Online ISSN : 2249 - 4618
Print ISSN : 0975 - 5888

Global Journal of Medical Research

DISCOVERING THOUGHTS AND INVENTING FUTURE

Highlights

- Study of Cytological Pattern
- Development of Animal Models
- Polymorphism with breast cancer
- Histopathological and Toxicological effects

The Blood Plasma

Volume 12 | Issue 1 | Version 1.0

ENG

Global Journal of Medical Research

Global Journal of Medical Research
Volume 12 Issue 1 (Ver. 1.0)

Open Association of Research Society

Incorporation No.: 0423089
License No.: 42125/022010/1186
Registration No.: 430374
Import-Export Code: 1109007027
Employer Identification Number (EIN):
USA Tax ID: 98-0673427

Global Journals Inc.

*(A Delaware USA Incorporation with "Good Standing"; **Reg. Number: 0423089**)*

Sponsors: Open Association of Research Society
Open Scientific Standards

Publisher's Headquarters office

Global Journals Inc., Headquarters Corporate Office, Cambridge Office Center, II Canal Park, Floor No. 5th, ***Cambridge (Massachusetts)***, Pin: MA 02141 United States
USA Toll Free: +001-888-839-7392
USA Toll Free Fax: +001-888-839-7392

Offset Typesetting

Open Association of Research Society, Marsh Road, Rainham, Essex, London RM13 8EU
United Kingdom.

Packaging & Continental Dispatching

Global Journals, India

Find a correspondence nodal officer near you

To find nodal officer of your country, please email us at *local@globaljournals.org*

eContacts

Press Inquiries: *press@globaljournals.org*
Investor Inquiries: *investers@globaljournals.org*
Technical Support: *technology@globaljournals.org*
Media & Releases: *media@globaljournals.org*

Pricing (Including by Air Parcel Charges):

For Authors:
22 USD (B/W) & 50 USD (Color)
Yearly Subscription (Personal & Institutional):
200 USD (B/W) & 250 USD (Color)

Editorial Board Members (HON.)

Dr. Bart Lambrecht
Director of Research in Accounting and FinanceProfessor of Finance
Lancaster University Management School
BA (Antwerp); MPhil, MA, PhD (Cambridge)

Dr. Carlos García Pont
Associate Professor of Marketing
IESE Business School, University of Navarra
Doctor of Philosophy (Management), Massachusetts Institute of Technology (MIT)
Master in Business Administration, IESE, University of Navarra
Degree in Industrial Engineering, Universitat Politècnica de Catalunya

Dr. Fotini Labropulu
Mathematics - Luther College
University of ReginaPh.D., M.Sc. in Mathematics
B.A. (Honors) in Mathematics
University of Windso

Dr. Lynn Lim
Reader in Business and Marketing
Roehampton University, London
BCom, PGDip, MBA (Distinction), PhD, FHEA

Dr. Mihaly Mezei
ASSOCIATE PROFESSOR
Department of Structural and Chemical Biology, Mount Sinai School of Medical Center
Ph.D., Etvs Lornd University
Postdoctoral Training,
New York University

Dr. Söhnke M. Bartram
Department of Accounting and FinanceLancaster University Management SchoolPh.D. (WHU Koblenz)
MBA/BBA (University of Saarbrücken)

Dr. Miguel Angel Ariño
Professor of Decision Sciences
IESE Business School
Barcelona, Spain (Universidad de Navarra)
CEIBS (China Europe International Business School).
Beijing, Shanghai and Shenzhen
Ph.D. in Mathematics
University of Barcelona
BA in Mathematics (Licenciatura)
University of Barcelona

Philip G. Moscoso
Technology and Operations Management
IESE Business School, University of Navarra
Ph.D in Industrial Engineering and Management, ETH Zurich
M.Sc. in Chemical Engineering, ETH Zurich

Dr. Sanjay Dixit, M.D.
Director, EP Laboratories, Philadelphia VA Medical Center
Cardiovascular Medicine - Cardiac Arrhythmia
Univ of Penn School of Medicine

Dr. Han-Xiang Deng
MD., Ph.D
Associate Professor and Research Department Division of Neuromuscular Medicine
Davee Department of Neurology and Clinical NeuroscienceNorthwestern University Feinberg School of Medicine

Dr. Pina C. Sanelli
Associate Professor of Public Health
Weill Cornell Medical College
Associate Attending Radiologist
NewYork-Presbyterian Hospital
MRI, MRA, CT, and CTA
Neuroradiology and Diagnostic Radiology
M.D., State University of New York at Buffalo,School of Medicine and Biomedical Sciences

Dr. Roberto Sanchez
Associate Professor
Department of Structural and Chemical Biology
Mount Sinai School of Medicine
Ph.D., The Rockefeller University

Dr. Wen-Yih Sun
Professor of Earth and Atmospheric SciencesPurdue University Director
National Center for Typhoon and Flooding Research, Taiwan
University Chair Professor
Department of Atmospheric Sciences, National Central University, Chung-Li, TaiwanUniversity Chair Professor
Institute of Environmental Engineering, National Chiao Tung University, Hsin-chu, Taiwan.Ph.D., MS The University of Chicago, Geophysical Sciences
BS National Taiwan University, Atmospheric Sciences
Associate Professor of Radiology

Dr. Michael R. Rudnick
M.D., FACP
Associate Professor of Medicine
Chief, Renal Electrolyte and Hypertension Division (PMC)
Penn Medicine, University of Pennsylvania
Presbyterian Medical Center, Philadelphia
Nephrology and Internal Medicine
Certified by the American Board of Internal Medicine

Dr. Bassey Benjamin Esu
B.Sc. Marketing; MBA Marketing; Ph.D Marketing
Lecturer, Department of Marketing, University of Calabar
Tourism Consultant, Cross River State Tourism Development Department
Co-ordinator , Sustainable Tourism Initiative, Calabar, Nigeria

Dr. Aziz M. Barbar, Ph.D.
IEEE Senior Member
Chairperson, Department of Computer Science
AUST - American University of Science & Technology
Alfred Naccash Avenue – Ashrafieh

Contents of the Volume

i. Copyright Notice
ii. Editorial Board Members
iii. Chief Author and Dean
iv. Table of Contents
v. From the Chief Editor's Desk
vi. Research and Review Papers

1. Study of Cytological Pattern of Tubercular Lymphadenitis. *1-3*
2. Evaluation of Immunosuppressive Regimens in Kidney Transplanted Patients in Iraq. *5-20*
3. Association of Leptin Receptor (LEPR) Q223R Polymorphism with Breast Cancer. *21-29*
4. Histopathological and Toxicological effects of crude saponin extract from Phyllanthus niruri, L (Syn. P. franternus. Webster) on Organs in animal studies. *31-37*
5. A Comprehensive Insight into the Development of Animal Models for Obesity Research. *39-44*

vii. Auxiliary Memberships
viii. Process of Submission of Research Paper
ix. Preferred Author Guidelines
x. Index

Global Journal of Medical research
Volume 12 Issue 1 Version 1.0 February 2012
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Study of Cytological Pattern of Tubercular Lymphadenitis

By Dr. Narayanamurthy C, D r. Kodanda Swamy C R
Hawler Medical University

Abstract – Background : Fine needle aspiration cytology is a diagnostic tool in which cells are extracted from a palpable swelling using FNAC gun, syringe and fine needle. It is a simple, speedy, safe, cost effective and accurate technique being used worldwide. [1, 2] Lymphadenopathy is one of the common conditions encountered in clinical practice with varied etiological predispositions.[3]

Keywords : *Cytological pattern, Lymphadenopathy, Tuberculosis and biopsy.*

GJMR-B Classification : *NLMC Code: WF 290, WH 700*

Strictly as per the compliance and regulations of:

Study of Cytological Pattern of Tubercular Lymphadenitis

Dr. Narayanamurthy C [α], Dr. Kodanda Swamy C R [σ]

Abstract – Background : Fine needle aspiration cytology is a diagnostic tool in which cells are extracted from a palpable swelling using FNAC gun, syringe and fine needle. It is a simple, speedy, safe, cost effective and accurate technique being used worldwide. [1, 2] Lymphadenopathy is one of the common conditions encountered in clinical practice with varied etiological predispositions.[3]

Aim : To know the cytological pattern of tubercular lymphadenitis of this region and correlation with biopsy whenever possible. To evaluate the utility of fine needle aspiration cytology of lymph nodes.

Study Setting & Design : This proospective study was conducted at the department of Pathology, of a tertiary healthcare teaching center for a period of three years from October 2000 to January 2003.

Materials And Methods : 22 to 23 gauze needle with 10ml disposable syringes were used. Minimum of three stains Hematoxylin & Eosin (H & E) stain, MGG S stain and AFB stain were done. Wherever possible Gram stain was also done. The cytological diagnoses were correlated whenever possible with histopathological examination.

Statistical Analysis : The data entry was carried out using Microsoft Office Excel worksheet and was analyzed.

Results : Cytological diagnosis of tubercular lymphadenitis was made for 109 cases. The smears were divided into four groups.

I. Epithelioid cell clusters with or without Langhan's giant cells with necrotic material.
II. Epithelioid cell clusters with or without Langhan's giant cell, without necrosis.
III. Occasional epithelioid cell collection without typical necrosis giant cells.
IV. Only necrotic material, without epithelioid cell clusters or giant cells.

The positivity was 16.67%, 7.3%, 11.12% and 50 % for group I, II, III and IV respectively.

Conclusion : Fine needle aspiration cytology is an useful technique for evaluation of patients with lympahdneopathies, because of lack of complication and excellent results. The present study is relative sensitive and specific. Diagnostic accuracy can be further improved by studying the cytological pattern of the tuberculosis, immunocytochemical stains and analysis male number of cases along with histopathological correlation.

Keywords : Cytological pattern, Lymphadenopathy, Tuberculosis and biopsy.

Author α: Associate professor, Department of Pathology, Basaveshwara Medical College and Hospital, S.J.M.I.T Campus, Chitradurga-577502 Karnataka, India Mobile: +91 9845316090
E-mail : murthypath@gmail.com.
Author σ : Professor and Head, Department of Pathology, Sree Siddhartha Medical College and Hospital, Tumkur, Karnataka, India

I. Introduction

Lymphadenopathy is one of the common conditions encountered in clinical practice with varied etiological predispositions.[1] it always poses dilemma for the clinician. Lymphadenopathy is one of the major causes of morbidity particularly in pediatric age group. Hence it is essential that a correct diagnosis is made as early as possible.

Fine needle aspiration cytology (FNAC) is a diagnostic tool in which cells are extracted from a palpable swelling using FNAC gun, syringe and fine needle. It is a simple, speedy, safe, cost effective and accurate technique being used worldwide.[2,3] Fine needle aspiration studies is being increasingly used for evaluation of lymphadenopathy.[4] lymph node FNAC can be performed routinely as a first approach to lymohadenopathies. The clinical value of FNAC is not limited to neoplastic conditions, but also in diagnosis of inflammatory, infectious and degenerative conditions. In most cases architectural distortion is minimal or absent, if biopsy becomes necessary. With adequate cytological examination and fallow-up, a large number of biopsies can be avoided.

II. Material and Methods

Study Setting & Design : The present prospective study was carried out in the department of pathology, Sree Siddhartha Medical College, Tumkur from Oct 2000 to Jan 2003.

Sample Collection and Laboratory Testing : 22 to 23 gauze needle with 10ml disposable syringes were used. Minimum of three stains H&E stain, MGG S stain and AFB stain were done. Wherever possible Gram stain was also done. The cytological diagnoses were correlated whenever possible with histopathological examination.

Data Management and Statistical Analysis : The data entry was carried out using Microsoft Office Excel worksheet and analyzed.

Ethical considerations: The protocol for this study was approved from the Chairman, and the secretary, institutional ethical committee (IEC). The approval was on the agreement that patient anonymity must be maintained, good laboratory practice/ quality control ensured, and that every finding would be treated

with utmost confidentiality and for the purpose of this research only. All work was performed according to the international guidelines for human experimentation in biomedical research.

III. Results

Cytological diagnosis of tubercular lymphadenitis was made 109 cases. The smears were divided into four groups.

I. Epithelioid cell clusters with or without Langhan's giant cells with necrotic material.
II. Epithelioid cell clusters with or without Langhan's giant cell, without necrosis.
III. Occasional epithelioid cell collection without typical necrosis giant cells.
IV. Only necrotic material, without epithelioid cell clusters or giant cells.

Groups I and II together amounting 65.13% of cases. The group-I consisted of 30 patients, group-II consisted of 41 patients. Group-III consisted of 18 patients while the remaining 20 patients belonged to group-IV.

AFB staining was done in all the cases. Group-I revealed AFB positively in 5 cases, group-II releaved AFB positively I 3 cases. Group-III releaved AFB positively in 2 cases, while the group-IV releaved AFB positively in 10 cases.

Biopsy correlation was possible in only 6 of group-1, 10 of the group-II, 4 of the group-III and 5 0f the group-1V.Amoung group-I, II, and IV all biopsies diagnosed as tubercular lymphadenitis on histopathological examination. In group-III, 2 cases were diagnosed as reactive lymphadenopathy and the remaining 2 cases as tubercular lymphadenitis.

IV. Discussion

Tubercular lymphadenitis constituted commonest group of lymphadenipathies diagnosed by fine needle aspiration, numbering 109 patients (36.33%) out of 300 patients of lymphadenopathy.

The results of our study were in accordance with many studies (Table-XIV0i.e.,Raghuveer et al 39(35.26%),Bharadwaj K.et al17 (40.95%).Prasad 16(45.76%).But studied by Khan et al 48 and Das Gupta et al 17 conducted by Aligarh and Calcutta respectively showed high incidence of tuberculosis. This must be in accordance with incidence of tuberculosis in that area 17.

Cytological features of tubercular lymph node were divided in to 4 groups (table-1).ZN staining was done in all the cases.ZN staining done in group-1 consisted of 30 patients, revealed positive in 5 patients. Group-II (fig -1) consisted 0f 41 patients revealed AFB positivity in 3 patients. Group II consisted of 18 patients' revealed AFB positivity in 2 patients. Group-IV consisted of 20 students revealed positivity in 10 patients.AFB positivity were maximum in the group with caseation only.(fig-2)

The result was in accordance with many studies 17, 35. For the bacilli to be demonstrated in the smears, their number should be between 10,000 to 1, 00,000 per ml of the material. If the number is less than this bacilli may be detected in the smears 19.the presence of AFB and its relation to immunological spectrum has been described by many authors. Lenzi and his colleagues (1977) proposed a point spectrum, at one pole were reactive cases showing a good cell mediated response, comparatively few bacteria and good response to treatment .At the other pole were unreactive cases, resembling lepreматous leprosy in showing numerous organisms in the apparent absence of all cell mediated immune response49.study by meter et al and Das et al found that 64-66% and 77.4% AFB positivity rates respectively in smeares containing only necrotic material or pus.Biopsy correlation was possible in 25 patients. Which included 6 patients of group-I, 10 of Group-II.4 of Group-III and 5of Group-IV.Biopsy results of group I, II, and iv confirmed the cytological diagnosis. Whereas biopsy of group-iii turned out to be reactive lymphodenopathy in two patients and remained as tubercular lymphadenitis in other two patients. There were difficulties in arriving at a definitive diagnosis in certain cases of tubercular lymphadenitis, when the aspirate shows a polymorphous picture with occasional epithelioid cells and obsence of typical Langhan's giant cells or caseous necrosis, making it necessary to resort to excisional biopsy for definitive diagnosis. This is particularly true in children, in whom a similar picture may be seen in cases of reactive lymphadenopathy due to viral or toxoplasma infection. Since the mere presence of occasional epithelioid cells is not diagnostic of any specific condition 35.In the present study 18 cases faced such difficulty. AFB was positive in only 2 cases. On biopsy examination 2 cases turned out to be reactive lymphadenopathy.

V. Conclusion

Fine needle aspiration cytology is an useful technique for evaluation of patients with lympahdneopathies, because of lack of complication and excellent results. The present study is relative sensitive and specific. Diagnostic accuracy can be further improved by studying the cytological pattern of the tuberculosis, immunocytochemical stains and analysis male number of cases along with histopathological correlation.

Table 1: Incidence of various types of cytologic picture with acid fast bacilll (AFB) positivity in patients of tubercular lymphadenitis.

Group No.	Cytological features on aspirated smear	No. Of cases	%	No.of cases positive for AFB	% of positivity
I	Epi.Cell clusters+Langhan;s giant cells+necrosis	30	27.52	5	16.67
II	Epi.Cell clusters+Langhan;s giant cells-necrosis	41	37.61	3	7.3
III	Occasional epithelioid cells only	18	16.51	2	11.12
IV	Necrotic material |pus only no epithelioid cells|no giant cells	20	18.34	10	50

Table 2 : Cyto-histopathological correlation of tubercular lymphadenitis.

Group. No.	Histopathological Diagnosis			
	No.of case in which biopsy done	Reactive lymphadenopathy	Tubercular lymphadenitis	Malignancy
I	6	-	6	-
II	10	-	10	-
III	4	2	2	-
IV	5	-	5	-

Reference Références Referencias

1. Egea AS,Gonzalez MAM,Barrios AP,Masgrau NA,Ausgstin PDAD.Usefulness of light microscopy in lymphnode fine needle aspiration biopsy.Acta Cytol 2002:16(2):364-368.
2. Naib ZM.Exfoliative cytopathology.4th Edn.New York :Little brown and company.1996:467-482.
3. Patra AK,Nanda BK, Mohapatra BK, Panda AK. Diagnosis of lymphadenopathy by fine needle aspiration cytology.Indian J Pathol Microbiol 1983 :26: 273-278.
4. Raghuveer CV,Pai MR, Monohar C. Role of fine needle aspiration cytology in disorder of lymph node.Journal of Cytol 1996 : 13:45-49.
5. Bharadwaj K,Bharadwaj BL,Goel T.Fine needle aspiration cytology in lymphnode disorders with special reference to tuberculosis.J of Cytology 200:17(3):155-159.
6. Prasad R,Gerg SK, Mukherji PK, Agarwal PK..Indian J chest Dis Allied Sci 1993:35(1):27-29.
7. Khan MG, Sherwani RK.Hasan SA, Ahmed S,Khan AA. Tubercular lymphadenitis.A cytologically detectable entity.Indian J Pathol Microbiol 1996:39:74-75.
8. Gupta AK,Nayar M,Chandra M.Critical appraisal of fine needle aspiration cytology in tubercular lymphadenitis. Acta Cytol 1992 :36(3):391-394.
9. Ssdanah MM,Gita Jayaram. Acid fast bacilli in aspiration smears from tuberculous lymphnodes.Analysis 0f 255 cases.Acto Cytol 1987:31(1):15-26.
10. Grange JM. Tuberculosis In: Editors.Parker MJ,Collier LH,Smith JR, Easman CSF.Topley &Wilson'sprinciples of bacteriology, virology and immunity.8th Ed.London:Edward Arnold,1990:93 122.
11. Olell SR, Sterrett GF, Waltons MN, Whitaker D. Mannual and atlas of fine needle aspiration cytology. Edinburg : Churchill livingstone 1997. 3036.
12. Gupta SK, Chungh TD, Shiekh ZA, Al Rubah NAR. Cytodiagnosis of tubercular lymphadenitis. A correlative study with microbiological examination. Acta cytol.

