG).

Fewer than two percent of the men in the study who reported that they had erection problems experienced them before age 40, and four percent had experienced problems between age 40 and 49. From age 50 upwards, the percentage of men reporting ED increased dramatically with 26 percent between the ages of 50 to 59, 40 percent aged 60 to 69 years and 61 percent for men older than 70 having experienced ED. The study demonstrated that erectile dysfunction is increasingly prevalent with age. At age 40, there is an approximately 40% prevalence rate, increasing to almost 70% in men at age 70. The prevalence of moderate erectile dysfunction increases from 17% to approximately 34%; the prevalence of complete erectile dysfunction increases from 5% to 15% as age increases from 40 to 70 years (Feldman HA, Goldstein I, Hatzichristou DG 1994).

The Massachusetts Male Aging Study reported a prevalence of 52% in men aged 40 to 70 (Feldman HA et al 1994). It is estimated that in 1995 there were over 152 million men worldwide who experienced erectile dysfunction. With the ageing worldwide population, it has been projected that by the year 2025, 322 million men will have some degree of erectile dysfunction (Ayta IA, McKinlay JB, Krane RJ 1999). Data on the prevalence of ED in Asia are limited. A recent study conducted in Thailand reported an overall prevalence rate of 37.5% amongst men 40 to 70 years of age (Kongkanand A 2000). No observational studies on erectile dysfunction have been done in Delhi previously. With an ageing population, erectile dysfunction may become a significant health problem.

Keeping this fact in mind and a high prevalence of erectile dysfunction, this Pilot study was conducted in Ayurvedic and Unani Tibbia College Hospital by Department of Physiology to find out the clinical efficacy of Tila e Hadaf in the management of erectile dysfunction. The objective of this study was to test the clinical efficacy of Tila e Hadaf a branded Unani medicine manufactured by Sangam Pharmacy, 2922, Kithore, Didtrict Meerut, (UP)-250104, India on erectile dysfunction. This was an 8 week, randomized, single blind, observational study.. A total of 100 subjects were randomly selected. Medicine was supplied by the hospital store.

II. Methodology

The "Clinical efficacy of Tila e Hadaf in the management of erectile dysfunction" was conducted at

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the hospital of Ayurvedic and Unani Tibbia College Delhi. This study was conducted from June 2012 to December 2012.

a) Criteria for Selection of patients

i. *Inclusion Criteria*

Patients of

- Male between the ages of 20 to 50 years.
- At least a 3-month history of erectile dysfunction (ED).
- Are able to read, understand and provide signed informed consent.
- Agree not to use any other erectile dysfunction (ED) treatment, including herbal therapy during the 8-week non-drug, run-in.

ii. *Exclusion Criteria*

- Patients below the age of 20 and above 50 years.
- Patients having anxiety associated with organic illnesses like hypertension, ischemic heart disease, diabetes mellitus, renal diseases, Tuberculosis etc.

b) Informed consent

Patients enrolled into study were given the information sheet having details about the nature of the study, the drug to be used, method of treatment and they were allowed to go through the contents of informed consent form accordingly to ask any question related to study, they were asked to sign the informed consent form.

c) Investigations

Investigations were carried out aiming:

- To exclude the patients with pathological conditions mentioned under exclusion criteria.
- To establish safety and validation of test drugs.

d) Study design

This study was designed as a Randomized single blind observational study.

e) Sample size

The sample size was fixed as 100 patients who fulfill the inclusive criteria for the study.

f) Study and data collection

The study is the test run study, conducted in different population with similar characteristic. The data for the study was collected from 100 married males based on objectives of the study; a structured questionnaire has been developed to assess the knowledge of regarding erectile dysfunction at A & U Tibbia college Hospital, Karol Bagh, New Delhi, from June 2012 to Dec 2012. A formal written permission was taken from Head of the Dept of physiology, A & U Tibbia College and Hospital to conduct the study.

i. *Family type*

The families were classified as follows:

Nuclear family : Married couple and their children, where the children are still regarded as dependant.