This page is intentionally left blank

Global Journal of Medical research
Volume 12 Issue 1 Version 1.0 February 2012
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Evaluation of Immunosuppressive Regimens in Kidney Transplanted Patients in Iraq

By Dr. Hemen Faik Mohammad, Prof. Dr. Kassim Al-Shamma & Dr. Ansam Naji Al-Hassani
Hawler Medical University

Abstract – Immunosuppressive regimens with the fewest possible toxic effects are desirable for transplant recipients. This study evaluated the efficacy and relative toxic effects of three immunosuppressive regimens used after kidney transplantation in Kirkuk city. 52 kidney transplanted patients were enrolled in this study and categorized into three treatment groups. The group I patients received standard-dose of CsA, MMF in combinations with prednisolone, and the group II patients received low-dose CsA, Aza in combinations with prednisolone, while the group III patients received low-dose Tac, MMF in combinations with prednisolone. The primary efficacy end point was the renal function; secondary end points were incidence of serious adverse effects and the complication of immunosuppression therapy in transplanted recipient.

Abbreviations : CNI= Calcineurin inhibitor, CsA= Cyclosporine A, MMF= Mycophenolate mofetil, Aza= Azathioprine, Tac= Tacrolimus.

GJMR-B Classification : NLMC Code: WJ 368, QY 175, WJ 378

Evaluation of Immunosuppressive Regimens in Kidney Transplanted Patients in Iraq

Dr. Hemen Faik Mohammad [α], Prof. Dr. Kassim Al-Shamma [σ] & Dr. Ansam Naji Al-Hassani [ρ]

Abstract - Immunosuppressive regimens with the fewest possible toxic effects are desirable for transplant recipients. This study evaluated the efficacy and relative toxic effects of three immunosuppressive regimens used after kidney transplantation in Kirkuk city. 52 kidney transplanted patients were enrolled in this study and categorized into three treatment groups. The group I patients received standard-dose of CsA, MMF in combinations with prednisolone, and the group II patients received low-dose CsA, Aza in combinations with prednisolone, while the group III patients received low-dose Tac, MMF in combinations with prednisolone. The primary efficacy end point was the renal function; secondary end points were incidence of serious adverse effects and the complication of immunosuppression therapy in transplanted recipient. The mean calculated serum urea and serum creatinine during study were significantly lower in patients receiving low-dose tacrolimus (4.26mmol/L, 112.01μmol/L for urea and creatinine respectively) than in patients receiving standard-dose cyclosporine (6.28 mmol/L, 133.57μmol/L for urea and creatinine respectively). The mean calculated creatinine clearance was significantly higher in patients receiving low-dose tacrolimus (88.50 ml/min) than in patients receiving standard-dose cyclosporine (73.26 ml/min). Whereas there were no significant differences in serum creatinine and creatinine clearance in patients receiving group III (low-dose tacrolimus) and those receiving group II (low-dose cyclosporine). The serum total cholesterol and serum triglyceride concentrations were significantly lower in the group III (low-dose tacrolimus) than in the other two groups. The serum total bilirubin and bilirubin indirect concentrations were significantly elevated in both group I & II receiving patients, while in the group III (low-dose tacrolimus) receiving patients there were no significant changes in serum bilirubin and hepatocellular enzyme. Neither group I (standard-dose cyclosporine) nor group II (low-dose cyclosporine) and group III (low-dose tacrolimus) were significantly effects on patients fasting blood glucose and patients serum electrolyte (Na& K). The most prominent adverse-effects associated with the all regimens were hypertension, whereas the use of cyclosporine based regimen is associated with a higher incidence of cosmetic adverse-effects (hirsutism & gum hyperplasia). Tremor and gastrointestinal adverse-effects are more frequent in tacrolimus-treated recipients than in cyclosporine-treated recipients. In conclusion, reduced the cyclosporine doses provided improvement in renal function, and immunosuppressive regimen of low-dose tacrolimus with mycophenolate mofetil in combinations with steroids provided significantly higher efficacy, advantageous for renal function, and associated with a more favourable lipid profile and liver function, as compared with regimens containing either standard-dose cyclosporine with mycophenolate mofetil or low-dose cyclosporine with azathioprine in combinations with prednisolone.

Abbreviations : CNI= Calcineurin inhibitor, CsA= Cyclosporine A, MMF= Mycophenolate mofetil, Aza= Azathioprine, Tac= Tacrolimus.

I. Introduction

Kidney transplant is the treatment of choice in end-stage renal disease (ESRD) patients, as it reduces morbidity and mortality rates and improves the quality of life (1). In the absence of the ideal immunosuppressive drug, maintenance immunosuppression is achieved with combinations of immunosuppressive agents at lower doses when the recipient requires less immunosuppression to prevent rejection (2). Standard protocols in use typically involve three immunosuppression drug groups each directed to a site in the T-cell activation or proliferation cascade which are the central to the rejection process: Calcineurin inhibitors (cyclosporine, tacrolimus), anti-proliferative agents (azathioprine, mycophenolate mofetil) and steroids (prednisolone) (3). Calcineurin inhibitors (CNIs) are considered the mainstay of immunosuppression in renal transplantation. Cyclosporine A (CsA) and tacrolimus (Tac) are currently the most widely used baseline immunosuppressant for prevention of acute rejection following kidney transplantation (4). Known adverse effects are similar for both calcineurin inhibitors, which are related to the concentration of the drug, the most prominent of which is nephrotoxicity (5, 6); much of this nephrotoxicity is mediated by impairment of renal hemodynamics (7). Tacrolimus has been associated with more diabetes and neurotoxic reactions, but with less hypertension, dyslipidaemia, hirsutism and gingival hyperplasia than cyclosporine (8, 9). Recent data suggest that calcineurin inhibitors may shorten graft half-life by their nephrotoxic effects (10). MMF is devoid of any diabetogenic, hyperlipidemic, or hypertensive effects (11). Leucopenia, anemia, and gastrointestinal side effects are common with MMF (12). Dose-limiting adverse effects of azathioprine are often hematologic. Leukopenia, anemia, and thrombocytopenia can occur within the first few weeks of therapy and can be managed by dose reduction or discontinuation of azathioprine (13). Corticosteroids have been an integral component of immunosuppressive regimens in renal transplantation for ≥ 50 yr. (14). Corticosteroids are associated with myriad complications. These include the development of obesity, hypertension, glucose intolerance,

hyperlipidemia, osteoporosis, glaucoma, cataracts, myopathy, Cushingoid habitus, and neuropsychiatric complications after transplantation (15). These distinct adverse effect profiles may impact on individual patient compliance and quality of life differently (16). Therefore when using immunosuppressant agents in renal transplantation, achieving low rejection rates while minimizing long term toxicities (eg, nephrotoxicity and cardiovascular disease) associated with these agents is the primary goal (17).

II. Subject and Methods

This retrospective study was carried out in Kirkuk governorate between the first of November 2010 to the end of May 2011. Patients were taken from the artificial Kidney Unit in Kirkuk General Hospital in Kirkuk. The study included 52 kidney transplanted patients (41 male and 11 female) with an age range from (17 to 60) year old 38.68 ± 1.6 (mean ± SE) were divided into three groups according to immunosuppression medication they received.

a) Group I (Standard-Dose Cyclosporine)

This group included thirty patients (26 male and 4 female) with an age range from 17 to 45 years (37.04 ± 2.1) who underwent kidney transplantation range from 2 months to 24 months (median 8 months) and were received: standard-dose of cyclosporine (microemulsion formulation), oral dose of 3 to 5 mg/kg, mean dose (214.42 ± 7.8) mg twice daily, mycophenolate mofetil at fixed doses (2g) per day and prednisolone in a mean dose (9.03 ± 0.66) mg per day in a single morning dose.

b) Group II (Low-Dose Cyclosporine)

This group included fifteen patients (10 male and 5 female) with an age range from 24 to 60 years (43.46 ± 3.2) who underwent kidney transplantation range from 2 years to 5 years (median 3 years) and were received: low-dose of cyclosporine (microemulsion formulation), oral dose of 1 to 2 mg /kg, mean dose (88.46 ± 6.08) mg twice daily, azathioprine at fixed doses (50mg) per day and prednisolone in a mean dose (5.7 ± 0.52) mg per day in a single morning dose.

c) Group III (Low-Dose Tacrolimus)

This group included seven patients (5 male and 2 female) with an age range from 28 to 46 years (32.6 ± 2.1) who underwent kidney transplantation range from 12 months to 24 months (median 14 months) and were received: low- dose of tacrolimus, oral dose of 0.1 mg /kg, mean dose (6.25 ± 0.69) mg twice daily, mycophenolate mofetil at fixed doses (2g) per day and prednisolone at fixed doses (10 mg) per day in a single morning dose.

d) Control Group

The control groups consist of 30 subjects. They were collected from medical staff and relatives who were free from signs and symptoms of renal disease, lipid disorders, diabetes mellitus and hypertension. 22 were males and 8 were females, and their ages ranged from 16 to 60 years (34.5 ± 2.1).

e) Exclusive Criteria

The exclusion criteria included patients with:

- Nephrotic syndrome.
- Primary hyperlipidemia.
- Liver dysfunction resulting from hepatitis, biliary obstruction or cirrhosis.
- Severe hypertension
- Diabetic patients
- Gastrointestinal disorder
- Overdose of cyclosporine dosages.

f) Collection Of Samples

Five milliliters of venous blood were drawn from each fasting patient (8-12 hours fasting). Slow aspiration of the venous blood sample via the needle of syringe to prevent hemolysis with tourniquet applies 15cm above the cubital fossa. The samples were dropped into clean disposable tubes, left at room temperature for 30 minutes for clot formation and then centrifuged for 3 minutes at 3000 run per minute. The serum was separated and used for estimating renal function (urea, creatinine), lipid profile (total cholesterol, triglyceride, HDL-c, LDL-c), liver function (ALP, ALT, AST, total bilirubin and bilirubin direct), fasting blood glucose and electrolyte (Na and K) by Auto analyzer (Flexor- E). Similarly the blood samples were taken from the control group.

g) Statistical Analysis

All data are expressed as mean ± standard error means (M ± SEM) and statistical analysis was carried out using statistically available software (SPSS Version 18). Statistical analyses were carried out using independent sample t-test to compare between mean values of parameters. Analysis of variance (ANOVA) was used for comparing the mean of different parameters used for evaluation of treatments between the treated groups. P value < 0.05 was considered statistically significant.

III. Results

a) Efficacy Measurements

i. *Kidney function parameters*

Significant elevations in the serum urea and serum creatinine were observed, whereas creatinine clearance (Ccl) had decreased significantly compared to the healthy controls in kidney transplanted patients treated with group I treatment regimen (standard-dose CsA/ MMF/ Pred.) measured for three consecutive months as shown in table 3-1.

Table 3-1 :

Parameter	at first month	at 2nd month	at 3rd month	Healthy control
Urea *(mmol/L)*	6.24 ± 0.39*	6.37 ± 0.36*	6.24 ± 0.34*	3.96 ± 0.18
Creatinine *(µmol/L)*	130.50 ± 7.48*	134.67 ± 7.99*	135.54 ± 7.60*	109.52 ± 3.40
Ccl *(ml/min)*	74. 53 ± 4.47*	73.58 ± 3.03*	71.69 ± 2.63*	91.53 ± 5.76

**P < 0.05 significant difference from the control*

Table 3.2 shows the effect of group II treatment regimen (low – dose CsA/ Aza/ Pred.) on renal function parameters in kidney transplanted patients measured for three consecutive months. Significant elevation was observed only in the serum urea value. Serum creatinine and creatinine clearance level showed no significant differences compared to the healthy controls.

Table 3-2 :

Parameter	at first month	at 2nd month	at 3rdmonth	Healthy control
Urea *(mmol/L)*	6.34 ± 0.36*	6.38 ± 0.35*	6.56 ± 0.39*	3.96 ± 0.18
Creatinine *(µmol/L)*	119.62 ± 7.06	123.65 ± 9.97	125.32 ± 9.72	109.52 ± 3.40
Ccl *(ml/min)*	83.81 ± 3.54	83.04 ± 3.96	81.64 ± 3.30	91.53 ± 5.76

** P < 0.05 significant difference from the control*

Table 3.3 shows the effect of group III treatment regimen (low – dose Tac/ MMF/ Pred.) on renal function parameters in kidney transplanted patients measured for three consecutive months. No significant changes were observed in the parameters measured.

Parameter	at first month	at 2nd month	at 3rd month	Healthy control
Urea *(mmol/L)*	4.30 ± 0.35	4.28 ± 0.19	4.22 ± 0.19	3.96 ± 0.18
Creatinine *(µmol/L)*	111.08 ± 2.92	112.43 ± 2.94	112.52 ± 2.99	109.52 ± 3.40
Ccl *(ml/min)*	88.92 ± 4.97	88.37 ± 4.24	88.23 ± 4.29	91.53 ± 5.76

Table 3-4 shows comparison between the effects of the three group's treatment regimen on renal function. There were significant differences between group I (standard-dose CsA) received patients and those on group III (low-dose Tac) at three months follow-up. The estimated serum urea and serum creatinine were significantly lower in the group III (low-dose Tac) than in group I (standard-dose CsA) and the estimated creatinine clearance was significantly higher in the group III (low-dose Tac) than in group I (standard-dose CsA). Whereas the changes where only significant in serum urea and not significant in serum creatinine and creatinine clearance between group II (low-dose CsA) received patients and those on group III (low-dose Tac).

Table 3-4 :

Serum urea								
at first month		**P value**	**at 2nd month**		**P value**	**at 3rd month**		**P value**
Group I	Group II	0.842 NS	Group I	Group 2	0.483 NS	Group I	Group II	0.822 NS
	Group III	0.040 S		Group III	0.004 S		Group III	0.005 S
Group II	Group III	0.037 S	Group II	Group III	0.002 S	Group II	Group III	0.003 S
Serum creatinine								
Group I	Group II	0.255 NS	Group I	Group II	0.252 NS	Group I	Group II	0.260 NS
	Group III	0.037 S		Group III	0.046 S		Group III	0.046 S
Group II	Group III	0.413 NS	Group II	Group III	0.586 NS	Group II	Group III	0.599 NS
Creatinine clearance								
Group I	Group II	0.147 NS	Group I	Group II	0.108 NS	Group I	Group II	0.142 NS
	Group III	0.027 S		Group III	0.015 S		Group III	0.019 S
Group II	Group III	0.525 NS	Group II	Group III	0.499 NS	Group II	Group III	0.502 NS

S: significant NS: no significant (P<0.05 for the comparisons between groups)

b) Safety Results

i. *Effect of treatment groups on lipid profile*

Table 3.5 shows the effect of group I treatment regimen (standard-dose CsA/ MMF/ Pred.) on lipid profile in kidney transplanted patients measured for three consecutive months. Both total cholesterol and triglyceride showed significant elevations compare to healthy control. However there were no significant changes in both serums HDL-c and LDL-c values in patients compared to the healthy control.

Table 3-5 :

Serum lipid	at first month	at 2nd month	at 3rd month	Healthy control
T. Cholesterol *(mmol/L)*	5.15 ± 0.25*	5.47 ± 0.27*	5.52 ± 0.28*	4.34 ± 0.13
Triglyceride *(mmol/L)*	2.17 ± 0.21*	2.29 ± 0.24*	2.31 ± 0.23*	1.33 ± 0.13
HDL-c *(mmol/L)*	1.13 ± 0.08	1.12 ± 0.07	1.14 ± 0.08	0.97 ± 0.03
LDL-c *(mmol/L)*	3.46 ± 0.25	3.22 ± 0.27	3.46 ± 0.26	2.87 ± 0.16

**P < 0.05 significant difference from the control*

Table 3-6 shows the effect of group II treatment regimen (low – dose CsA/ Aza/ Pred.) on lipid profile in kidney transplanted patients measured for three consecutive months. Both cholesterol and triglyceride showed significant elevations compare to healthy control. However there were no significant changes in both serums HDL-c and LDL-c value in patients compared to the control.

Table 3-6 :

Serum lipid	at first month	at 2nd month	at 3rd month	Healthy control
T. Cholesterol *(mmol/L)*	5.31 ± 0.32*	5.20 ± 0.31*	5.17 ± 0.26*	4.34 ± 0.13
Triglyceride *(mmol/L)*	2.55 ± 0.36*	2.50 ± 0.35*	2.57 ± 0.35*	1.33 ± 0.13
HDL-c *(mmol/L)*	1.16 ± 0.11	1.10 ± 0.08	1.10 ± 0.08	0.97 ± 0.03
LDL-c *(mmol/L)*	3.08 ± 0.24	3.37 ± 0.39	3.37 ± 0.39	2.87 ± 0.16

** P < 0.05 significant difference from the control*

Table 3.7 shows the effect of group III treatment regimen (low – dose Tac/ MMF/ Pred.) on lipid profile in kidney transplanted patients measured for three consecutive months. No significant differences were observed in all values of total cholesterol, triglyceride, HDL-c, and LDL-c of the patients at all intervals compared to healthy controls.

Table 3-7 :

Serum lipid	at first month	at 2nd month	at 3rd month	Healthy control
T. Cholesterol *(mmol/L)*	4.48 ± 0.31	4.51 ± 0.27	4.46 ± 0.27	4.34 ± 0.13
Triglyceride *(mmol/L)*	1.51 ± 0.22	1.58 ± 0.27	1.53 ± 0.28	1.33 ± 0.13
HDL-c *(mmol/L)*	0.84 ± 0.13	0.90 ± 0.11	0.90 ± 0.11	0.97 ± 0.03
LDL-c *(mmol/L)*	2.71 ± 0.23	2.97 ± 0.21	2.97 ± 0.21	2.87 ± 0.16

Table 3-8 shows comparison between the effects of the three group's treatment regimen on lipid profile. There were significant differences in serum total cholesterol and triglyceride between groups I (standard-dose CsA) and group II (low-dose CsA) received patients and those on group III (low-dose Tac) at three months follow- up. The estimated serum total cholesterol and serum triglyceride were significantly lower in the group III (low-dose Tac) than in other two groups. Whereas no significant changes in serum total cholesterol and triglyceride were observed between group I (standard-dose CsA) received patients and those on group II (low-dose CsA). Also no significant changes were observed in serum HDL-c and serum LDL-c among all groups treatment regimen.

Table 3-8 :

Total Cholesterol								
at first month		P value	at 2nd month		P value	at 3rd month		P value
Group I	Group II	0.533 NS	Group I	Group II	0.483 NS	Group I	Group II	0.822 NS
	Group III	0.005 S		Group III	0.004 S		Group III	0.005 S
Group II	Group III	0.046 S	Group II	Group III	0.02 S	Group II	Group III	0.03 S
Triglyceride								
Group I	Group II	0.556 NS	Group I	Group II	0.552 NS	Group I	Group II	0.550 NS
	Group III	0.030 S		Group III	0.047 S		Group III	0.034 S
Group II	Group III	0.014 S	Group II	Group III	0.022 S	Group II	Group III	0.016 S
HDL-c								
Group I	Group II	0.796 NS	Group I	Group II	0.668 NS	Group I	Group II	0.642 NS
	Group III	0.111 NS		Group III	0.142 NS		Group III	0.122 NS
Group II	Group III	0.218 NS	Group II	Group III	0.284 NS	Group II	Group III	0.240 NS
LDL-c								
Group I	Group II	0.817 NS	Group I	Group II	0.782 NS	Group I	Group II	0.689 NS
	Group III	0.295 NS		Group III	0.215 NS		Group III	0.245 NS
Group II	Group III	0.445 NS	Group II	Group III	0.435 NS	Group II	Group III	0.489 NS

S: significant NS: no significant (P<0.05 for the comparisons between groups)

ii. *Effect Of Treatment Groups On Liver Function*

Table 3.9 shows serum liver function parameters in kidney transplanted patients treated with group I treatment regimen (standard-dose CsA/ MMF/ Pred.) for three consecutive months. No significant differences were observed in the serum values of ALP, ALT and AST of the patients at all intervals compared to the healthy controls. Total bilirubin values were significantly increased compare to the healthy control, this increases in the total bilirubin value properly came from the indirect bilirubin values which were also increases compare to the healthy control. However the direct bilirubin values were not significantly changed.