Joint family : Consists of number of married couples and their children who live in the same household.

ii. *Occupation of subjects*

The occupation of the parents was recorded as the determinant of the SES.

iii. *Literacy of the subjects*

The data related to the literacy of the subjects was assessed to know the educational status and the socioeconomic status.

iv. *Socioeconomic status (SES)*

The SES was assesses by using the Kuppuswami's SES Scale for Urban population, 1976. Due to changes in the economy to year, the classification or scale was modified accordingly. The latest SES scale is of 2012.

g) Duration of protocol

The treatment period of test formulation was determined as 60 days.

h) Trial formulation

Tila e Hadaf as local application on penis; composition of as follows.

Kharateen (Earth worm)200 mg (Guerrero RD III. 2009).

Beerbahuti(Coccus cacti) 200 mg (Nobel, Park S. 2002).

Jund Bedustar (Castoreum)100 mg (Müller-Schwarze, D and Houlihan 1991).

Laung (Cloves)100 mg (Tajuddin , Ahmad S. 2004).

Aqarqarha (Anacyclus pyrethrum)100 mg.

Roghan Zaitun (Olive oil) qs.

i) Method of preparation and mode of administration of test drug

The Tila e Hadaf for trial formulation was provided by Sangam Pharmacy Meerut. Tila Hadaf was dispensed to the patients allocating in test group in the transparent glass bottle in sufficient quantity to last for 7 days.

i. *Dosage*

To be rubbed on the penis. Just 4-5 drops are to be massaged on the male organ 1- 2 times a day.

j) Follow up during treatment

Two month study was divided into eight visits of follow up, which were made at an interval of 7 days. At every visit, patients were asked about the progression or regression in their symptoms, and subjected to assess the clinical findings. Concomitant treatment was not allowed during study.

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k) Efficacy assessment

The efficacy of the treatment in test group was assessed on the basis of subjective and objective parameters.

l) Withdrawal criteria

a. Failure to follow the protocol
b. Any adverse event
c. Drug defaulters

m) Adverse drug reaction documentation

Any adverse event or reaction that appears during the study.

n) Data analysis

Data were tabulated in a systematic way for presentation and analysis on the basis of recorded parameters.

o) Documentation

The case record form and consent forms properly documented throughout the study.

In view of the nature of the problem selected for the study and the objectives to be accomplished, this study designed as a Randomized single blind observational study was found appropriate.

i. *Mechanism of action of Tila e Hadaf in Erectile dysfunction*

Tila e Hadaf manufactured by Sangam Pharmacy, is special oil with all unique natural ingradients known for ages as per unani ssystem of medicines to treat the erectile dysfunction. Tila e Hadaf helps supply the penis with all the essential nutrients it needs. Besides offering natural gains in the length and girth of the penis they improve many aspects of penis health. It is natural penis enhancement oil that increases blood flow to the penile erectile chambers which leads to a harder and long-lasting erection when aroused. Gives enhanced sexual pleasure and more intense orgasm. It has been proven to be safe and without any known side-effects. It is an effective remedy to normalize the hypersensitization of male organ due to spermatorrhea, premature ejaculation or excess of coitus.

The sensitivity of the sexual organs increases abnormally as a result of masturbation, hyper sensitiveness, premature ejaculation, nocturnal emission (wet dream), spermatorrhoea and excessive sexual intercourse. It becomes essential to bring the sensitivity of the sexual organs to normal level in order to cure the said diseases. Excessive nocturnal emission (wet dream) and spermatorrhoea cause pricking on the gland which produces Mazi (a fine liquid that flows before the discharge of semen), such a pricking causes frequent erection and pain in testicles. Such conditions adversely affect the growth of the penis.

Tila Hadaf is the compound of such components which give potency to the male sexual organs and bring their sensitiveness to a normal level. They also regulate the circulation of blood and give energy to the nerves and muscles of penis. Its use makes away with the harmful effects of masturbation. Tila Hadaf also potentiates nerves and muscles of the male organ.

III. Observation and Result

The sample of 100 married male from A & U Tibbia College Hospital, Karol Bagh Delhi was taken from the population, by using convenient sampling. The personal data obtained include age, educational status, occupation, family income, religion, type of family, duration of married life, and place of residence.