Table 3-9 :

Parameter	at first month	at 2nd month	at 3rd month	Healthy control
ALP *(U/L)*	240.03 ± 11.96	239.23 ± 11.30	240.19 ± 12.42	206.52 ± 12.97
ALT *(U/L)*	24.83 ± 2.64	23.71 ± 1.83	24.71 ± 1.83	19.90 ± 1.52
AST *(U/L)*	20.85 ± 1.25	20.76 ± 1.51	21.41 ± 1.51	19.76 ± 0.69
T. Bilirubin *(umol/L)*	16.39 ± 1.25*	16.31 ± 1.15*	16.06 ± 1.11*	12.57 ± 1.10
Bilirubin(direct) *(umol/L)*	10.29 ± 0.87	10.08 ± 0.70	9.91 ± 0.69	8.50 ± 0.72
Bilirubin(indirect) *(umol/L)*	6.10 ± 0.82*	6.23 ± 0.62*	6.15 ± 0.58*	4.07 ± 0.53

**P < 0.05 significant difference from the control*

Table 3.10 shows the effect of group II treatment regimen (low – dose CsA/ Aza/ Pred.) on serum liver function parameter in kidney transplanted patients measured for three consecutive months. No significant differences were observed in the values of serum ALP, ALT and AST of the patients at all intervals compare to the healthy controls. Total bilirubin values were significantly increased compare to the healthy control, this increases in the total bilirubin value properly came from the indirect bilirubin values which were also increases significantly compare to the healthy control. However the direct bilirubin values were not significantly changed.

Table 3-10 :

Parameter	at first month	at 2nd month	at 3rd month	Healthy control
ALP *(U/L)*	221.53 ± 15.49	218.86 ± 15.09	229.91 ± 15.60	206.52 ± 12.97
ALT *(U/L)*	21.66 ± 1.10	20.53 ± 1.15	21.40 ± 1.19	19.90 ± 1.52
AST *(U/L)*	20.45 ± 1.23	21.10 ± 1.33	20.65 ± 1.46	19.76 ± 0.69
T. Bilirubin *(µmol/L)*	16.40 ± 1.76*	16.94 ± 1.81*	16.77 ± 1.79*	12.57 ± 1.10
Bilirubin (direct) *(µmol/L)*	8.98 ± 0.71	9.15 ± 0.71	9.15 ± 0.69	8.50 ± 0.72
Bilirubin(indirect) *(µmol/L)*	7.42 ± 0.54*	7.79 ± 0.49*	7.62 ± 0.58*	4.07 ± 0.53

** P < 0.05 significant difference from the control*

Table3.11 shows the effect of group III treatment regimen (low – dose Tac/ MMF/ Pred.) on serum ALP, serum ALT, serum AST and total bilirubin (direct & indirect) in kidney transplanted patients measured for three consecutive months. No significant differences were observed in the values of serum ALP, serum ALT and serum AST of the patients at all intervals compare to the healthy controls. And no significant differences were observed in the values of total bilirubin, bilirubin direct and bilirubin indirect of the patients at all intervals compare to the healthy controls.

Table 3-11:

Parameter	at first month	at 2nd month	at 3rd month	Healthy control
ALP *(U/L)*	224.62 ± 13.76	226.87 ± 14.03	228.81 ± 14.03	206.52 ± 12.97
ALT *(U/L)*	20.09 ± 3.56	23.50 ± 2.32	22.50 ± 3.12	19.90 ± 1.52
AST *(U/L)*	19.79 ± 2.24	20.23 ± 4.15	20.75 ± 4.15	19.76 ± 0.69
T. Bilirubin *(µmol/L)*	15.44 ± 1.94	15.95 ± 2.31	15.66 ± 1.99	12.57 ± 1.10
Bilirubin (direct) *(µmol/L)*	9.58 ± 1.81	10.72 ± 1.63	10.40 ± 1.53	8.50 ± 0.72
Bilirubin(indirect) *(µmol/L)*	5.86 ± 0.78	5.23 ± 1.46	5.26 ± 0.84	4.07 ± 0.53

Table 3-12 shows comparison between the effects of the three group's treatment regimen on liver function. There were no significant differences in serum ALP, ALT, AST and total bilirubin among all groups treatment regimen at the three months follow- up.

Table 3-12 :

Serum alkaline phosphatase								
at first month		**P value**	**at 2nd month**		**P value**	**at 3rd month**		**P value**
Group I	Group II	0.264 NS	Group I	Group II	0.283 NS	Group I	Group II	0.222 NS
	Group III	0.405 NS		Group III	0.414 NS		Group III	0.425 NS
Group II	Group III	0.929 NS	Group II	Group III	0.922 NS	Group II	Group III	0.931 NS
Serum alanine aminotransferase								
Group I	Group II	0.203 NS	Group I	Group II	0.252 NS	Group I	Group II	0.250 NS
	Group III	0.708 NS		Group III	0.747 NS		Group III	0.734 NS
Group II	Group III	0.652 NS	Group II	Group III	0.622 NS	Group II	Group III	0.616 NS
Serum aspartate aminotransferase								
Group I	Group II	0.829 NS	Group I	Group II	0.848 NS	Group I	Group II	0.842 NS
	Group III	0.969 NS		Group III	0.942 NS		Group III	0.922 NS
Group II	Group III	0.920 NS	Group II	Group III	0.984 NS	Group II	Group III	0.940 NS
Serum total bilirubin								
Group I	Group II	0.804 NS	Group I	Group II	0.812 NS	Group I	Group II	0.789 NS
	Group III	0.783 NS		Group III	0.715 NS		Group III	0.745 NS
Group II	Group III	0.604 NS	Group II	Group III	0.635 NS	Group II	Group III	0.689 NS

S: significant NS: no significant (P<0.05 for the comparison between groups)

iii. *Effect Of Treatment Groups On Fasting Blood Glucose*

Table 3.13 shows fasting blood glucose in kidney transplanted patients treated with different groups treatment regimen measured for three consecutive months. No significant differences were observed in the serum fasting glucose of the patients at all intervals compared to the healthy control. And when comparing among the three treatment groups there were no significant differences in serum fasting glucose among the groups treatment at three months follow- up (Table 3-14).

Table 3-13 :

Glucose	at first month	at 2nd month	at 3rd month	Healthy control
Group I n = 30	5.32 ± 0.23	5.31 ± 0.27	5.30 ± 0.27	4.80 ± 0.19
Group II n = 15	5.66 ± 0.49	5.77 ± 0.70	5.92 ± 0.68	
Group III n = 7	4.86 ± 0.27	5.02 ± 0.51	5.10 ± 0.50	

Table 3-14 :

Serum fasting glucose								
at first month		P value	at 2nd month		P value	at 3rd month		P value
Group I	Group II	0.400 NS	Group I	Group II	0.388 NS	Group I	Group II	0.398 NS
	Group III	0.567 NS		Group III	0.514 NS		Group III	0.522 NS
Group II	Group III	0.182 NS	Group II	Group III	0.122 NS	Group II	Group III	0.131 NS

S: significant NS: no significant (P<0.05 for the comparisons between groups)

iv. *Effect Of Treatment Groups On Serum Electrolyte (Na, K)*

Table 3.15 shows serum electrolyte (Na, K) in kidney transplanted patients treated with different groups treatment regimen measured for three consecutive months. No significant differences were observed in the serum electrolyte (Na, K), of the patients at all intervals compared to the healthy controls in all groups. Also when comparing among the three treatment groups there were no significant differences in serum electrolyte (Na, K) among the groups treatment at three months follow-up (Table 3-16) .

Table 3-15 :

Na *(mmol/L)*	at first month	at 2nd month	at 3rd month	Healthy control
Group I n = 30	139.84 ± 0.52	139.74 ± 0.59	139.85 ± 0.61	139.36 ± 0.43
Group II n = 15	140.83 ± 0.60	141.05 ± 0.58	140.90 ± 0.61	
Group III n = 7	139.75 ± 1.65	139.60 ± 1.55	139.70 ± 1.65	
K *(mmol/L)*				
Group I n = 30	4.37 ± 0.10	4.36 ± 0.07	4.40 ± 0.07	4.32 ± 0.11
Group II n = 15	4.37 ± 0.12	4.48 ± 0.09	4.44 ± 0.09	
Group III n = 7	4.33 ± 0.23	4.36 ± 0.17	4.36 ± 0.22	

Table 3-16 :

Serum Na								
at first month		P value	at 2nd month		P value	at 3rd month		P value
Group I	Group II	0.139 NS	Group I	Group II	0.183 NS	Group I	Group II	0.122 NS
	Group III	0.997 NS		Group III	0.914 NS		Group III	0.925 NS
Group II	Group III	0.389 NS	Group II	Group III	0.322 NS	Group II	Group III	0.331 NS
Serum K								
Group I	Group II	0.410 NS	Group I	Group II	0.452 NS	Group I	Group II	0.450 NS
	Group III	0.968 NS		Group III	0.947 NS		Group III	0.934 NS
Group II	Group III	0.600 NS	Group II	Group III	0.622 NS	Group II	Group III	0.616 NS

S: significant NS: no significant (P<0.05 for the comparisons between groups)

v. *Adverse Effects Of Treatment Groups Observed In Kidney Transplanted Patients*

It is obvious from the below table that the group I treatment regimen (standard-dose CsA/ MMF/ Pred.) had the greatest incidence adverse effects including: (83%) of patients had hypertension, (26%) had tremors, (23%) had gastrointestinal upset, (43%) had hirsutism, and (16 %) had gum hyperplasia. While the group II treatment regimen (low – dose CsA/ Aza/ Pred.) had a similar percent of adverse effect regarding hypertension and tremor (80% and 20%) respectively and lower percent of adverse effects regarding hirsutism (33%), GI upset(13%) and gum hyperplasia (13%). However group III treatment regimen (low – dose Tac/ MMF/ Pred.) had the lowest adverse effects with hypertension (71%), tremor (42%) and GI upset (28%) with no other adverse effects.

Table 3-17 : Adverse effects associated with different group's treatment in kidney transplanted patients.

Adverse Effects	Group I (n =30)		Group II(n =5)		Group III (n =7)	
	No.	(%)	No.	(%)	No.	(%)
Hypertension	25	83%	12	80%	5	71%
Tremor	8	26%	3	20%	3	42%
GI upset	7	23%	2	13%	2	28 %
Hirsutism	13	43%	5	33%	0	---
Gum hyperplasia	5	16 %	2	13%	0	---

IV. Discussion

The primary efficacy end point in this study was renal function. Therefore standard analysis such as serum urea, serum creatinine and creatinine clearance measurement are used to monitor the renal function that changes only after significant kidney injury (18). The glomerular filtration rate (GFR), the underlying indicator of renal function, is inversely proportional to the concentration of creatinine in plasma (19). Creatinine clearance gives an acceptable estimate of the glomerular filtration rate. The most widely used equations for calculation creatinine clearance are the Cockcroft-Gault equations (20).

On the basis of our results and literature review it was shown that nephrotoxicity (functional changes) induced by calcineurin inhibitor drug (CsA) is characterized by dose-dependent functional changes of the kidney function, which are reversible with a decrease in the dose or drug withdrawal (21, 22, 23, 24, 25).

In this study, table 3.1 showed the effects of group I treatment regimen (standard-dose CsA/ MMF/ Pred.) on renal function in thirty kidney transplanted patients. There were significant increases in serum urea, serum creatinine and significant decreased in creatinine clearance level when compared to the healthy control for three month consecutively. These results are in agreement with results of other studies conducted by Van Buren et al., 1994 (26); Lassila, 2000 (27); puigmule et al., 2009 (18) who found that there were a significant increases in serum urea and serum creatinine, and a significant decreases in creatinine clearance after standard doses of cyclosporine administered in kidney transplanted patients. Since MMF has favorable safety profile and not adversely affect kidney function (28, 29). Therefore we suggested that the standard doses of cyclosporine causes significant changes in renal function (30).

Table 3.2 showed the effects of group II treatment regimen (low-dose CsA/ Aza/ Pred.) on renal function in fifteen kidney transplanted patients. Serum urea was only significantly increased, and serum creatinine and creatinine clearance level were slightly increased and decreased respectively compared to the healthy control for three consecutive months (not significant). These results are in agreement with the results of other studies conducted by Wissmann et al., 1996 (22); Moroni, et al, 2006 (31); Bobadilla and Gamba, 2007 (32) who found that the cyclosporine nephrotoxicity is dose –dependent and the low doses of cyclosporine did not significantly changes renal function. Therefore we suggest that to find a significant association between CsA and changes in renal function may depend on the dosage used in the regimen. The explanation for the only significant increase in serum urea in this group is probably that, serum urea concentration may increase out of proportion with a change in serum creatinine (33), and the rate of urea production is not constant, urea can be grossly modified by a high protein intake, critical illness (i.e. sepsis, burns, and trauma), or drug therapy such as use of corticosteroids or tetracycline, and the rate of renal clearance of urea is also not constant, an estimated 40–50% of filtered urea is passively reabsorbed by proximal renal tubular cells (33).

Table 3.3 showed the effects of group III treatment regimen (low dose Tac/ MMF/ Pred.) on renal function in seven kidney transplanted patients. No significant increases in serum urea & serum creatinine, and no significant decreased in creatinine clearance level were observed when compared to healthy control for three consecutive months. These results are in agreement with the results of other studies conducted by Artz et al., 2003 (34); Kramer et al., 2005 (4); Naesens et al., 2009 (35) who found less calcineurin-

inhibitor nephrotoxicity with the use tacrolimus in kidney transplanted patients. This may reflect a lower nephrotoxicity of tacrolimus-based immunosuppressive regimens and also may reflect a lower immunologic damage of the graft (36).

When comparing renal function as efficacy end point among the three groups treatment regimen. The mean calculated serum urea and serum creatinine during study were significantly lower in patients receiving low-dose tacrolimus (4.26mmol/L, 112.01μmol/L for urea and creatinine respectively) than in patients receiving standard-dose cyclosporine (6.28 mmol/L, 133.57μmol/L for urea and creatinine respectively). The mean calculated creatinine clearance was significantly higher in patients receiving low-dose tacrolimus (88.50 ml/min) than in patients receiving standard-dose cyclosporine (73.26 ml/min). Whereas there were no significant differences in serum creatinine and creatinine clearance in patients receiving group III (low-dose tacrolimus) and those receiving group II (low-dose cyclosporine). Therefore the reduced doses of cyclosporine improve renal function, and low-dose tacrolimus based regimen provided better renal function when compared with standard-dose cyclosporine based regimens as shown in (Table 3-4). The results of this study is in agreement with other studies Jurewicz, 2003 (37); Ekberg et al., 2007 (30); Bobadilla and Gamba, 2007 (32) who found improvement in renal function with reducing cyclosporine dosage, and the uses of low-dose tacrolimus based regimens in kidney transplanted patients had advantageous for renal function than standard-dose of cyclosporine based regimen.

The causes of post transplant dyslipidemia include increased nutrient intake after transplantation (38), and adverse effects of steroids or cyclosporine used for immunosuppression (39, 40, 41).

In this study, Table 3.5 and Table 3-6, there were mild significant elevations of plasma total cholesterol and triglyceride concentrations compared to healthy control. This results is in agreement with other studies conducted by Ilgenli et al., 1999 (42); Vaziri et al., 2000 (43); Ichimaru et al., 2001 (39); Abramowicz et al.,2005 (28); Hami et al., 2010 (44) who revealed that long-term administrations of CsA and steroid were significantly raise plasma total cholesterol and triglyceride concentrations in renal transplanted patients. This reported changes in serum lipids has been found to be related with the mechanism of CsA adverse effects, since neither azathioprine (45) nor mycophenolate mofetil (28 , 46) and corticosteroids (in daily dose of 12.5 mg or less) (42) are known to be associated with changes of serum lipid profile. Although the mechanism of calcineurin inhibitor induced hyperlipidemia is not well understood. Calcineurin inhibitors may decrease the activity of lipoprotein lipase (47). Hypercholesterolemia may be due to down-regulation of enzyme cholesterol 7α-hydroxylase. This enzyme is the rate-limiting step in cholesterol conversion to bile acid, which is the principal pathway of cholesterol catabolism (43). Hypertriglyceridemia may be due to lipoprotein lipase and triglyceride hydrolase deficiency (39). Corticosteroids causes decrease in lipoprotein lipase activity, as well as excessive triglyceride production. But a daily dose of 12.5 mg or less of corticosteroid as in patients in this study has only a minimal effect on cholesterol (42). Also both serum (HDL-c) and (LDL-c) in both groups I & II treatment regimens were slightly increases but not significantly compared to control healthy individual. This finding has been reported only in study of Vaziri et al., 2000 (43) who revealed that the hepatic LDL receptor (play an important role in LDL metabolism) and HDL receptor (which facilitates transport of cholesterol esters from HDL to hepatocytes) expressions were not altered by CsA therapy.

Table 3.7 showed the effects of group III treatment regimen on lipid profile. No significant changes were observed on lipid profile when compared to healthy control, since the tacrolimus have less potential to induce hyperlipidemia than cyclosporine (48). These results are in agreement with other studies conducted by Pirsch et al, 1997 (49); McCune et al., 1998 (50); Ligtenberg et al., 2001 (51); Artz et al., 2003 (34); Morales and Domınguez-Gil, 2006 (36) who revealed no significant effects of tacrolimus on lipid metabolism in renal transplanted recipient.

When comparing serum lipid profile among the three group treatment regimens, there were statistically significant differences among groups treatment at three months follow- up (table 3-8). The serum total cholesterol and serum triglyceride concentrations were significantly lower in the group III (low-dose tacrolimus) than in the other two groups. Therefore the use of low dose tacrolimus based immunosuppressive regimen is associated with a more favourable lipid profile than the use of different cyclosporine dosage based immunosuppressive regimens. The results of this study are in agreement with other studies conducted by Scott et al., 2003 (48); Kramer, et al., 2005 (4); Becker-Cohen et al., 2006 (38) who found better lipid profile with the use of tacrolimus based regimen than cyclosporine based regimen. Whereas there were no significant differences between group I (standard-dose cyclosporine) and group II (low-dose cyclosporine), thus the reduced doses of cyclosporine did not improve the changes in lipid profile. Therefore replacement of cyclosporine with tacrolimus reduced the high level of total cholesterol and triglyceride in patients taking cyclosporine (50, 52).

Calcineurin inhibitor (CsA & Tac) hepatotoxicity has been reported in few case reports after organ transplantation (53, 54). The exact mechanism of CsA induced hepatotoxicity is not completely understood, numerous current findings suggest that oxidative stress mechanism playing an important role in its pathology.

CsA therapy induces overproduction of reactive oxygen species (ROS) in hepatocytes and lowers their antioxidant capacity) 55).