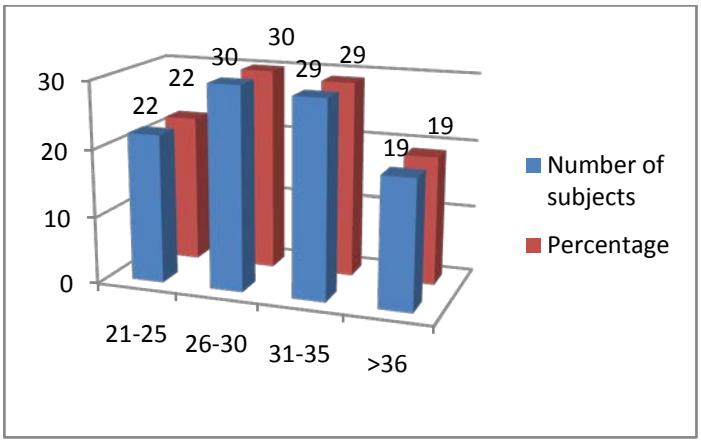

Figure 1 : Distribution of patients according to age.

Figure 1 reveals the percentage distribution of the married males according to the age group. Majority of the married males (30.00%) were between the age group of 26-30 years, 23.00% were in age group between 21-25 years, 29.00% were between the age group of 31-35 years and only 19.00% were in the age group of more than 36 year. Thus it is seen most of the married males participated in the study were below 50 years of age. Most of the males were between 25-35 years of age which could be attributed to performance anxiety and other psychogenic factors.

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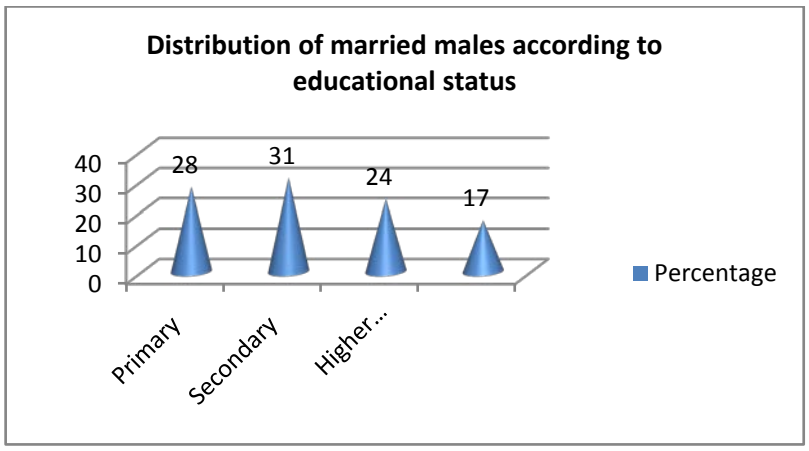

Figure 2 : Distribution of the married males according to educational status.

Figure 2 shows that in educational status majority of married males (31.00%) had studied upto secondary, 28% had primary education, 24% had higher secondary education and 17% had graduate and above educational qualification. This shows that majority of participants were having secondary education.

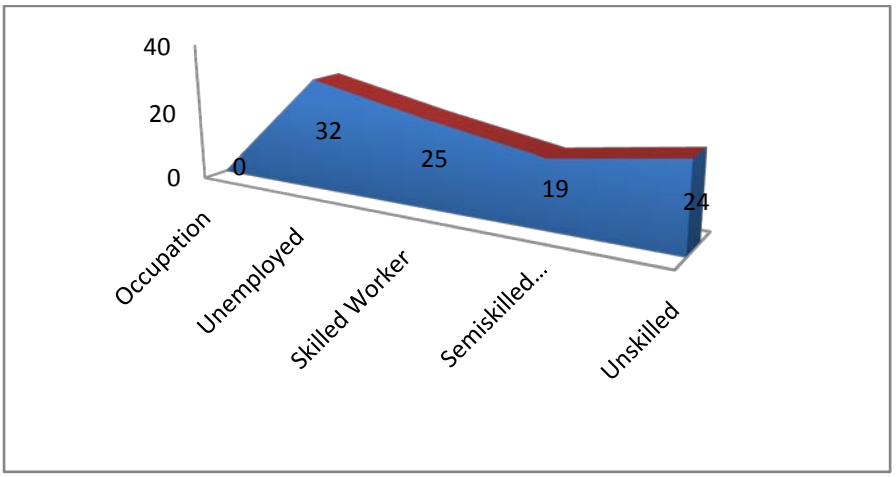

Figure 3 : Distribution of married males according to occupation.

Figure 3 shows that majority of married males 32% were unemployed, 25% were skilled, 19% were semiskilled, and 24% were unskilled worker respectively. Thus it is seen that most of the married males had an occupation.

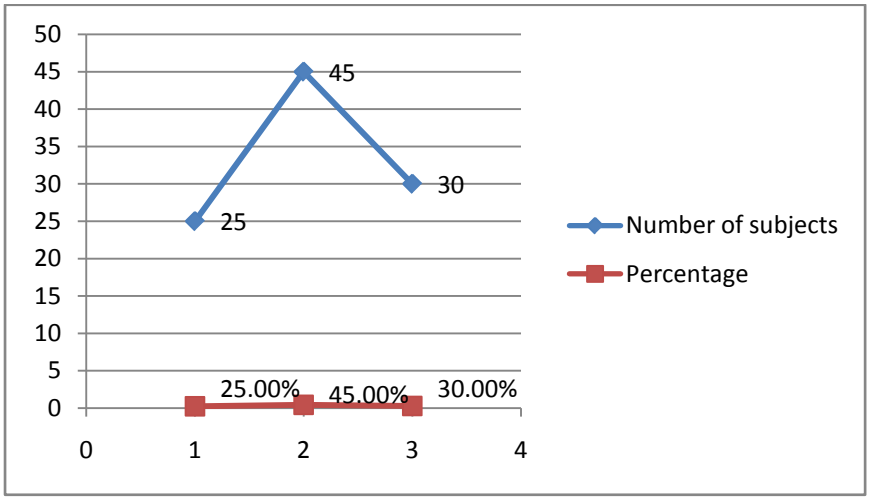

Figure 4 : Distribution of married males according to socio-economic status.

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Figure 4 shows that out of total 100 married males in the study 25% were from upper middle (2) SES, 45% were from lower middle (3) SES, and 30% were from upper lower (4) SES. Maximum numbers, 45 (45.00%) were from lower middle (3) SES. This can be attributed to lower educational and occupational status of subjects (Figure 4).

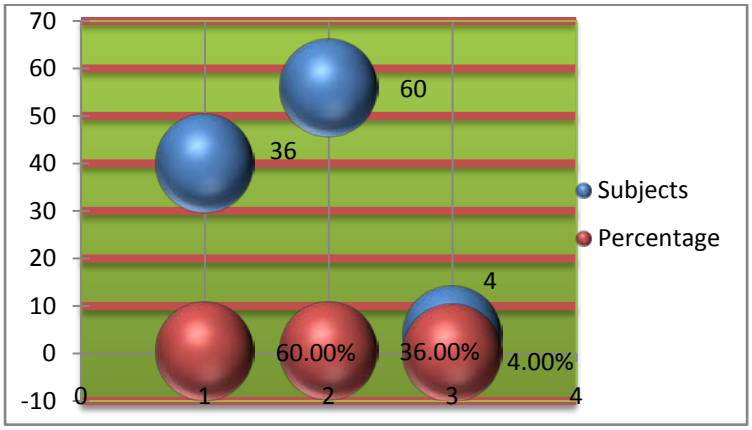

Figure 5 : Distribution of subjects according to religion.

Figure 5 shows that 60% of the subjects were from Hindu community, 36% subjects were from Muslim community and 4% from other communities. This shows that majority of the subjects were from Hindu community.