In this study, Table 3.9 and Table 3-10, significant mild elevations were observed only in total bilirubin and bilirubin indirect levels compared to control healthy individual. These results (elevations of total bilirubin and bilirubin indirect) are in agreement with results of other studies conducted by Schade et al., 1983 (56); Kahan, 1987 (21); Cadranel, et al, 1992 (57); Hecking, et al, 2008 (58) who revealed that there is a significant elevations in total bilirubin after cyclosporine treatment. This elevation of total bilirubin seen after cyclosporine treatment is most probably related to a cholestasis (59). This could be due to the toxic metabolite of cyclosporine (AM19 and AM1A) (60), and since the bilirubin and cyclosporine metabolites are eliminated by the same transport system through the biliary membrane, therefore the elevated total bilirubin level suggested impaired cyclosporine elimination (61). Hepatocellular enzymes ALP, ALT and AST in this study in both group I and group II showed no significant differences compared to control healthy individual for three consecutive months. The explanation for that could be attributed to the doses of CsA used. Also many other articles and case reports conducted by Lorber et al, 1987 (62); Gulbis, et al, 1988 (63); Taniai et al, 2008 (54); Oto et al, 2010 (53) revealed that the reduction of the cyclosporine doses was sufficient to resolve the presumed hepatotoxicity (elevated level of hepatocellular enzymes).

Table 3.11 showed the effects of group III on liver function, no significant changes in hepatocellular enzymes ALP, ALT and AST and in total bilirubin and (bilirubin direct & bilirubin indirect) were observed in any of the patients in the group compared to control healthy individual. Such results were also reported in case reports conducted by Taniai, et al, 2008 (54); Oto, et al, 2010 (53) who found that the tacrolimus hepatotoxicity is seemed to be dose-dependent and low doses of tacrolimus did not significantly changes liver function as this study shows.

When comparing liver function among the three group treatment regimens, there were no statistically significant differences among groups treatment at three months follow- up (table 3-12). Also patients receiving group II (low-dose cyclosporine) had a mean serum total bilirubin and bilirubin indirect close to those of patients receiving group I (standard-dose cyclosporine). Therefore we suggest the reduced doses of cyclosporine did not resolved the mild elevated values of total bilirubin and bilirubin indirect, and group III (low_ dose tacrolimus) regimen has favorable liver function.

New-onset diabetes after renal transplantation (NODAT) represents a serious metabolic complication with a negative impact on graft and patient survival, as well as on cardiovascular morbidity and mortality (64).

Among immunosuppressant, there are no alterations in glucose metabolism due to the use of MMF (65). The use of steroids causes in dose-dependent an increase in peripheral insulin resistance and increasing hepatic glucose production (66, 67). However, daily prednisone doses (5 mg/day) may not influence insulin sensitivity at all (68). Calcineurin inhibitors contribute to the development of (NODAT) by directly inhibiting insulin secretion from the pancreatic β-islet cell. This effect is dose-dependent, reversible and more pronounced for patients who are treated with tacrolimus than cyclosporine (69, 52). Consistent with this, a meta-analysis of randomized controlled trials of cyclosporine versus tacrolimus after renal transplantation found a higher incidence of diabetes among those treated with tacrolimus suggesting that the use of cyclosporine rather than tacrolimus may be an effective strategy to prevent NODAT (70). However, tacrolimus has been reported to be diabetogenic, this risk is predominantly present in the initial period after transplantation and in patients who already had an impaired glucose tolerance before treatment (34).

In this study, table 3.13 showed the effects of all groups' treatment regimen (I & II & III) on fasting blood glucose in kidney transplanted patients. No significant changes in blood glucose level in either group were observed compared to control healthy individual, and also there were no statistically significant differences among groups treatment at three months follow- up (table 3-14). This results is not in parallel with other studies results conducted by Filler et al., 2000 (71); Vincenti et al., 2007 (72); Johnston et al., 2008 (73); Hornum et al., 2010 (74) who revealed a highest incidence of new-onset post transplantation diabetes mellitus in patients treated with CsA in combination with MMF or Aza and steroid, and in patients treated with tacrolimus in combination with MMF/steroid. The probable explanation is that cyclosporine and tacrolimus influences glucose metabolism by reducing pancreatic insulin secretion in a dose-dependent manner (65, 75, and 69) and patients in this study predominantly received low doses of these drugs. Also other studies conducted by Ligtenberg et al., 2001 (51); Hooda et al., 2007 (76) suggested that low dose tacrolimus significantly reduces incidence of new-onset post transplantation diabetes mellitus and do not impair glycemic control.

In this study, table 3.15 showed the effects of all groups' treatment regimen (I & II & III) on serum electrolyte (Na & K) in kidney transplanted patients. No significant changes in either group compared to control healthy individual were observed, and also there were no statistically significant differences among groups treatment at three months follow- up (table 3-16). This could indicate no significant effects of the three group's treatment regimen on serum Na and serum K.

In this study, Table 3-17 showed the most common adverse effects for all groups treatment regimen (I & II & III) experienced in patients, and ranged from hypertension (83%) to gum hyperplasia (13%). Hypertension is usually reversible after discontinuation of short-term CsA therapy (77). The effect seems to be more dependent on vasoconstriction than salt retention as demonstrated by hypertension present in an anuric transplant on cyclosporine therapy (78).

In this study, among patients receiving calcineurin inhibitor, those receiving cyclosporine A based regimen were more prone to develop hypertension (83%) & (80%) in group I & II respectively than those receiving tacrolimus based regimen (71%) in group III. This adverse hypertension effects was also reported by others studies conducted by Lassila, 2000 (27); Castillo-Lugo and Vergne-Marini, 2005 (79); Catarsi et al., 2005 (80). Therefore the use of tacrolimus may lead to less risk for hypertension when compared with treatment with CsA and conversion from treatment with CsA to treatment with tacrolimus may leads to a slight decline in blood pressure (51). Although there were no significant difference in blood pressure between groups treatment regimen (4) .

In this study the blood pressure remained unchanged in the CsA receiving groups; although the low doses of CsA in group II treatment regimens had been received during the study period. Similar results also reported by Schnuelle et al., 2002 (81); Jose, 2007 (52) who found continued treatment with CsA even at reduced doses frequently results in sustained hypertension.

The other adverse-effects (tremor, GI upset, hirsutism & gum hyperplasia) have been also recorded in other studies Kasiske et al, 2000 (16); Ciavarella et al., 2007 (82); Webster et al., 2009 (3). In this study apart from hypertension, these adverse-effects are considered mild. The incidences of these cosmetic conditions (hirsutism and gingival hyperplasia) were predominant in patients taking cyclosporine, hirsutism (43% in group I & 33% in group II) and gum hyperplasia (16% in group I & 13% in group II), than in patients taking tacrolimus (no case reported). Similar results are also reported in other studies Jose, 2007 (52); Chan et al., 2008 (9). CsA induced gingival hyperplasia is connected with increased collagen levels due to the CsA mediated inhibition of collagen phagocytosis (83). Neurological effects (tremor) and gastrointestinal effects (diarrhea, vomiting and dyspepsia) were more frequent in tacrolimus-treated recipients, tremor (42% in group III than 26% & 20% in group I & II respectively) and gastrointestinal effects (28% in group III than 23% & 13% in group I & II respectively). Similar results are also reported in other study Morales et al., 2001 (24). These reported gastrointestinal effects were being due to concurrent mycophenolate mofetil use more than to the calcineurin inhibitor associated gastrointestinal effects (84).

V. Conclusion

- Immunosuppressive regimen of low-dose tacrolimus with mycophenolate mofetil in combination with steroids and regimen of low-dose cyclosporine with azathioprine in combinations with steroids provided significantly higher efficacy by improvement in renal function, as compared with regimen containing standard-dose cyclosporine with mycophenolate mofetil in combinations with steroids.
- Immunosuppressive regimen of low-dose tacrolimus with mycophenolate mofetil in combination with steroids associated with a more favourable lipid profile and liver function, as compared with regimens containing either standard-dose cyclosporine with mycophenolate mofetil or low-dose cyclosporine with azathioprine in combinations with steroids.
- Neither low-dose tacrolimus/ mycophenolate mofetil/ steroid, standard-dose cyclosporine/ mycophenolate mofetil/ steroid nor low-dose cyclosporine/ azathioprine/ steroid immunosuppressive regimens are associated with post transplant diabetes mellitus and disturbance in serum electrolyte (Na& K).
- Cyclosporine nephrotoxicity is dose-dependent and reduce the dose of cyclosporine lead to less nephrotoxicity and improvement in renal function.
- The use of cyclosporine based immunosuppressive regimen is associated with elevations in serums total cholesterol, triglyceride and total bilirubin in dose-independent manner, compared with the use of tacrolimus based immunosuppressive regimen which show no changes in post renal transplant.
- The most prominent adverse-effects associated with the all immunosuppressive regimens were hypertension. Whereas the use of cyclosporine is associated with a higher incidence of cosmetic adverse-effects (hirsutism & gum hyperplasia), and neurological (tremor) adverse-effects are more common in tacrolimus-treated recipients than in cyclosporine-treated recipients.

References Références Referencias

1. Saracino A, Santarsia G, Latorraca A, Gaudiano V, 2006. Early assessment of renal resistance index after kidney transplant can help predict long-term renal function. Nephrol Dial Transplant; 21: 2916 2920
2. Khurana A and Brennan DC, 2011. Current concept of immunosuppression and side effect. Pathology of Solid Organ Transplantation; 6(6): 337–340.
3. Webster AC, Taylor RR, Chapman JR, Craig JC, 2009. Tacrolimus versus cyclosporine as primary immunosuppression for kidney transplant recipients (Review). The Cochrane Library; Issue 1.

4. Kramer BK, Montagnino G, Castillo D, Margreiter R, et al., 2005. Efficacy and safety of tacrolimus compared with cyclosporine A microemulsion in renal transplantation: 2 year follow-up results. Nephrol Dial Transplant; 20: 968–973.
5. Halloran PF, 2004. Immunosuppressive drugs for kidney transplantation. N Engl J Med; 351: 2715–2729.
6. Rainienë T, 2005. Immunosuppression in the past and today. ACTA Medica Lituanica; 12(3):10–17.
7. Bennett WM, De Mattos A, Meyer MM, Andoh T, Barry JM, 1996. Chronic cyclosporine nephropathy in renal transplantation. Transplant Proc; 28: 2100–2103.
8. Denton MD, Magee CC, Sayegh MH, 1999. Immunosuppressive strategies in transplantation. Lancet; 353: 1083–1091.
9. Chan A, Stüve O, von Ahsen N, 2008. Immunosuppression in clinical practice: approaches to individualized therapy. J Neurol; 255(Suppl6): 22-29.
10. Nankivell BJ, Richard J, Borrows R, Fung CL-S, et al., 2003. The natural history of chronic allograft nephropathy. N Engl J Med; 349: 2326–2333.
11. Srinivas TR, Kaplan B, Meier-Kriesche HU, 2003. Mycophenolate mofetil in solid-organ transplantation. Expert Opin Pharmacother; 4: 2325–2345.
12. Sollinger HW, 1995. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients: U.S. Renal Transplant Mycophenolate Mofetil Study Group. Transplantation; 60(3): 225–232.
13. Levy GL, 2001. Long-term immunosuppression and drug interactions. Liver Transplantation; 11: S53–S59.
14. Srinivas TR and Meier-Kriesche HU, 2008. Minimizing immunosuppression, an alternative approach to reducing side effects: objectives and interim result. Clin J Am Soc Nephrol; 3: S101–S116.
15. Danovitch GM, 2005. Immunosuppressive medications and protocols: Handbook of Kidney Transplantation. Philadelphia, Lippincott, Williams &Wilkins: 72–134.
16. Kasiske BL, Vazques MA, Harmon WE, et al., (2000). Recommendations for the Outpatient Surveillance of Renal Transplant Recipients. J Am Soc Nephrol; 11: S1–S86.
17. Stephen J, Pearson T, Gallon L, 2007. Evolving strategies for immunosuppression in renal transplantation: A review of recent clinical trials. Adv Stud Med; 7(9): 275-280.
18. Puigmule M, Lopez-Hellin J, Sune G, Tornavaca O, et al., 2009. Differential proteomic analysis of cyclosporine A-induced toxicity in renal proximal tubule cells. Nephrol Dial Transplant; 24: 2672–2686
19. Trull A, Hue K, Tan K, Gore S, et al, 1990. Cross – correlation of cyclosporine concentration and biochemical measures of kidney and liver function in heart and heart-lung transplant recipients. Clinical Chemistry; 36(8): 1474-1478.
20. Bauer JH, Brooks CS, Burch RN, 1982. Clinical appraisal of creatinine clearance as a measurement of glomerular filtration rate. Am J Kidney Dis; 2: 337-347.
21. Kahan BD, 1987. Immunosuppressive therapy with cyclosporine for cardiac transplantation. Circulation; 75: 40-56.
22. Wissmann C, Felix J, Ferrari P, Uehlinger D, 1996. Acute cyclosporine_induced nephrotoxicity in renal transplant recipients: the role of the transplanted kidney. Journal of the American Society of Nephrology; 7: 2677-2681.
23. Karamehic J, Asceric M, Rakic S, 1997. Adverse reactions of cyclosporine in patients after kidney transplantation. Acta Medica Saliniana; 26(2): 3-6.
24. Morales JM, Andres A, Rengel M, Rodicio JL, 2001. Influence of cyclosporine, tacrolimus and rapamycin on renal function and arterial hypertension after renal transplantation. Nephrol Dial Transplant; 16 (suppl1): 121-124.
25. Ponticelli C, 2005. Cyclosporine: from renal transplantation to autoimmune diseases. Ann. N. Y. Acad. Sci.; 1051: 551–558.
26. Van Buren DH, Burke JF, Lewis RM, 1994. Renal function in patients receiving long-term cyclosporine therapy. J Am Soc Nephrol; 4: s17-s22.
27. Lassila M, 2000. Cyclosporine A-induced hypertension and nephrotoxicity in spontaneously hypertensive rats on high- sodium diet. Transplantation; 45: 1171–1181.
28. Abramowicz D, Rial M, Vitko S, et al., 2005. Cyclosporine withdrawal from a mycophenolate mofetil-containing immunosuppressive regimen: Results of a five-year, prospective, randomized study. J Am Soc Nephrol; 16: 2234–2240.
29. Takemoto SK, Pinsky BW, Schnitzler MA, et al., 2007. A Retrospective analysis of immunosuppression compliance, dose reduction and discontinuation in kidney transplant recipients. American Journal of Transplantation; 7: 2704–2711.
30. Ekberg H, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, 2007. Reduced exposure to calcineurin inhibitors in renal transplantation. N Engl J Med; 357: 2562-2575.
31. Moroni G, Doria A, Mosca M, et al., 2006. A randomized pilot trial comparing cyclosporine and azathioprine for maintenance therapy in diffuse Lupus Nephritis over four years. Clin J Am Soc Nephrol; 1: 925–932.
32. Bobadilla NA and Gamba G, 2007. New insights into the pathophysiology of cyclosporine

nephrotoxicity: A role of aldosterone. Am J Physiol Renal Physiol; 293: F2–F9.
33. Finn WF and Porter GA, 2008. Urinary biomarkers and nephrotoxicity: Clinical Nephrotoxins: Renal Injury from Drugs and Chemicals 3th Edition. Springer Science; 92-117.
34. Artz MA, Boots JM, Ligtenberg G, et al, 2003. Improved cardiovascular risk profile and renal function in renal transplant patients after randomized conversion from cyclosporine to tacrolimus. J. Am. Soc. Nephrol; 14:1880–1888.
35. Naesens M, Kuypers D, Sarwal M, 2009. Calcineurin Inhibitor Nephrotoxicity. Clinical Journal of the American Society of Nephrology; 4: 481–508.
36. Morales JM and Domınguez-Gil B, 2006. Impact of tacrolimus and mycophenolate mofetil combination on cardiovascular risk profile after kidney transplantation. J Am Soc Nephrol; 17: S296–S303.
37. Jurewicz AW, 2003. Tacrolimus versus cyclosporine immunosuppression: long-term outcome in renal transplantation. Nephrol Dial Transplant; 18 (Suppl1): i7–i11
38. Becker-Cohen R, Nir A, Rinat C, Feinstein S, Algur N, et al, 2006. Risk factors for cardiovascular disease in children and young adults after renal transplantation. Clin J Am Soc Nephrol; 1: 1284–1292.
39. Ichimaru N, Takahara S, Kokado Y, Wang J, Hatori M, Kameoka H, InoueT, Okuyama A, 2001. Changes in lipid metabolism and effect of simvastatin in renal transplant recipients induced by cyclosporine or tacrolimus. Atherosclerosis; 158: 417–423.
40. Wanner C and Quaschning T, 2001. Abnormal lipid metabolism after renal transplantation. Annals of Transplantation; Vol.6: No.1.
41. Kasiske BL, Cosio FG, Beto J, et al., 2004. Clinical practice guidelines for managing dyslipidemias in kidney transplant patients: A report from the managing dyslipidemias in chronic kidney disease work group of the national kidney foundation kidney disease outcomes quality initiative. Am J Transplant; 4 (Suppl7):13–53.
42. Ilgenli T, Atilla G, Cirit M, Azmak N, 1999. The role of serum lipids on Cyclosporine-induced gingival overgrowth in renal transplant patients. Tr. J. of Medical Sciences; 29: 297-301.
43. Vaziri N, Liang K, Azad H, 2000. Effect of Cyclosporine on HMG-CoA Reductase, Cholesterol 7α-Hydroxylase, LDL Receptor, HDL Receptor, VLDL Receptor, and Lipoprotein Lipase Expressions. The journal of Pharmacology and Experimental Therapeutics; 294: 778–783.
44. Hami M, Mojahedi M, Naghibi M, Shakeri M, Sharifipour F, 2010. Cyclosporine trough levels and its side effects in kidney transplant recipients. Iranian Journal of Kidney Diseases; Vol. 4: No. 2.
45. Hilbrands L, Demacker P, Holtsma A, et al., 1995. The effects of cyclosporine and prednisolone on serum lipid and (Apo) lipoprotein level in renal transplant recipients. J Am Soc Nephrol; 5: 2073-2081.
46. Hamdy A, Bakr M, Ghoneim M, 2008. Long-term efficacy and safety of a Calcineurin Inhibitor-free regimen in live-donor renal transplant recipients. J Am Soc Nephrol.; 19: 1225–1232.
47. Moore R, Hernandez D, Valantine H, 2001. Calcineurin inhibitors and post transplant hyperlipidemias. Drug Saf; 24: 755–766.
48. Scott LJ, McKeage K, Keam SJ, Plosker GL, 2003. Tacrolimus: A further update of its use in the management of organ transplantation. Drugs; 63: 1247–1297.
49. Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS, 1997. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation: FK506 kidney transplant study group. Transplantation; 63(7): 977–983.
50. McCune T, Thacker L, Peters T, et al., 1998. Effects of tacrolimus on hyperlipidemia after successful renal transplantation. Transplantation; 65: 87-92.
51. Ligtenberg G, Hene RJ, Blankestijn PJ, Koomans HA, 2001. Cardiovascular risk factors in renal transplant patients: cyclosporin A versus tacrolimus. J Am Soc Nephrol; 12(2): 368–373.
52. Jose M, 2007. Calcineurin inhibitors in renal transplantation: Adverse effects. NEPHROLOGY; 12: S66–S74.
53. Oto T, Okazaki M, Takata K, Egi M, Yamane M, et al., 2010. Calcineurin inhibitor-related cholestasis complicating lung transplantation. Ann Thorac Surg; 89: 1664-1665.
54. Taniai N, Akimaru K, Ishikawa Y, Kanada T, 2008. Hepatotoxicity caused by tacrolimus and cyclosporine after living donor liver transplantation. J Nippon Med Sch; 75 (3).
55. Shakiba Y, Mostafaie A, Arshadi D, Sabayan B, 2009. Application of garlic organosulfur compounds in prevention of cyclosporine A-induced hepatotoxicity. Iranian Journal of Medical Hypotheses and Ideas; 3: 3.
56. Schade R, Guglielmi A, Van Thiel DH, et al., 1983. Cholestasis in heart transplant recipient treated with cyclosporine. Transplan. Proc; 4: 57-61.
57. Cadranel JF, Swindle J, Machnicki G, et al., 1992. Chronic administration of cyclosporine A induces a decrease in hepatic excretory function in man. Digestive Diseases and Sciences; 37(16):1422 1430.
58. Hecking M, Kainz A, Schillinger M, Posch C, et al., 2008. Analysis of liver function in renal transplant recipients undergoing C2-monitoring for cyclosporine. Transpl Int.; 21(3): 223-233.