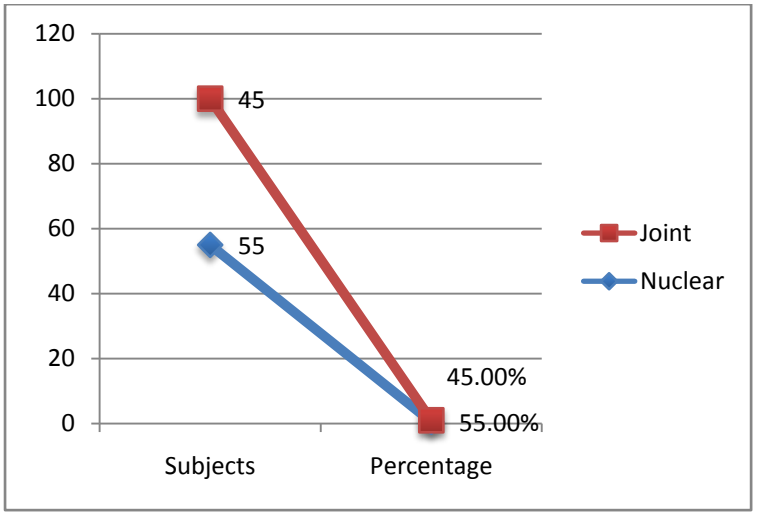

Figure 6 : Distribution of subjects according to family type.

Figure 6 depicts that 55% of the subjects were from nuclear family and 45% were from joint family. Hence it is clear majority of the subjects who participated in the study were from nuclear family.

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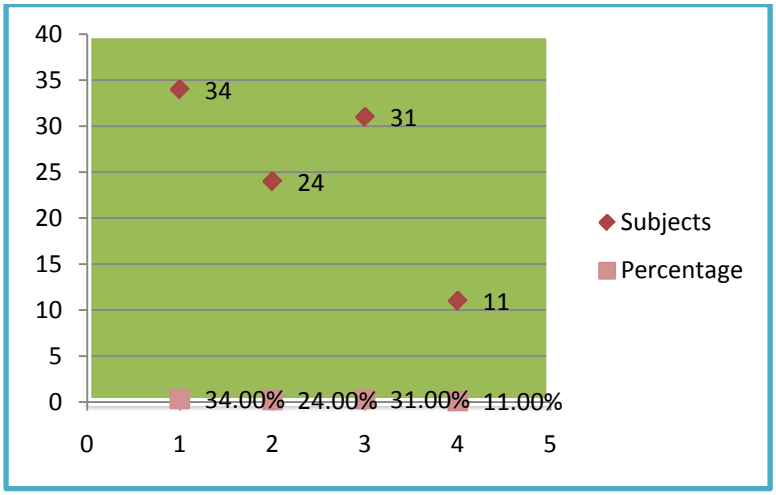

Figure 7 : Distribution of subjects according to duration of married life.

Figure 7 shows that 34% of the subjects had 0-5 years duration of married life, 24% subjects had 6-10 years duration of married life, 31% subjects had 11-15 years duration of married life, and 11% subjects had more than 15 years duration of married life.

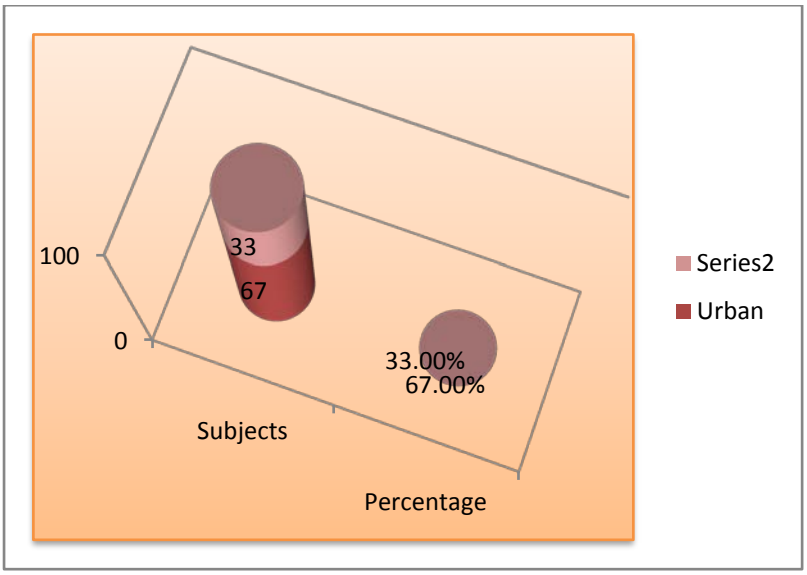

Figure 8 : Distribution of subjects according to area of residence.