59. Kassianides C, Schechter P, Mekki QA, et al, 1990. Liver injury from cyclosporine A. Digestive Diseases and Sciences; 35(6): 693-697.
60. Christians U, Kohlhaw K, Surig T, et al., 1995. Parallel blood concentration of second generation cyclosporine metabolites and bilirubin in liver graft recipients. Therapeutic Drug Monitoring; 17: 487-498.
61. Christians U and Sewing KF, 1995. Alternative cyclosporine metabolic pathways and toxicity. Clin Biochem.; 28(6): 547-559.
62. Lorber MI, Cambar J, Wolf A, et al., 1987. Hepatobiliary and pancreatic complication of cyclosporine therapy in 466 renal transplant recipients. Transplantation; 43: 35-40.
63. Gulbis B, Adler M, Ooms H, et al., 1988. Liver function studies in heart-transplant recipients treated with cyclosporine. Clinical Chemistry; 34(9): 1772-1774.
64. Delgado P, Diaz J, Silva I, Osorio J, et al., 2008. Unmasking Glucose metabolism alterations in stable renal transplant recipients: A multicenter study. Clin J Am Soc Nephrol; 3: 808-813.
65. Boots JM, Van Duijnhoven EM, Christiaans MH, Wolffenbuttel BH, Van Hooff JP, 2002. Glucose metabolism in renal transplant recipients on tacrolimus: The effect of steroid withdrawal and tacrolimus trough level reduction. J Am Soc Nephrol; 13: 221–227.
66. Crutchlow MF and Bloom DR, 2007. Transplant-associated hyperglycemia: A new look at an old problem. Clin J Am Soc Nephrol; 2: 343–355.
67. Chadban S, 2008. New-onset diabetes after transplantation—should it be a factor in choosing an immunosuppressant regimen for kidney transplant recipients. Nephrol Dial Transplant; 23: 1816–1818.
68. Teutonico A, Schena P, Di Paolo S, 2005. Glucose metabolism in renal transplant recipients: Effect of Calcineurin Inhibitor withdrawal and conversion to sirolimus. J Am Soc Nephrol; 16: 3128–3135.
69. Burroughs T, Lentine K, Takemoto S, et al., 2007. Influence of early Post transplantation Prednisone and Calcineurin Inhibitor dosages on the incidence of New- Onset Diabetes. Clin J Am Soc Nephrol; 2: 517-523
70. Webster AC, Woodroffe RC, Taylor RS, et al, 2005. Tacrolimus versus cyclosporine as primary immunosuppression for kidney transplant recipients: meta-analysis and meta-regression of randomized trial data. BMJ; 331: 810-818.
71. Filler G, Neuschulz I, Vollmer I, et al., 2000. Tacrolimus reversibly reduces insulin secretion in paediatric renal transplant recipients. Nephrol Dial Transplant; 15: 867- 871.
72. Vincenti F, Friman S, Scheuermann E, Rostaing L, et al., 2007. Results of an international randomized trial comparing glucose metabolism disorders and outcome with cyclosporine versus tacrolimus. Am J Transplant; 7: 1506–1514.
73. Johnston O, Rose C, Webster A, Gill J, 2008. Sirolimus Is Associated with New-Onset Diabetes in Kidney Transplant Recipients. J Am Soc Nephrol; 19: 1411–1418.
74. Hornum M, Jørgensen K, Hansen J, Nielsen F, Christensen K, Mathiesen E, Feldt-Rasmussen B, 2010. New-Onset Diabetes Mellitus after kidney transplantation in Denmark. Clin J Am Soc Nephrol; 5: 709–716.
75. Van Hooff JP, Christiaans MH, van Duijnhoven EM, 2004. Evaluating mechanisms of post-transplant diabetes mellitus. Nephrol Dial Transplant; 19: vi8–vi12.
76. Hooda A, Kumar A, Varma P, 2007. Tacrolimus dose in renal transplantation – do we have an answer. Indian Journal of Nephrology; Vol. 17: Issue 3.
77. Taler SJ, Textor SC, Canzanello VJ, Schwartz L, 1999. Cyclosporine-induced hypertension: incidence, pathogenesis and management. Drug Saf; 20: 437−449.
78. Wahba IM and Bennett WM, 2007. Increased vascular resistance and not salt retention characterizes cyclosporine A-induced hypertension: report in an anuric patient. Am J Transplant; 7(8): 2042–2046.
79. Castillo-Lugo J and Vergne-Marini P, 2005. Hypertension in Kidney Transplantation. Seminars in Nephrology; 25: 252-260.
80. Catarsi P, Ravazzolo R, Emma F, Fruci D, et al., 2005. Angiotensin-converting enzyme (ACE) haplotypes and cyclosporine A (CsA) response: a model of the complex relationship between ACE quantitative trait locus and pathological phenotypes. Human Molecular Genetics; 14 (16): 2357–2367.
81. Schnuelle P, Van der heide J, Tegzess A, et al., 2002. Open randomized trial comparing early withdrawal of either Cyclosporine or Mycophenolate Mofetil in stable renal transplant recipients initially treated with a triple drug regimen. J Am Soc Nephrol; 13: 536–543.
82. Ciavarella D, Guiglia R, Campisi G, Di Cosola M, et al., 2007. Update on gingival overgrowth by cyclosporine A in renal transplants. Med Oral Patol Oral Cir Bucal; 12: E19-E25.
83. Erdmann F, Weiwad M, Kilka S, Karanik M, et al., 2010. The Novel Calcineurin Inhibitor CN585 has potent immunosuppressive properties in stimulated human T Cells. The journal of biological chemistry; 285: 1888–1898.
84. Ko HH, Greanya E, Lee TK, et al., 2008. Mycophenolate mofetil in liver transplant patients with calcineurin-inhibitor-induced renal impairment. Annals of Hepatology; 7(4): 376-380.

Global Journal of Medical research
Volume 12 Issue 1 Version 1.0 February 2012
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Association of Leptin Receptor (LEPR) Q223R Polymorphism with Breast Cancer

By C.Anuradha, P.Madanranjit, D.Surekha, D.Raghunadharao, N.Santhoshi Rani & S.Vishnupriya

Osmania University, Hyderabad

Abstract – The leptin receptor (LEPR) is a member of the cytokine receptor family with two cytokine domains and a single trans membrane domain, which plays an important role in body weight homeostasis through regulation of food intake and energy expenditure. It also influences other pathways like hematopoiesis, reproduction, angiogenesis, and immune processes upon interacting with ligand leptin. Leptin is an adipocytokine produced in adipose tissue. Since obesity is one of the known risk factor as well as the LEPR expression and leptin levels were shown to be associated with the development of mammary ductal carcinoma, an attempt has been made to evaluate the role of LEPR Q223R polymorphism with breast cancer susceptibility in south Indian population as well as with confounding epidemiological and clinical factors. The present study included 194 breast cancer cases and 186 age matched control samples for the analysis of LEPR Q223R polymorphism through PCR-RFLP method.

Keywords : *Leptin, LEPR, PCR RFLP, Breast cancer.*

GJMR-B Classification : *FOR Code: 111201, 730108, NLMC Code: QZ 20.5, QZ 275*

Strictly as per the compliance and regulations of:

Association of Leptin Receptor (LEPR) Q223R Polymorphism with Breast Cancer

C.Anuradha[α], P.Madanranjit[σ], D.Surekha[ρ], D.Raghunadharao[ω], N.Santhoshi Rani[¥] & S.Vishnupriya[§]

Abstract -The leptin receptor (LEPR) is a member of the cytokine receptor family with two cytokine domains and a single trans membrane domain, which plays an important role in body weight homeostasis through regulation of food intake and energy expenditure. It also influences other pathways like hematopoiesis, reproduction, angiogenesis, and immune processes upon interacting with ligand leptin. Leptin is an adipocytokine produced in adipose tissue. Since obesity is one of the known risk factor as well as the LEPR expression and leptin levels were shown to be associated with the development of mammary ductal carcinoma, an attempt has been made to evaluate the role of LEPR Q223R polymorphism with breast cancer susceptibility in south Indian population as well as with confounding epidemiological and clinical factors. The present study included 194 breast cancer cases and 186 age matched control samples for the analysis of LEPR Q223R polymorphism through PCR-RFLP method. The frequency of RR genotype was significantly elevated in breast cancer compared to control subjects ($\chi 2$= 6.567; df=2, p= 0.037)). Similar trend was also observed with respect to high BMI and post menopausal status. No significant association was found with stage of the disease.

Keywords : *Leptin, LEPR, PCR RFLP, Breast cancer.*

I. Introduction

The leptin receptor (LEPR) is a single transmembrane protein, belongs to class I cytokine receptor family. Due to alternative splicing during transcription of LEPR gene different isoforms of LEPR are formed with varied length of intracellular domain, but all the isoforms have identical extracellular and transmembrane domains [Lee et al., 1996; Tartagia, 1997]. Six isoforms derived from LEPR transcription have been identified so far, and a long isoform, LEPR-b, is reported to be responsible for signal transduction [Zabeau et al., 2003].

Leptin, a 16 kDa cytokine acts as a regulator of body weight and energy balance, a product of ob gene synthesized from white adipose tissue which influences food intake and energy expenditure [Campfield et al., 1995; Halaas et al., 1995]. Leptin exerts its physiological action through binding to leptin receptor (LEPR) and exerts negative feedback mechanism to control food intake and body weight [Malik et al., 1996]. However, its expression was identified in other tissues and cells, including immune cells, placenta, endometrium, stomach and lung [Caldefie-Chezet et al., 2001 Ebenbichler et al., 2002; Kitawaki et al., 2000; Breidert et al., 1999; Tsuchiya et al., 1999]. In addition, leptin receptor expression has been detected in pathological conditions such as acute myeloid leukemia, hepatocelluar carcinoma, gastric cancer cells and breast cancer [Konopleva et al., 1999; Wang et al., 2004; Mix et al., 2004; Ishikawa et al., 2004; Garofalo et al., 2006]. The binding of leptin to LEPR activates the JAK/STAT (Janus kinase signal transducer and activates of transcription), the Ras/ERK1/2 signaling cascade (a member of the MAPK family), and the PI-3K/Akt/GSK3 growth/anti-apoptotic pathways [Zabeau et al., 2003]. In addition, leptin binding to receptor has shown to transactivate HER2/neu, and also increase the expression of vascular endothelial growth factor (VEGF) [Eisenberg et al., 2004; Garofalo et al., 2006].

In humans, several single nucleotide polymorphisms (SNPs) have been described in the LEPR gene [Chung et al., 1997]. An A to G transition at nt 668 from the start codon that converts a glutamine to an arginine at codon 223 (Q223R) in the LEPR gene [Gotoda et al., 1997] alters amino acid charge from neutral to positive that could affect the functionality of the receptor and modifies its signaling capacity, which is associated with higher mean circulating levels of leptin [Quinton et al 2001., Yiannakouris et al., 2001]. This polymorphism is located within the region encoding the extracellular domain of the leptin receptor; the amino acid change affects all forms of the receptor. It has been demonstrated that individuals homozygous for the G (R223R) allele is associated with variation in ligand binding activity than the carriers of the A (Q223Q) [Quinton et al 2001]. Several studies had shown the relationship between LEPR Q223R gene polymorphism with BMI, insulin resistance, Prostate cancer, Postmenopausal breast cancer

[Snoussi et al., 2006]. It can activate ERα and ERα-dependent transcription in a ligand-independent manner [Catalano et al., 2004]. Since BMI is known to be associated with breast cancer as well as with LEPR polymorphism, the present study has been planned to evaluate the role of LEPR polymorphism in the pathogenesis of breast cancer development.

Author [ρ, ¥] : Department of Genetics, Osmania University, Hyderabad 500007, India.
Author [α, σ] : AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam530003, India.
Author [ω] : Department of Medical Oncology, Nizams Institute of Medical sciences, Hyderabad 500082, India.
Author [§] : Ph.D. Head & Professor Department of Genetics Osmania University Hyderabad 500007, Andhra Pradesh, INDIA Phone (Off) 91-40-2768-2335,91-40-2709-5178 Fax: 91-40-2709-5178
Corresponding Author : *E-mail : sattivishnupriya@gmail.com*

II. Material and Methods

a) Study population

The study comprises of 194 breast cancer patients from Nizam's institute of medical sciences (NIMS) and 186 age matched women with out any familial history of malignancies to serve as controls. Clinical information such as stage of the breast cancer, menopausal status at the time of onset, hormonal receptor status (ER, PR), Her2 status and BMI was noted down from the hospital records with the help of medical oncologist Ethical committee approval from the institute is obtained before initiation of the study. All the participants were informed about the study and consent was taken. 5ml of blood sample was collected into 10 ml EDTA vacutainer tube from both patients and controls. All the samples were stored at -20◦ C. Genomic DNA was extracted from leukocyte nuclei by the salting-out method [Lahiri et al., 1991] and used for amplification of Q223R polymorphism in exon 6 of LEPR gene.

b) LEPR Q223R Polymorphism

PCR-RFLP was carried out for the identification of LEPR Q223R polymorphism using gene specific oligonucleotide primers previously described [Gotoda et al., 1997]. The amplified product was digested with 5 units of Msp1enzyme (New England Biolabs, USA) at 37°C for overnight and electrophoresed on 3% agorose gel. A–G substitution at nucleotide 668 in exon six of LEPR gene introduces a recognition site for MspI. Digestion by MspI produces one band of 80 bp in the normal LEPR sequence (QQ). Three separate bands of 80, 58 and 22 bp in the heterozygous individual (QR), and two separate bands of 58 and 22 bp in mutant homozygotes (RR) were observed.

c) Statistical Analysis

All the statistical analyses were performed using SPSS software15.0 (Statistical Package for the Social Science). Chi square test was done to test the significance of genotype association with the occurrence of disease. Odds ratio was estimated to calculate the relative risk for each genotype to develop disease. All the P values were two sided and the level of significance was taken as $P < 0.05$.

III. Results and Discussion

LEPR a cytokine receptor involved in the homeostatic control of appetite, weight, metabolism and reproductive functions in women [Friedman et al., 1998], via activating signal transduction pathways through ligand binding with leptin [Banks et al., 2000]. LEPR spans over 70 kb and includes 20 exons which encode for 1,165 amino acid protein that belongs to the superfamily of cytokine receptors and requires all its extracellular subdomains functional for transmitting the signal. LEPR presents two basic isoforms, a short intracellular domain variant, unable to transmit a signal, which is present in a variety of tissues; and a second one with a long intracellular functional domain, capable of activating JAK2 and STAT3, and perhaps other signaling pathways [Sweeney et al., 2002].

The LEPR Q223R polymorphism corresponding to A to G transition in exon 6 of the gene is known to result in several functional consequences due to altered charge distribution from neutral to positive charge. The Q223R polymorphism lies within the first of two putative leptin-binding regions in the extracellular domain of the receptor and therefore, the amino acid change affects all isoforms of the receptor and may be associated with impaired LEPR signaling capacity [Yiannakouris et al., 2001].

The genotype and allele frequencies of the LEPR Q223R SNP in 194 breast cancer patients and 186 healthy controls are shown in Table1. The frequency of LEPR 223 RR genotype was significantly elevated in breast cancer as compared to control group ($\chi 2 = 6.567$; df=2, p= 0.037) (Table 1) LEPR 223RR genotype with more efficient binding capacity to leptin might trigger cellular JAK2 /STAT3, the Ras/ERK1/2 signaling pathways there by initiating the tumorogenesis. Leptin when binds to receptor also acts synergistically with VEGF (vascular endothelial growth factor) and fibroblast growth factor 2 (FGF- 2) to promote angiogenesis [Sierra-Honigmann et al., 1998]. It has effect on the expression of several genes involved in the angiogenesis (MMP-2 and MMP-9) [Park et al., 2009; Zhang et al., 2007].

The homozygous RR genotype frequency was elevated considerably in post menopausal patients (11.34%) compared to pre-menopausal patients (6.19%) (Table2.1). There was no significant association of LEPR Q223R polymorphism with Estrogen and Progesterone receptor status among breast cancer patients (Table2.2, 2.3), however the RR genotype frequencies were elevated in both ER-ve (8.2%) and PR-ve cases (8.97%) compared to ER+ve(6.85%) and PR+ve cases(5.88%) (Table2.2, 2.3).

With reference to BMI of patients (Table.3), a considerable elevation in the RR genotype and R allele frequency in obese patients (10.66%, 36.07%) compared to patients with lower BMI (3.45%, 27.59%) was observed. Further when non-carriers of R allele (i.e Q/Q) and carriers of R allele (i.e. Q/R+R/R) were compared, R allele carrier frequency was significantly higher (61.48%) in obese subjects when compared to less weight group (33.34%). Many reports had suggested that Q223R LEPR polymorphism was associated with BMI and overweight. Thompson et al, 1997 first reported this polymorphism was associated with obesity [Bruce Thompson et al., 1997].

When the data was stratified based on the stage of the disease and genotype frequency (Table. 5). A higher percentage of patients with stage II breast

cancer carried RR genotype (12.5%) than patients with advanced stage cancer (6.98%) (OR, 0.39; 955 CI, 0.14-1.08) although the OR was not statistically significant due to a small sample size.

The impact of Q223R polymorphic variants on human body composition had also been reported in two recent studies [Chagnon et al., 1999; Chagnon et al., 2000]. In the Que´bec Family Study, Chagnon et al., 1999 observed a significant sibling pair linkage between the Q223R polymorphism and fat mass (FM). Although no association between body composition variables and the Q223R polymorphism was observed, there was a weak, but significant, association between the Q223 allele and free fat mass (FFM) in lean males when the analysis was performed by BMI and gender groups (Chagon et al., 1999). In the Heritage Family Study cohort, stronger evidence of an association between the Q223R polymorphism and human adiposity was reported among Caucasians, although no reciprocal linkages were detected [Chagnon et al., 2000]. In particular, middle-aged Caucasian males who were carriers of the R223 allele had significantly higher BMI, %FM, and plasma leptin levels than noncarriers.

Nikos et al 2001 reported that, a higher percentage of homozygotes for R223 allele were found among the heavier subjects. R/R homozygotes had higher BMI (2±U; P=0.01) and higher fat mass values than the carriers of Q223 allele, the Q223R polymorphism was a significant predictor of 5% of the body composition variability [Yiannakouris et al., 2001]. Our study is in accordance with earlier studies, which infers that the presence of R 223 allele has a higher BMI than non carriers leading to obesity. Further obesity is a known epidemiological risk factor in the breast cancer development.

On the other hand, negative results also have been reported for the Q223R polymorphism with BMI in different Caucasian population including American [Silver et al., 1997], British [Gotoda et al., 1997] and Danish groups [Echwald et al., 1997].

However, the proximity and similarity of the Q223R polymorphism to the Q269P mutation, which causes obesity in the Zucker mouse model, raise the distinct possibility that the Q223R polymorphism may lead to subtle changes in signaling pathways that also predispose to a leptin-resistant state. Although the higher leptin levels in RR homozygotes provide supportive evidence for this hypothesis, future in vitro experiments involving expression of wild-type and mutant leptin receptors in cell lines are needed to evaluate the effect of the Q223R substitution on the functionality of the long isoform of the human LEPR [White et al., 1997; Chua et al., 1996].

Our study provides the support for the hypothesis that the Q223R polymorphism of LEPR gene is associated with obesity and BMI in accordance with earlier reports.

IV. Conclusion

In conclusion, our results suggest that the RR genotype of the LEPR Q223R polymorphism of the leptin receptor gene might be considered as a risk genotype for development of breast cancer. Thus, this study has demonstrated a modestly increased risk of breast cancer in obese women harboring the LEPR 223R allele of the LEPR Q223R polymorphism of the leptin receptor gene. To the best of our knowledge, ours is the first study to provide information on the role of LEPR Q223R polymorphism in breast cancer risk in Indian women, a population characterized by paucity of epidemiological literature on the determinants of breast cancer and other malignancies.

V. Declaration of Interest

No conflicts of Interest

VI. Acknowledgement

This work was financially supported by DBT ISLARE, New Delhi, India.

References Références Referencias

1. Banks AS, Davis SM, Bates SH, Myers MG Jr (2000). Activation of Downstream Signals by the Long Form of the Leptin Receptor. J Biol Chem 275:14563-72.
2. Breidert M, Miehlke S, Glasow A, et al (1999). Leptin and its receptor in normal human gastric mucosa and in Helicobacter pylori associated gastritis. Scand J Gastroenterol 34: 954-61.
3. Bruce Thompson D, Eric Ravussin, Peter H. Bennett and Clifton Bogardus(1997). Structure and sequence variation at the human leptin receptor gene in lean and obese Pima Indians, Human Molecular Genetics 6(5): 675–79.
4. Caldefie-Chezet F, Poulin A, Tridon A, Sion B and Vasson MP (2001). Leptin: a potential regulator of polymorphonuclear neutrophil bactericidal action? J Leukoc Biol 69: 414-18.
5. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P (1995). Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 269: 546- 49.
6. Catalano S, Mauro L, Marsico S, Giordano C, Rizza P, Rago V,Montanaro D, Maggiolini M, Panno ML, Ando S(2004). Leptin induces, via ERK1/ERK2 signal, functional activation of estrogen receptor alpha in MCF-7 cells. J Biol Chem 279:19908-15 Chagnon. Y. C, WK Chung, L Pearusse1, M Chagnon1, RL Leibel and C Bouchard(1999). Linkages and associations between the leptin receptor (LEPR) gene and human body composition in the Quebec Family Study. International Journal of Obesity 23: 278-86 Chagnon. Y. C, jack H. Wilmore,

lingrid B. Borecki, Jacques Gagnon, Louis pe´ Russe, M Chagnon,et al(2000). Associations between the Leptin Receptor Gene and Adiposity in Middle-Aged Caucasian Males from the Heritage Family Study. The Journal of Clinical Endocrinology & Metabolism 85(1).
7. Chua SC, White DW, Wu-Peng XS, et al (1996). Phenotype of fatty due to Gln269Pro mutation in the leptin receptor (Lepr). Diabetes 45:1141–43.
8. Chung WK, Power-Kehoe L, Chua M, Chu F, Aronne L, Huma Z, et al(1997). Exonic and intronic sequence variation in the human leptin receptor gene (LEPR). Diabetes 46:1509-11.
9. Ebenbichler CF, Kaser S, Laimer M, Wolf HJ, Patsch JR and Illsley NP (2002). Polar expression and phosphorylation of human leptin receptor isoforms in paired, syncytial, microvillous and basal membranes from human term placenta. Placenta 23: 516-21.
10. Echwald SM, Sorensen TD, Sorensen TIA, et al (1997). Amino acid variants in the human leptin receptor: lack of association to juvenile onset of obesity. Biochem Biophys Res Commun 233:248 – 252
11. Eisenberg A, Biener E, Charlier M, Krishnan RV, Djiane J, Herman B, Gertler A(2004). Transactivation of erbB2 by short and long isoforms of leptin receptors. FEBS Lett. 565:139-42.
12. Friedman JM, Halaas JL(1998). Leptin and the regulation of body weight in mammals. Nature 395:763-70.
13. Garofalo C, Koda M, Cascio S, et al (2006). Increased expression of leptin and the leptin receptor as a marker of breast cancer progression: possible role of obesity-related stimuli. Clin Cancer Res 12: 1447-53.
14. Garofalo C, Surmacz E(2006). Leptin and cancer. J Cell Physiol 207:12-22.
15. Gotoda T, Manning BS, Goldstone AP, et al (1997). Leptin receptor gene variation and obesity: lack of association in a white British male population. Hum Mol Genet 6:869–76
16. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, et al(1995). Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269:543-46.
17. Ishikawa M, Kitayama J and Nagawa H (2004). Enhanced expression of leptin and leptin receptor (OB-R) in human breast cancer. Clin Cancer Res 10: 4325-31.
18. Kitawaki J, Koshiba H, Ishihara H, Kusuki I, Tsukamoto K and Honjo H(2000). Expression of leptin receptor in human endometrium and fluctuation during the menstrual cycle. J Clin Endocrinol Metab 85: 1946-50.
19. Konopleva M, Mikhail A, Estrov Z, et al (1999). Expression and function of leptin receptor isoforms in myeloid leukemia and myelodysplastic syndromes: proliferative and anti-apoptotic activities. Blood 93: 1668-76.
20. Lahiri DK, Nurnberger JI (1991). A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Research 19(19): 5444.
21. Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI, et al(1996). Abnormal splicing of the leptin receptor in diabetic mice. Nature 379: 632-35.
22. Malik KF, Young WS 3rd(1996). Localisation of binding sites in the central nervous system for leptin (OB protein) in normal, obese (ob/ob), and diabetic (db/db) C57BL/6J mice. Endocrinology 137:1497-500.
23. Mix H, Widjaja A, Jandl O, et al (2000). Expression of leptin and leptin receptor isoforms in the human stomach. Gut 47: 481-86.
24. Park KS, Shin HD, Park BL, Cheong HS, Cho YM, Lee HK, Lee JY, Lee JK, Oh B, Kimm K (2009). Polymorphisms in the leptin receptor (LEPR)-putative association with obesity and T2DM. J Hum Genet 51:85-91.
25. Quinton ND, Lee AJ, Ross RJ, Eastell R, Blakemore AI (2001). A single nucleotide polymorphism (SNP) in the leptin receptor is associated with BMI, fat mass and leptin levels in postmenopausal Caucasian women. Hum Genet 108:233-23.
26. Sierra-Honigmann MR, Nath AK, Murakami C, García-Cardeña G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR(1998). Biological action of leptin as an angiogenic factor. Science 281:1683-86.
27. Silver K, Walston J, Chung WK, et al (1997). The Gln223Arg and Lys656Asn polymorphisms in the human leptin receptor do not associate with traits related to obesity. Diabetes 46:1898 –900.
28. Snoussi K, Strosberg AD, Bouaouina N, Ben Ahmed S, Helal AN, Chouchane L(2006). Leptin and leptin receptor polymorphisms are associated with increased risk and poor prognosis of breast carcinoma. BMC Cancer 6:38.
29. Sweeney G (2002). Leptin signalling. Cell Signal 14: 655-63.
30. Tartaglia LA (1997).The leptin receptor. J Biol Chem 272: 6093-96.
31. Tsuchiya T, Shimizu H, Horie T and Mori M(1999). Expression of leptin receptor in lung: leptin as a growth factor. Eur J Pharmacol 365: 273-79.
32. Wang XJ, Yuan SL, Lu Q, Lu YR, Zhang J, Liu Y and Wang WD (2004). Potential involvement of leptin in carcinogenesis of hepatocellular carcinoma. WorldJ Gastroenterol 10: 2478-81.
33. White DW, Kuropatwinski KK, Devos R, Baumann H, Tartaglia LA (1997). Leptin receptor (OB-R)

signaling. Cytoplasmic domain mutational analysis and evidence for receptor homo-oligomerization. J Biol Chem 272(7):4065–71.
34. Yiannakouris N, Yannakoulia M, Melistas L, Chan JL, Klimis-Zacas D, Mantzoros CS (2001). The Q223R polymorphism of the leptin receptor gene is significantly associated with obesity and predicts a small percentage of body weight and body composition variability. J Clin Endocrinol Metab 86:4434-39.
35. Zabeau L, Lavens D, Peelman F, Eyckerman S, Vandekerckhove J, Tavernier J(2003). The ins and outs of leptin receptor activation. FEBS Lett 546(1):45-50.
36. Zhang YY, Gottardo L, Mlynarski W, Frazier W, Nolan D, Duffy J, Marescotti MC, Gervino EV, Johnstone MT, Mantzoros CS, Avogaro A, Doria A(2007). Genetic variability at the leptin receptor (LEPR) locus is a determinant of plasma fibrinogen and C-reactive protein levels. Atherosclerosis 191:121-27.

Table 1 : Genotype distribution of LEPR Q223R polymorphism in Breast Cancer cases and Controls

	Cases	Controls	OR(95% CI)	P-value	Z statistics	χ P-value
All Women	(n =194)	(n =186)				
Allele frequencies						
LEPR (Q)	262(67.52)	246(66.12)	1.0			
LEPR (R)	126(32.47)	126(33.87)	0.94(0.69-1.27)	0.68	0.41	0.110 df= 1 0.74
Genotype frequencies						
LEPR (Q/Q)	85(43.8)	68(36.6)	1.0			
LEPR (Q/R)	92(47.4)	110(59.1)	0.7 (0.44-1.02)	0.0622	1.865	
LEPR(R/R)	17(8.8)	8(4.3)	1.70(0.69-4.18)	0.2472	1.157	6.567* df=2 0.0375*
LEPR (Q/Q)	85(43.8)	68(36.6)	1.0			
LEPR (Q/R) + (R/R)	109(56.2)	118(63.4)	0.74(0.49-1.12)	0.1498	1.440	1.788 df=1 0.18

* $\chi 2$ value significant

Table 2 : Association of LEPR Q223R polymorphism with respect to breast cancer and Menopausal status, Estrogen receptor and Progesterone receptor status at disease onset.

			OR(95% CI)	P-value	Z statistics	$\chi 2$	P-value
1. Menopausal Status	Pre	Post					
(N=194)	(n =97)	(n =97)					
Allele frequencies							
LEPR (Q)	135(69.59)	127(65.46)	1.0				
LEPR (R)	59(30.41)	67(34.54)	0.83(0.54-1.27)	0.39	0.87	0.58	
df=1 0.45							
Genotype frequencies							
LEPR (Q/Q)	44(45.36)	41(42.27)	1.0				
LEPR (Q/R)	47(48.45)	45(46.39)	0.97(0.54-1.76)	0.93	0.09		
LEPR(R/R)	6 (6.19)	11(11.34)	0.51(0.17-1.50)	0.22	1.23	1.620	
df=2 0.45							
LEPR (Q/Q)	44(45.36)	41(42.27)	1.0				
LEPR (Q/R) + (R/R)	53(54.63)	56(57.73)	0.88 (0.5-1.56)	0.66	0.43	0.08	
df=1 0.77							
2. Estrogen status	+ve	-ve					
(N=146)	(n =73)	(n =73)					
Allele frequencies							
LEPR (Q)	100(68.49)	100(68.49)	1.0				
LEPR (R)	46(31.51)	46(31.51)	1.0(0.61-1.64)	1.00	0.00	0.02	
df=1 0.89							

Genotype frequencies						
LEPR (Q/Q)	32(43.84)	33(45.2)	1.0			
LEPR (Q/R)	36(49.3)	34(46.6)	1.10(0.56-2.15)	0.80	0.26	
LEPR(R/R)	5(6.85)	6(8.2)	0.86(0.24-3.10)	0.82	0.23	0.163
df=2 0.92						
LEPR (Q/Q)	32(43.84)	33(45.2)	1.0			
LEPR (Q/R) + (R/R)	41(56.16)	40(54.79)	1.06(0.55-2.03)	0.87	0.17	0.00
df=1 1.0						
3. Progesterone status	+ve	-ve				
(N=146)	(n =68)	(n =78)				
Allele frequencies						
LEPR (Q)	91(66.91)	109(69.87)	1.0			
LEPR (R)	45(33.09)	47(30.13)	1.15(0.7-1.9)	0.59	0.54	0.174
df=1 0.677						
Genotype frequencies						
LEPR (Q/Q)	27(39.7)	38(48.72)	1.0			
LEPR (Q/R)	37(54.4)	33(42.31)	1.58(0.80-3.12)	0.19	1.31	
LEPR(R/R)	4(5.88)	7(8.97)	0.80(0.21-3.0)	0.75	0.32	2.234
df=2 0.327						
LEPR (Q/Q)	27(39.7)	38(48.72)	1.0			
LEPR (Q/R) + (R/R)	41(60.2)	40(51.2)	1.44(0.75-2.79)	0.28	1.09	0.858
df=1 0.354						

Table 3 : Genotype distribution of LEPR Q223R polymorphism in Breast Cancer cases with BMI

			OR(95% CI)	P-value	Z statistics	$\chi 2$	P-value
BMI	**Less weight**	**Obese**					
(N=151)	(n = 29)	(n = 122)					
Allele frequencies							
LEPR (Q)	42(72.41)	156(63.93)	1.0				
LEPR (R)	16(27.59)	88(36.07)	0.68 (0.36-1.27)	0.224	1.22	1.140	
df=1 0.29							
Genotype frequencies							
LEPR (Q/Q)	14(48.28)	47(38.52)	1.0				
LEPR (Q/R)	14(48.28)	62(50.82)	0.76(0.33-1.74)	0.51	0.65		
LEPR(R/R)	1(3.45)	13(10.66)	0.26(0.031-2.15)	0.21	1.25	1.894	
df=2 3.88							
LEPR (Q/Q)	14(48.28)	47(38.52)	1.0				
LEPR (Q/R) + (R/R)	15(51.72)	75(61.48)	0.67 (0.3-1.52)	0.34	0.96	0.565	
df=1 0.45							

Table 4 : Genotype distribution of LEPR Q223R polymorphism in Breast Cancer cases with Stage of the disease

				χ^2	P-value
Stage of the Disease	**I**	**II**	**III &IV**		
Allele frequencies					
LEPR (Q)	12(75)	103(64.38)	118(68.60)		
LEPR (R)	4(25)	57(35.62)	54(31.40)		1.161
df=1 0.56					
Genotype frequencies					
LEPR (Q/Q)	5(62.5)	33(41.25)	38(44.19)		
LEPR (Q/R)	2(25.0)	37(46.25)	42(48.84)		3.016
df=4 0.56					
LEPR(R/R)	1(12.5)	10(12.5)	6(6.98)		
LEPR (Q/Q)	5(62.5)	33(41.25)	38(44.19)		
LEPR (Q/R) + (R/R)	3(37.5)	47(58.75)	48(55.81)		1.353
df=2 0.51					

Table 5 : Association of LEPR Q223R polymorphism and breast cancer risk by breast cancer stage

Genotype	Controls(n)	Stage I(n)	OR(95% CI)	P	Stage II(n)	OR(95% CI)	P	StageIII& IV(n)	OR(95% CI)	P
LEPR (Q/Q)	68(36.6)	5(62.5)	1.0		33(41.25)	1.0	38(44.19)	1.0		
LEPR (Q/R)	110(59.1)	2(25)	4.04(0.8-21.43)	0.10	37(46.25)	1.44 (0.83-2.52)	0.2	42(48.84)	1.46(0.92-2.49)	0.16
LEPR(R/R)	8(4.3)	1(12.5)	0.59(0.06-5.69)	0.65	10(12.5)	0.39(0.14-1.08)	0.07	6(6.98)	0.75(0.24-2.31)	0.61
LEPR (Q/Q)	68(36.6)	5(62.5)	1.0			33(41.25)	1.0 1.0	38(44.19)		
LEPR (Q/R) + (R/R)	118(63.4)	3(37.5)	2.89(0.67-12.48)	0.15	47(58.75)	1.22(0.71-2.08)	0.47	48(55.81)	1.37(0.82-2.31)	0.23

This page is intentionally left blank

Global Journal of Medical research
Volume 12 Issue 1 Version 1.0 February 2012
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

Histopathological and Toxicological effects of crude saponin extract from Phyllanthus niruri, L (Syn. P. franternus. Webster) on Organs in animal studies

By Ajibade V. A., Famurewa, O
The Federal Polytechnic, P.M.B 5351, Ado-Ekiti, Nigeria

Abstract – The histopathological view of liver, intestines and kidney of bacterial infected rabbits, fed with 100mg/ml saponin extracted from Phyllanthus niruri over a period of seven days was carried out to determine the effect of the plant extract on these organs after treatment. Saponin was administered as strawberry suspension at a dose of 10mg per day (divided into four doses) to ten rabbits, nine of which were fed with food contaminated with 0.5mL bacterial suspension obtained by McFarland standardization (10% Barium sulfate) after starvation for 6hrs . Multiple foci of tubular necrosis and haemorrhages in the kidney, marked hyperplasia of the mucosal layer of the small intestine, and a mild periportal lymphocytic cellular infiltration of the liver of the treated rabbits were observed. Plasma urea, uric acid, creatinine and blood glucose levels increased significantly ($p < 0.05$) in the treated rabbits.

Keywords : *Histopathological, Phyllanthus niruri, Saponin, Toxicological.*

GJMR-B Classification : *NLMC Code: WX 207, QV 290*

Strictly as per the compliance and regulations of:

Histopathological and Toxicological effects of crude saponin extract from *Phyllanthus niruri*, L (Syn. *P. franternus*. Webster) on Organs in animal studies

Ajibade V. A.[α], Famurewa, O[σ]

Abstract – The histopathological view of liver, intestines and kidney of bacterial infected rabbits, fed with 100mg/ml saponin extracted from *Phyllanthus niruri* over a period of seven days was carried out to determine the effect of the plant extract on these organs after treatment. Saponin was administered as strawberry suspension at a dose of 10mg per day (divided into four doses) to ten rabbits, nine of which were fed with food contaminated with 0.5mL bacterial suspension obtained by McFarland standardization (10% Barium sulfate) after starvation for 6hrs . Multiple foci of tubular necrosis and haemorrhages in the kidney, marked hyperplasia of the mucosal layer of the small intestine, and a mild periportal lymphocytic cellular infiltration of the liver of the treated rabbits were observed. Plasma urea, uric acid, creatinine and blood glucose levels increased significantly ($p < 0.05$) in the treated rabbits. Plasma protein, hemoglobin, red blood cell and leukocyte counts were not altered adversely. No significant changes were observed in the enzymes' activity in all the groups of rabbits tested. The extract seems to show therapeutic actions on infections caused by *E. coli* and *Salmonella typhi* without any adverse effect on the organs.