Figure 8 depicts that 67% of the subjects were from urban area whereas 33% of the subjects were from rural area. This shows that majority of the cases were from urban area.

Table 1 : Distribution of subjects according to situation in terms of a full erection.

Situation in terms of full erection	Number of subjects	Percentage
Never get a full rigid erection	51	51.00%
Full rigid erection in some situations	49	49.00%
Total	100	100.00%

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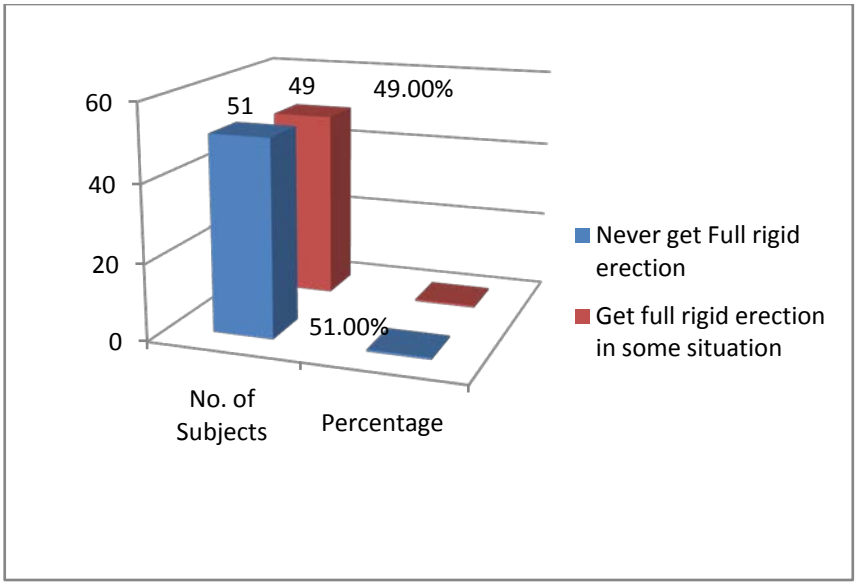

Figure : 9

Table 1 and figure 9 depicts that 51% of the subjects had never got a full rigid erection and 49% had got full rigid erection in some situations. Hence it is clear majority of the subjects 51.00% participate in the study never got a full rigid erection.

Table 2 : Effect of Tila e Hadaf on Erectile dysfunctions at end of study.

Grade	Number of subjects	Percentage
Grade 0	8	8.00%
Grade I	25	25.00%
Grade II	50	50.00%
Grade III	17	17.00%