Keywords : Histopathological, Phyllanthus niruri, Saponin, Toxicological

I. Introduction

Before the advent of modern medicine which witnessed synthetic production of many drugs including antimicrobial agents, extract of plants were known to elicit certain reactions in human body when applied in a prescribed manner. Among such plant is *Phyllanthus niruri* L., (Syn. *P. fraternus*.Webster). It belongs to the *Euphorbiaceae* family and has been claimed to be an excellent remedy for jaundice and hepatitis (Qudhia and Tripathi, 2002; Tabasum *et al.*, 2005). Based on its long documented history of uses in the Amazonian region, the plant is believed to be helpful in treating oedema, anorexia and diabetes (George and Roger, 2002; Khanna *et al.* , 2002.). The bark yields a bitter principle, phyllanthin, while the infusion of the root and leaves is a good tonic and diuretic when taken cold in repeated doses (Unander, 1990). Many of the active constituents found in the plant are biologically active lignands, glycosides, flavonoids, saponins, alkaloids, ellagitannins and phenylpropanoids (Dhir *et al.*, 2002 ; Tabasum *et al.,* 2005).), common lipids sterols and flavonoids also occur in the plant (Barros *et al.*, 2003).

Saponins are glycosides with a distinctive foaming characteristic. They are found in various parts of the plant leaves, stems, roots, bulbs, blossom, and fruit. The name originated from soapwort plant (saponaria), the root of which was used historically as a soap. Saponins are believed to be useful in the human diet for controlling cholesterol, but some including those produced by the soapberry are poisonous if swallowed and can cause urticaria (skin rash) in many people (Otsuka, 2005). Digistalis type of saponin strengthens heart muscle contractions, causing the heart pump to work more efficiently (Desert, 2007). They inhibit some kind of cancer cell tumor growth in animals particularly in the lungs and blood cancers, without killing normal cells (Unander, 1990; Ray, 2007). These effects point to the potentials of saponin, including those present in the diet, as a remedy against two of the major health hazards in many countries, namely obesity and cancer (Otsuka, 2005). Saponin from *P. niruri* has been observed, within the range of standard antibiotics like Chloramphenicol and Gentimycin used as control; showed high potency *on E. coli* and *Salmonella typhi* (Ajibade and Famurewa, 2010). Histopathological studies evaluate the conditions of organs of the body after the use of some therapeutic agents (Ambi *et al.,* 2007). The study estimates the toxic stage and damages that could come from the use of these agents. This study is designed to determine the toxicological and histopthatological effect of crude saponin extract from *Phyllanthus niruri* on some organs excised from bacterial infected rabbits.

Author α : The Microbiology Unit, Department of Science Technology, Federal Polytechnic, P. M. B 5351, Ado-Ekiti, Ekiti-State, Nigeria. Tel: +2348035781628 E-mail : ajibvijay@yahoo.com, (corresponding author) .

Author σ : Department of Microbiology, Ekiti State University, P.M.B 5363, Ado- Ekiti, 360101, Nigeria. College of Science, Engineering and Technology, Osun- State University P.M.B4494, Oke- Baale, Oshogbo 230212, Nigeria.

II. Materials and Methods

a) Collection of Plant Material

Phyllanthus niruri was collected from shrubs around the Federal Polytechnic compound, Ado- Ekiti, Nigeria between the months of July and September, 2008 and identified at the Department of Plant Science, University of Ado-Ekiti, Ekiti- State, Nigeria. A voucher specimen (STD/MIC/PLT.0982) was deposited at the herbarium of the Department of Science Technology, Federal Polytechnic, and Department of Plant Science, University of Ado-Ekiti.

b) Extraction of crude saponin

The sample used for the analysis was air-dried at room temperature of $\pm 28^0$C and pulverized. The saponin was extracted according to the method described by Otsuka *et al.* (2005). The milled plant (170g) was defatted using 700ml of Petroleum ether for 72h with the aid of Soxhlet. Seven hundred (700ml) milliliter of methanol was used to extract saponin from defatted sample and the residue was left overnight under reflux at 70°C. It was then filtered and the filtrate evaporated to dryness. The yield was dissolved in 300ml distilled water-butanol (1:1 v/v) in a separating funnel. The set up was left for three days after which two layers were formed. The upper layer was precipitated with di-ethyl ether to obtain 20mg of crude saponin; this was poured into an evaporating dish and dried by evaporation for 2 weeks at room temperature.

c) Experimental animals

Rabbits of both sexes were maintained under standard environmental conditions at room temperature of (± 28 - ± 1°C) in the animal house of the Department of Science Technology, Federal Polytechnic, Ado-Ekiti. The rabbits had free access to feed and water. Prior to the experiment, rabbits were fed with standard feed for 1 week in order to adapt to the laboratory conditions. Seven days after acclimatization, the rabbits were divided into five groups of six rabbits each ($n \geq 6$/group): the groups of rabbits were treated as follows: one negative control group, (water; 5ml/kg body weight); one positive control group (200mg amoxicillin; 5ml/kg body weight,) and three saponin treated groups (25-400mg saponin; 5ml/kg body weight). Prior to test, on day 1, the rabbits were fasted for 6hr, but allowed free access to water.

d) Effect and toxicity of crude saponin on rabbits infected with Escherichia coli and Salmonella typhi.

The toxicity effect was studied using the methods of Anupama *et al.,* (2011). Saponin was administered as strawberry suspension at a dose of 10mg per day (divided into four doses) to ten rabbits, nine of which were fed with feed contaminated with 0.5mL bacterial suspension of *Salmonella typhi* and *Escherichia coli* obtained by McFarland standardization (10% Barium sulfate) containing 10^3 (forming units/ml) cfu/ml after starvation for 6hrs .Toxicity studies were done on white blood, diff count, urine and haemoglobin analysis, blood urea nitrogen (BUN), creatinine, serum alanine transaminase (ALT) and aspartate transaminase (AST). It was performed before administration of saponin, on the third day of therapy, and at 9^{th} day of therapy. The levels of intact saponin were determined in specimen of urine and blood collected from the rabbits by spectrofluorometric analysis described by Schwartz *et al.* (1999).

e) Behavioural and toxic effects

The acute oral toxicity study was evaluated in the rabbits according to the standard methods of Litchfield and Wilcoxon (1949) described in Adesokan and Akanji (2004) and Aziza *et al,* (2008). Four groups of five rabbits were administered with 25, 50, 100, 200 and 400mg/kg of the saponin extract orally, while one group with the same number of rabbits served as control. The animals were observed continuously for 1hr for any gross behavioral changes, symptoms of toxicity and mortality if any, and intermittently for 6hr and 24hr after dosing with saponin extract. After 24hr, animals were sacrificed following chloroform anesthesia. Blood was collected by heart puncture. Blood samples were collected from each animal and allowed to clot for 45min at room temperature. Serum was separated by centrifugation at 600rpm for 15min and analyzed for various biochemical parameters including serum alanine transaminase (ALT) and serum aspartate transaminase (AST) (Ahmed *et al.,* 2003).

f) Histopathological examination

Experimental rabbits were dissected on the 9th day after administration of saponin. The method describe by Patel *et al.* (2010) was employed for the dissecting. They were killed by chloroform anesthesia and dissected. The small and large intestine, liver and kidneys were removed separately and cut into sections, The sections were fixed directly on a slide, stained with haematoxylin and eosin, examined and photographed.

g) Statistical analysis

The data were expressed as mean $\pm$ S.D., while biochemical and physiological parameters were analyzed statistically using one way ANOVA followed by Dunnet-+-test using the Statistical Package for Social Sciences for comparison with control group and saponin treated group. $P < 0.05$ was considered as significant while $P < 0.01$ and $P < 0.001$ were considered as insignificant.

III. Results and Discussion

The mean blood parameters of groups of six rabbits each treated with saponin is shown in Table 1. The table depicts the effect of saponin on blood parameters. Urea, uric acid, creatinine and blood

glucose levels were significantly ($p < 0.05$) increase in group V rabbits when compared with group 1. Plasma protein, haemoglobin, red blood cell (RBC) and leukocyte counts were not significantly different in all the groups.

The percentage of saponin in urine 72hr after oral administration is shown in Table 2. The mean percentage of the saponin in the urine of the rabbits is 52.36μg/ml

The urine analysis of rabbits treated with saponin for a period of 9days is shown in Table 3. The urine area, uric acid and creatinine levels decreased significantly ($p < 0.05$) in group V animals. Urinary protein and alkaline phosphate activity were not significantly different.

The effect of saponin on the activity of serum, liver and kidney enzymes in controlled and experimental groups of rabbits is shown in Table 4 indicating the activity of marker enzymes (AST and ALT). Slight differences were observed in the activity of enzymes in all the groups of rabbits tested.

Table 1 : Blood parameters of rabbits treated with saponin at dose of 100mg/day for a period of 30 days

Parameters	Groups				
	I	II	III	IV	V
Urea(mg/dl)	16.82±2.45	17.53±3.84	16.59±3.51	18.53±2.92	22.29±2.31**
Uric acid(mg/dl)	7.53±1.67	7.42±0.85	7.22±1.56	8.76±1.56	10.42±1.90*
Creatinine(mg/dl)	1.32±0.41	1.26±0.49	1.35±0.38	1.65±0.38	2.08±0.46**
Protein (g/dl)	8.62±2.25	9.48±1.92	9.52±2.01	8.34±2.68	9.06±2.87
Blood glucose (mg/dl)	40.12±5.59	40.86±5.25	41.60±5.21	47.57±4.00**	52.50±4.25***
Hb (g/dl)	12.27±3.36	11.58±2.96	12.87±3.55	11.91±1.81	11.08±1.91
RBC X 10^6 mm^3	2.45±0.15	2.57±0.09	2.53±0.08	2.35±0.16	2.60±0.26
WBC	4410±182	4429±179	4317±191	4215±186	4388±183

Values are expressed as mean ± SD for six rabbits
Comparisons were between groups I(control) with II, III, IV and group V
*X $p < 0.05$, **XX $p < 0.01$,***XXX $p < 0.001$

Table 2 : Percentage of saponin in urine (0-72hr) after oral administration (100mg/day)

Rabbit Groups No	(% Mean (μg/ml) of the groups)
1	63.8
2	63.5
3	59.7
4	29.0
5	57.9
6	40.3
TOTAL MEAN	52.36
Standard Error (SE)	4.8

Table 3 : Urine analysis of rabbits treated with saponin for a period of 9 days

Parameters	Groups				
	I	II	III	IV	V
Urea	3.18±0.43	3.06±0.51	3.51±0.45	2.99±0.31	2.56±0.47**
Uric acid	0.73±0.12	0.78±0.24	0.69±0.17	0.61±0.13	0.46±0.04*
Creatinine	0.81±0.27	0.87±0.35	0.83±0.31	0.62±0.21	0.52±0.19**
Protein	5.02±1.85	5.56±1.4	6.06±1.22	6.18±1.55	5.47±0.97
Alkaline phosphate	0.45±0.08	0.43±0.07	0.45±0.07	0.52±0.06	0.54±0.12

Values are expressed as mean ± SD for 6 rabbits
Comparisons were made between groups I (control) with II, III, IV and group V
* p< 0.05, ** p< 0.01 ,*** p< 0.001

Table 4 : Effect of saponin on serum, liver and kidney enzymes activity in controlled and experimental groups

Parameters	Groups				
	I	II	III	IV	V
Serum (units/ml)					
AST	25.62±5.2	27.14±5.5	26.60±4.98	22.6±4.81	24.95±2.82***
ALT	36.25±3.5	39.41±6.04	34.16±5.62	34.16±5.62	34.94±4.94***
Liver (units/mg protein)					
AST	173.3±18.01	174.6±12.5	179.7±16.2	164.12±14.6	169.12±16.3***
ALT	37.02±5.31	37.34±5.20	39.89±3.80	39.91±6.20	41.80±5.25**
Kidney (units/mg protein)					
AST	29.10±5.10	26.60±3.51	33.25±5.05	26.43±5.08	24.6±4.3***
ALT	26.82±3.27	25.32±2.61	29.44±3.50	24.71±2.96	24.33±4.6***

Values are expressed as mean ± SD for six rats Comparisons were between groups I with II, III, IV and group V

*p<0.05, **p<0.01, ***p<0.001

a) Results of histopathological examination

The result of the histopathological studies of the liver, kidney and small intestine of treated and untreated rabbits is shown in figs 1, 2 and 3 respectively. The liver, small intestine and kidney of the untreated rabbits showed no visible lesion, but there were sectioning artifacts (figs 1(a), 2(a) and 3 (a)). In the treated liver, there was a mild periportal lymphocytic and histiocytic cellular infiltration (Fig 1b). In the kidney, there are multiple foci of haemorrhages into the intertitium. There were few loci of tubular necrosis and presence of hyaline casts with interstitial cellular infiltration by macrophages (fig 2b), and small intestine of the treated rabbits there were marked hyperplasias of the mucosal layer (Fig 3b).

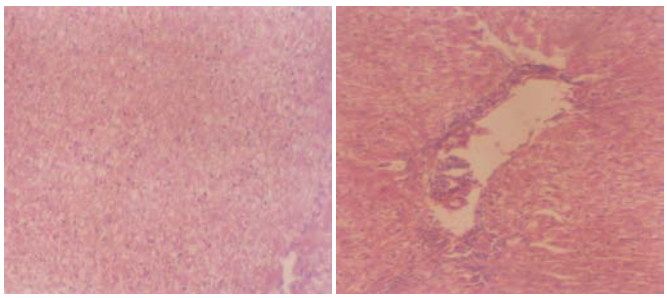
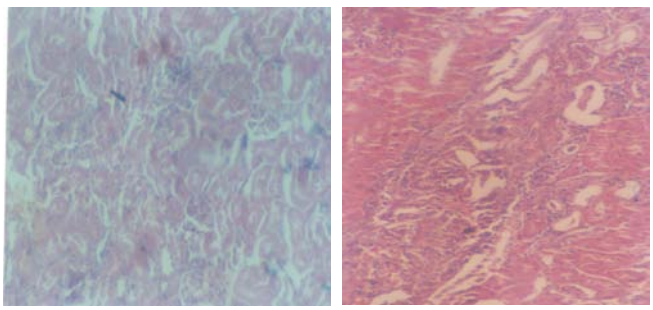


Fig. 1 : Liver of untreated (a) and saponin treated (b) rabbits.



a b

Fig. 2 : Kidney of untreated (a) and saponin treated (b) rabbits

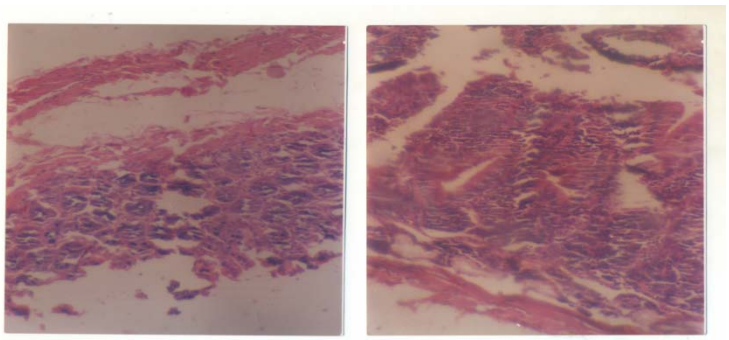


Fig. 3 : Small intestine of untreated (a) and saponin treated (b) rabbit

IV. Discussion

Tolerance and toxicity studies of the treated rabbits included analyzing levels of crude saponin in blood between 1 and 24hr, investigating blood parameters e.g., urea, uric acid, creatinine, protein, glucose, white blood differential, haemoglobin, urinalysis, alanine transaminase (ALT), aspartate transaminase (AST) in serum, liver and kidney before and after administration of saponin. None of the experimental rabbits exhibited microbiologically active or chemically detectable saponin in the serum or urine before therapy. The mean level of crude saponin in the blood after administration to the rabbits reduced between 1h and 24h. This observation substantiates the constancy of absorption, distribution, metabolism and excretion of the saponin. Ingested saponins are exposed to many potential lignands in the intestine such as bile salts, dietary cholesterol and membrane sterols of the mucosal cells, and nutrients or antinutrients in body of ruminants (Flaoyen *et al.* 2001; Meagher *et al.*, 2001) and human subjects (Lee *et al.,* 2000) has however been demonstrated. Absorption was further

substantiated in the mean percentage of detectable saponin in urine at 72h after administration. The percentage of saponin reduced significantly.

The analysis of the blood parameters of the experimental rabbits treated with saponin showed that there was a significant increase in the values of urea, uric acid, creatinine, plasma protein and blood glucose with significant decrease in urea, uric acid and creatinine levels in urine. This may explain the use of *P. niruri* saponin to remove uric acid from urine (Nishiura *et al*., 2005). Plasma protein, hemoglobin, red blood cell and leukocyte counts were not significantly different. This findings correlates with that of Lee *et al*. (2000) and Yoshikawa *et al*.(2001) where it was reported that the use of *P. niruri* do not affect the blood cells adversely.. The saponin was also found to significantly and dose-dependently inhibit gastric emptying. This observation was earlier reported by Oda *et al*. (2000), Shim *et al*. (2000), and Zhongguo *et al*. (2005) who opined that the inhibitory activity of saponin on gastric emptying was dependent on the level of serum glucose and mediated at least in part by the capsaicin-sensitive sensory nerves and the central nervous system.

It has been reported that renal dysfunction may be the cause of raised plasma, urea, uric acid and creatinine level accompanied by lowered urine urea, uric acid and creatinine level in high dose of drug treated rabbits (Adesokan and Akanji, 2004). Raised urea and non-protein nitrogen level in blood have been observed with impaired renal function or in acute renal failure (Adebayo *et al.,* 2003). In the present study, the observed differences in the urinary contents are not significant. This difference may be due to the concentration of saponin used in the treatment. Zhongguo *et al*. (2005) found that concentration-dependent response was noticed when Quallaja saponin was used to treat *E. coli* K-12- infected wistar rats and that saponin from various sources differ in their biological activity. The initial increase observed in the blood glucose level was suspected to be due to the high percentage of sugar moiety that makes the chemical structure of saponins (Francis *et al*., 2002). There was however a gradual reduction of the blood glucose to an insignificant level ($p < 0.001$) after 9 days. This could be due to constancy of distribution, metabolism and excretion of the saponin (Zhongguo *et al*., 2005). The presences of transaminase (AST and ALT) are good indices of liver and kidney damage (Nishiura *et al*., 2005). In this study, saponin did not induce any damage to any of the organs which could be inferred from the normal values of these enzymes. Reduction in the level of AST and ALT is an indication of the stabilization of plasma membranes as well as repair of hepatic tissue damage. This in effect conforms with the commonly accepted view reported earlier by Francis *et al.* (2002) that serum levels of transaminase return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes. It thus, means that in this study, the saponin therapy did not lead to liver inflammation and/or kidney dysfunction.

The appearance of mild periportal lymphocytic and histiocytic cellular infiltration in the liver of the saponin-treated rabbits is an indication of a cellular immunological response brought about by infiltration of polymorphornuclear leucocytes to the site of infection induced by *Salmonella typhi* (Pooneh *et al.,* 2010).

The presence of hyaline cast in the kidney is normal and has been ascribed to the use of medicines (Medline Plus Medical Encyclopaedia). The appearance of a few loci tubular necrosis in the kidney has been observed to be a reflection of the initial pathogenesis of the infection; indicating damage to the renal tubular epithelial cells. This condition is normal and not caused by the saponin therapy but a clinical manifestation of the disease. The description of renal tubular necrosis as one of the pathogenesis of clinical manifestation of typhoid fever has been made (Nishiura *et al*., 2005) and this substantiates the observations made in this study. The binding of saponins to bile acids in the intestine could reduce the availability of bile acids to the microbial population, thus reducing the formation of carcinogenic substances in the colon (Nishiura *et al*., 2005) that may lead to necrosis.