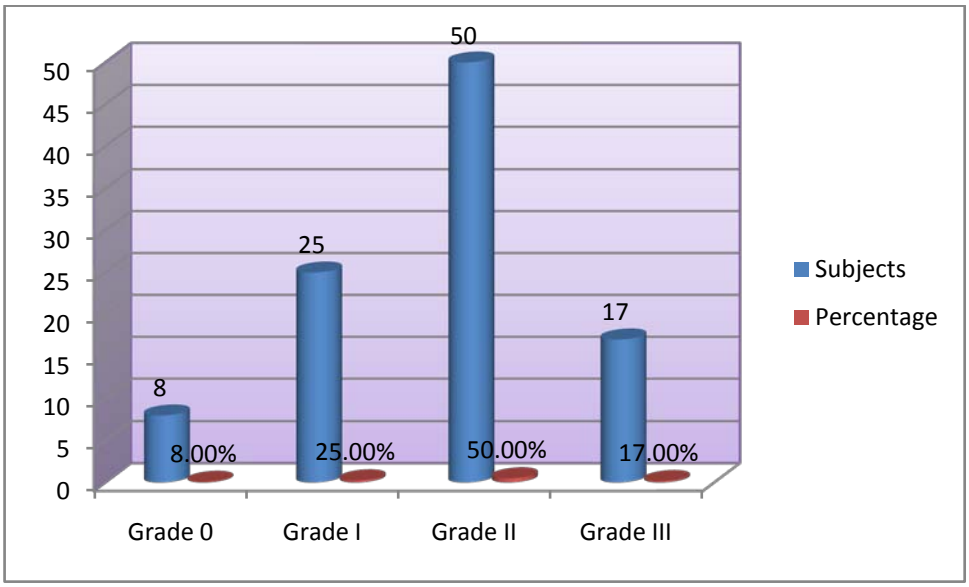

Figure : 10

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Table 2 and figure 10 depicts that at the end of treatment, 8% of the subjects had got grade 0 meanwhile no improvement, whereas 25% of the subjects had got grade I, 50% of the subjects had got grade II, and 17% of the subjects got grade III improvement respectively. This shows majority of the cases had got grade II and were having satisfactory improvement.

IV. Conclusion

Satisfactory sex life is an important influencing factor for a harmonious marriage and relationship. Sexual problems like erectile dysfunction deeply effects personal life causing isolation, frustration and decreased self-esteem, which may extend into their job performance and interaction with others.

Keeping this fact of high prevalence of ED in mind, A clinical study was carried out under Prof. Dr Yusuf Jamal, Department of Physiology in Ayurvedic and Unani Tibbia College & Hospital to evaluate the efficacy of a Unani formulation named Tila e Hadaf in the management of erectile dysfunction. The results of the study revealed that:

Majority of the married males (30.00%) were between the age group of 26-30 years, 23.00% were in age group between 21-25 years, 29.00% were between the age group of 31-35 years and only 19.00% were in the age group of more than 36 year. Thus it is seen most of the married males participated in the study were below 50 years of age.

The present study shows that in educational status majority of married males (31.00%) had studied up to secondary, 28% had primary education, 24% had higher secondary education and 17% had graduate and above educational qualification. This shows that majority of participants were having secondary education.

The present study reveals that majority of married males 32% were unemployed, 25% were skilled, 19% were semiskilled, and 24% were unskilled worker respectively. Thus it is seen that most of the married males had an occupation.

The present study shows that out of total 100 married males in the study 25% were from upper middle (2) SES, 45% were from lower middle (3) SES, and 30% were from upper lower (4) SES. Maximum numbers, 45 (45.00%) were from lower middle (3) SES. This can be attributed to lower educational and occupational status of subjects.

The present study reveals that 60% of the subjects were from Hindu community, 36% subjects were from Muslim community and 4% from other communities.. This shows that majority of the subjects were from Hindu community.

The present study depicts that 55% of the subjects were fromo nuclear family and 45% were from joint family. Hence it is clear majority of the subjects participating in the study were from nuclear family.

The present study shows that 34% of the subjects had 0-5 years duration of married life, 24% subjects had 6-10 years duration of married life, 31% subjects had 11-15 years duration of married life, and 11% subjects had more than 15 years duration of married life.

The present study depicts that 67% of the subjects were from urban area whereas 33% of the subjects were from rural area. This shows that majority of the cases were from urban area.

The present study depicts that 51% of the subjects had never got a full rigid erection and 49% had got full rigid erection in some situations. Hence it is clear majority of the subjects 51.00% participating in the study never got a full rigid erection.

The present study depicts that at the end of study, 8% of the subjects had got grade 0 meaning no improvement, whereas 25% of the subjects had got grade I, 50% of the subjects had got grade II, and 17% of the subjects got grade III improvement respectively. This shows majority of the cases had got grade II and were having satisfactory improvement.

The present study reveals that overall effect of Tila e Hadaf was found quite encouraging in the treatment of erectile dysfunction and significant improvement was observed in subjective parameters. No clinically significant side effects were observed in test group and overall compliance to the treatment was found excellent. These results conclude that the test drug Tila e Hadaf is a cheap, safe, and effective treatment for erectile dysfunctions.

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9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

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15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

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17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

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21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. **Think technically:** Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be

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33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

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To make a paper clear

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- Insertion a title at the foot of a page with the subsequent text on the next page

- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

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· Use past tense to describe specific results

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· Shun use of extra pictures - include only those figures essential to presenting results

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An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

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- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

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- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
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- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

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- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

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The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
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What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.

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- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

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- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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