V. Conclusion

With the information available and the observation recorded in this study, the extract seems not to show any adverse effect on the organs despite its positive therapeutic actions on infections caused by *E. coli* and *Salmonella typhi*.

References Références Referencias

1. Adebayo, J. O., Yakubu, M. T., Egwin, E. C., Owoyele, B. V and Enaibe, B. U (2003). The effect of extract of Khaya senegalensis on some biochemical parameters of rat kidney J. Ethnopharmacol 88:69-72.
2. Adesokan, A.A and Akanji, M.A. (2004). Responses of selected rat kidney enzyme activities to administration of aqueous extract of Enantia chloranta. Nig. J. Pure and Appl. Sci 19:1592-1596.
3. Ahmed, B., Al-Hoiriny, T.A., and Siddigui, A.B. (2003). Antihepatotoxic activity of seeds of Chichorium intybus Journal of Ethnopharmacology.87:237-240
4. Ajibade, V. A and Famurewa, O (2011). Antibacterial activity of saponin and alkaloid extraction from whole plant of Phyllanthus niruri L. (Syn. P. franternus Webster) Pak. J. Sci Ind. Res. 54 (1): 47-52.
5. Ajibade, V.A., Adanlawo, I.G and Komolafe, O.D. (2006).Pharmacokinetic studies of saponin extracted from Phyllanthus niruri in rabbits with

gastroenteritis. Biomedical Research 17(1): 1725-1730.
6. Anupama T, Mani C and Rajest S (2011) A 28-days Sub-Acute Toxicity Study in some Albino mice to evaluate toxicity profile of Neurotol plus (Mannitol and Glycerol combination) International Journal of Biomedical Science 5(4):428-433.
7. Ambi A .A, Abdurrahmal E.M, Sule M.I, Patch U.U, Abdurrahman Y.R and Ibrahim N.D.G (2007) Phytochemical Screening and histopathological Studies on the seed of Colocynthis citrullus in Albino rats Nigeria Journal of Pharmaceutical Sciences 6 (2): 7-13
8. Aziza M.H., Wagdy K.B>K and Kawkab A.A (2008) Genetic and histopathology studies on mice; effect of Fenugreek oil on the efficiency of Ovarian and liver tissues African journal of Biotechnology 6(6) 477-483.
9. Barros, M. E., Schor, N and Boin, M. A. (2003) The effect of an aqueous extract from Phyllanthus niruri on calcium oxalate crystallization in vitro. Urol. Res. 30(6):367-373.
10. Dersert, K. C. (2007. Triterpenoid saponins from Acacia victoriae (Bentham) induce apoptosis by mitochondrial perturbation. Proceedings of the Nat Acad of Sci 98:103-107
11. Flaoven, A., Wilkins, A.L., Deng, D. and Brekket, T. (2001) Ovine metabolisms of saponin: evaluation of a method for estimating the ovine uptake of steroridal saponins from Narthecium ossifragnum. Veterinary Research Communications 25: L 225-238.
12. Francis, G.,Kerem, Z., Makkar, H.P.S and Becker, K. (2002). The biological action of saponins in animal system: a review. Br. J. of Nutri 88 (6): 587-605.
13. George, D. P and Roger, M. D .(2002). Encyclopaedia of Medicinal Plant 5th print of the original edition Spanish1.
14. Khanna, A.W., Rizvi, F and Chander, R. (2002). Lipid lowering activity of Phyllanthus niruri in hyperlipemic rats J. Ethnopharmacol, 82 (1): 19-22.
15. Lee, K.T., Sohn I.C., Kim, D.H., Choi, J.W and Kwon, S.H. (2000) Hypoglycaemic and hypolipidemic effects of tectorigenin and kaika-saponin III in the streptozotocin-induced diabetic rat and their antitoxidant activity in vitro. Arch Pharmacol. Res. 23:461-466.
16. Meagher, L.P., Smith, B.L and Wilkins, A.L. (2001). Metabolism of diosgenin-derived saponins: implications for hepatogenous photosensitization diseases in ruminants. Animal Feed Science and Technology 91:157-170.
17. Nishiura, J; Campus, A; Boim, M and Schor, N .(2005). Effect of Phyllanthus niruri on urinary calcium levels in calcium stone forming patients Journal of Clinical and Laboratory Investigation of Urolothies and Related Areas 32 (15):362-366.
18. Oda, K; Matsuda, H; Murakami, T; Katayama, S; Ohgifani, T. and Yoshikawa, M. (2000) Adjuvant and haemolytic activities of 47 saponins derived from medicinal and food plants. Biological Chemistry 381:67-74.
19. Otsuka, E. A. (2005). Analysis and isolation of saponin from plant materials. Saponin in food, feedstuffs and medicinal plants. Annual proceeding of the phytochemical society pg 1-12 (W. Oleszed and A. Mars ton, edition Oxford and London, Clarendon process.
20. Patel, P; Sweiss, K; Shatavi, S; Peace, D; Clark, N; Rondelli, D (2010). The impart of novel influenza A (H1N1) after hematopoietic SCT. Bone Marrow Transplant, J. Exp. Med. 45: 181-187.
21. Pooneh, R; Amir, S; Javad, A; Rosita, E; Mehran, A; Mohammadhossein, M; Saied, M; Kayhan, A (2010) Emergence of African swine fever virus, Northwestern Iran. 16(12): 1946-1949.
22. 22. Qudhia, P and Tripathi, R. S (2002) Prospect of cultivation of medicinal plant in Chattisgarh, Indian. P211-236. in: recent progress in medicinal plants. Vol 5, Crop Improvement, Production Technology, Trade and commerce. Sci Tech Public, U.S.A
23. Ray, S. M. D .(2007). The sapogenin of the seed of flour lupin species. Journal of Plant Food 3: 181-186.
24. Shim, I; Javaid, J.I and Kim, S.E. (2000). Effect of ginseng total saponin on extracellular dopamine released elicited by local infusion of nicotine into the striatum of freely moving rats. Planta medica 66:705-708.
25. Tabasum, N., Chatterrredi, S.,Agrawal, S. S and Ahmed, N. (2005). Hepatoprotective studies of Phyllanthus niruri on Paracetamol- induced liver cell damage in albino mice. J. Exp. Med. 12 (14): 211-213.
26. Unander, D. W., VenKateswaran, P. S., Mill man, I., Bryan, H. H and Blumberg, B. S. (1990). Phyllanthus species: Sources of new antiviral compounds. Advances in new crops. Timber Press, Portland, p518-521.
27. Yoshikawa, M; Murakami, T; Kishi, A; Kageura, T and Matsuda, H. (2001). Medicinal flowers 111, marigold (1): hypoglycaemic gastric emptying inhibitory, and gastroprotective principles and new oleanane-type triterpene oligoglycosides, calendasaponins A,B,C and D, from Egyptian Calendala officinalis, Chemical and Pharmacecitical Bullentin 49:863-870.
28. Zhong Yao Za Zhi. (2005). Antispasmodic saponins from bulbs of red onion Allium cepa (L.var.Tropea) J Agric Food Chem 53 (4): 935-940

This page is intentionally left blank

Global Journal of Medical research
Volume 12 Issue 1 Version 1.0 February 2012
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN : 0975-5888

A Comprehensive Insight into the Development of Animal Models for Obesity Research

By Amit Goyal, Anamika Gupta Dureja, Devinder Kumar Sharma & Kunal Dhiman

Abstract – Obesity, a multifactorial, metabolic disorder, involves complex interaction between genetic and environmental factors. With an alarming increase in the prevalence of obesity worldwide, it has become a major health care burden not just in terms of the increased risk of type 2 diabetes, cardiovascular morbidity, cholelithiasis, arthiritis and certain malignancies, but also in the economic costs to healthcare providers. The great similarity and homology between the genomes of rodents and humans make these animal models an important tool in unraveling the mechanisms involved in the etiology, prevention and treatment of obesity. This review summarizes the various approaches for the induction of obesity in the rodent models via genetic manipulation, hypercaloric diets and neuroendocrine perturbations.

Keywords : *Obesity; Animal models; Rodents; Hypercaloric diet; Genome.*

GJMR-G Classification : *NLMC Code: WD 210, W 20.55.A5*

Strictly as per the compliance and regulations of:

A Comprehensive Insight into the Development of Animal Models for Obesity Research.

Amit Goyal[α], Anamika Gupta Dureja[σ], Devinder Kumar Sharma[ρ] and Kunal Dhiman[ω]

Abstract - Obesity, a multifactorial, metabolic disorder, involves complex interaction between genetic and environmental factors. With an alarming increase in the prevalence of obesity worldwide, it has become a major health care burden not just in terms of the increased risk of type 2 diabetes, cardiovascular morbidity, cholelithiasis, arthiritis and certain malignancies, but also in the economic costs to healthcare providers. The great similarity and homology between the genomes of rodents and humans make these animal models an important tool in unraveling the mechanisms involved in the etiology, prevention and treatment of obesity. This review summarizes the various approaches for the induction of obesity in the rodent models via genetic manipulation, hypercaloric diets and neuroendocrine perturbations.

Keywords : Obesity; Animal models; Rodents; Hypercaloric diet; Genome.

I. INTRODUCTION

Obesity is a metabolic disorder characterized by an excessive accumulation of fat in the body to a sufficient magnitude which adversely affects the health of an individual. It is a direct consequence of perpetual imbalance between energy intake and expenditure with storage of extra calories in the form of triglycerides in the adipose tissue[1-2]. It is increasing probably, as a consequence of easily available hypercaloric diet and an increasingly sedentary lifestyle. Thus it can be appropriately termed as New World Syndrome or Disease of Civilization[3]. In obesity, there is an increase in intake of high fat and high energy food and a decrease in daily energy expenditure[4]. Diet and physical reduce appetite or to inhibit fat absorption[5]. However in exercise remain as main stay in obesity management; nonetheless antiobesity drugs may be required either to gastric balloon may be placed to reduce stomach volume and or bowel length, leading to earlier satiation and reduced ability to absorb nutrients from food[6-7].

Animal models of obesity not only allow us to investigate the basic mechanisms by which food intake is regulated but also act as tool for investigation of mechanism of antiobesity drugs. These models also provide significant insights into the etiology of human obesity consequently aiding in the development of pharmaceuticals for treatment of obesity. The animal models used for study of obesity either have a spontaneous origin or the result of experimental manipulation of the environmental or hypothalamic center that regulate food intake and energy balance or gene expression[8]. The rodent models are advantageous also in terms of their size, ease of handling, fast reproduction rate, shorter generation time availability of accurate and reliable metabolic tests[2,5,9]. This review summarizes the various approaches for induction of obesity in the rodent models via dietary manipulations, genetic interventions or neuroendocrinological perturbations.

II. DIET INDUCED MODELS

Diet induced obesity models are best suited and simplest obesity induction models and possibly the one that most closely resembles the reality of obesity in humans. Hypercaloric value diets varying between 3.7Kcal/g and 5.4Kcal/g have proved effective for induction of obesity[10]. The environmental conditions including temperature, duration of light and dark period, number of animals per cage or the feeding system used for the cages are important to develop these types of models[11]. Hence, to minimize the data variability, it is important to control the environmental conditions. Another factor that has to be considered is the age of the animals at which study is conducted. It is most effective to start high fat diet feeding at a young age, but it is also important to take into consideration that the energy balance differs in young compared to older animals. For example rats in their pubertal age rapidly gain lean mass and show completely different metabolic features compared to aged rats, which may in turn be losing lean mass and gaining fat mass. Another important variable is the duration of an obesity producing diet, that is the longer the feeding period, the greater the increment of bodyweight gain and presumably body fat.

a) High Fat Diet (HFD) Induced Models

Diet-induced obesity (DIO) has a late onset and is developed after feeding mice with high-fat diet which includes (powdered normal chow, 365 g; lard, 310 g; casein, 250 g; cholesterol, 10 g; vitamin and mineral mix, 60 g; dl-methionine, 03 g; yeast powder, 01 g; and NaCl, 01 g for 1.0 kg of diet)[12] for 10 weeks. Prolonged

Author α : Lecturer, Department of Pharmacology, Surya School of Pharmacy, Bapror, 140417, Punjab, India.
Phone :+919914100231 E-mail : goyal141186@yahoo.com.
Author σ, ρ, ω : Surya School of Pharmacy, Bapror, 140417, Punjab, India.

Exposure to HFD results in positive energy balance and obesity in certain rodent models that can be considered an adequate model of human obesity[13-14]. The male C57BL/6J mouse is the gold standard for a diet induce obese model. The C57BL/6J mouse develops obesity only when allowed ad libitum access to a high-fat diet whereas on a low-fat diet, C57BL/6J mice remain normal. In comparison to C57BL/6J, other strains such as the A/J mouse or the C57BL/KsJ are relatively resistant to these effects when fed a HFD[15]. The adipocyte hypertrophy and hyperplasia are responsible for obese phenotype in the C57BL/6J mouse. The outbred Sprague-Dawley rat is markedly more sensitive to HFD-induced obesity than other common rat strains. However, when outbred Sprague-Dawley rats are placed on a high-energy diet, only a subset of them overeat and develop diet induced obesity (DIO) whereas others remain lean (diet resistant, DR)[16-17]. The obesity prone subset of rats becomes obese, hyperphagic, hyperleptinemic, hyperinsulinemic, hyperglycemic, and hypertriglyceridemic[17]. The obese phenotype results from hyperphagia which is caused by an increase in meal size and increased energy efficiency[18]. The DIO rat displays a central resistance to circulating leptin indicating that reduced central leptin signaling may be involved in the etiology of hyperphagia in the obesity proneness. DIO rats exhibit a positive energy balance and a significantly higher respiratory quotient than DR rats, indicating a lower usage of fat as energy substrate. The physiological aspects of this model replicate many of the features observed with the human obesity syndrome: a polygenic mode of inheritance, a persistence of the phenotype once it is established, and dysregulated glucose homeostasis[19].

b) High Fructose Fed Animal Models

Fructose has several adverse metabolic effects, including hypertriglyceridemia, hyperinsulinemia and hypertension in rodents and induces moderate obesity[20]

The abnormalities and the disease progression in fructose fed rats resemble the human condition of metabolic syndrome, and are important risk factors for coronary heart disease. As to the metabolic mechanisms underlying the effects of dietary fructose the general notion is that hepatic intermediary metabolism is more affected by ingestion of fructose than of glucose. Fructose bypasses the phosphofructokinase regulatory step and enters the pathway of glycolysis or gluconeogenesis at the triose phosphate level, resulting in increased hepatic triglyceride production[21]. Recent findings also provide a novel hypothesis: Fructose raises uric acid, which in turn inhibits nitric oxide availability. Since insulin requires nitric oxide to stimulate glucose uptake it can be speculated that fructose-induced hyperuricemia may have a pathogenic role in promoting insulin resistance and metabolic syndrome[22].

c) Cafeteria Rats

The mixture of palatable commercially available supermarket food can act as a diet to stimulate energy intake in rodents. This diet is known as cafeteria diet, It is the combination of the high fat content with a high carbohydrate content[23]. These diets can be implicated in the development of obesity, leading to significant body weight gain, fat deposition and also insulin resistance[24]. It has been suggested that rats become more obese with cafeteria diets than with pure high fat diets, indicating a greater hyperphagia arising from the food variety, texture and palatability[25].

III. Venteromedial Hypothalamic (VMH) Nucleus Lesion

a) Monosodium Glutamate (MSG)

The ventromedial hypothalamic and arcuate nuclei is considered to be the area which controls the food intake and energy expenditure. The administration of monosodium glutamate to newborn rats is responsible for the destruction of the ventromedial hypothalamic and arcuate nuclei which leads to development of obesity. The subcutaneous or intraperitoneal route can be used for administration of MSG[26-27]. The dose that varies by 2-4 g/kg of body weight of the rat for 5 times every other day, during the neonatal period of rat causes obesity[28-31]. Overeating is not responsible for the obesity in neonatal MSG treated rodents. MSG obesity is associated with high level of corticosteroids[28]. The increase in the level of glucocorticoids is due to the chronic exposure of the adrenal gland to high serum levels of leptin, which occurs in rats treated with MSG[32-33].

b) Electrical VMH Lesion

The Electrical VMH lesion can be used to induce obesity. A bilateral destruction of hypothalamic nuclei, which leads to obesity can be caused by passing a current of 1.2 mA for 4 seconds, repeated thrice at an interval of 30-second each after adjusting the position of electrodes[34]. The stereotactic instruments can be used to cause injury with a single electrical current of 2.5 mA for 15 seconds by placing the tip of the rat nose 3.3 mm below the interaural line and positioning the tip of a stainless steel electrode 2.6 mm behind the bregma, 0.5-0.6 mm lateral to the midline and below the base of the brain and raised 0.5mm[35]. The irritative theory suggests that the hypothalamic nuclei gets destroyed due to the deposition of iron ions in the hypothalamus with the introduction of electrodes, the ablative theory is of the view that the cause of injury is electric current only. Studies were performed comparing electric injury with radiofrequency (without ion deposition) using the conventional technique and the results obtained were a lower index of obesity using radio frequency. Therefore, both mechanisms are involved in the development of obesity[36-39].

IV. Ovariectomy

The initial leptin level drops by the removal of gonads from female rats, which causes hyperphagia and marked weight gain. Seven weeks after ovariectomy, the leptin levels rise again reaching much higher levels that the preoperative ones. It is not known whether this increase is due to resistance to leptin, and could involve hypothalamic receptors[40-41]. More recent studies have tried to find changes in the expression of genes related to energy expenditure in ovariectomized rats to account for weight gain[42]. It appears that leptin and estradiol do not regulate themselves directly, because administration of these in intact female rats did not show that it altered either of them, and the reciprocal is true[43-44]. Therefore it is believed that there is a factor responsible for alerting the hypothalamus to the fact that estrogen production has ceased. A few studies speculate on the participation of neuropeptide Y. It appears to serve as a signal to the hypothalamus when the estrogen levels have dropped, since it would be raised after ovariectomy and would remain at the same levels if hormone replacement occurred in the female rats[41,45].

V. Genetic Models of Obesity

The genetic models of obesity are very useful and can be easily developed. The use of these models to study obesity increased in the 1990s because of cloning and identification of the product of five different genes causing obesity. Furthermore, it was discovered that by crossing quantitative trait loci (QTL) with known genes, i.e., obese phenotypes vs identified genotype, the influence of quantitative gene loci, and its penetrance in the quantity of body fat and its distribution[46-47].

a) Monogenic Model Of Obesity

The diabetic (db/db), obese(ob/ob), Tubby (tub), "Agouti" yellow(Ay) and fat (fat) were first five monogenic models of obesity. Over a century ago The "Agouti" rat was described for the first time and it was the first obesity gene to be cloned and characterized at the molecular level in1992. Agouti is expressed in adipose tissue as well as in several other tissues in humans, suggesting that it could be involved in regulating the energy balance. The over expression of agouti in adipose tissue, by genetic modification in rats, results in increased body weight than the non genetically modified ones, without any change in the amount of intake. This suggest that the increase of fatty mass in these rats could be the result of changes in the energy expenditure [46-47]. The gene of the obese rat (ob/ob) was cloned at the end of 1994, followed a year later by cloning the diabetic one (db/db). In experimental studies, it was found that a circulating factor of a normal rat or a db/db rat, when administered in an ob/ob rat, normalized its weight. But when this factor of a normal rat or an ob/ob was placed in a db/db rat, there was no weight change. These results strongly suggested that both genes were from the same metabolic pathway, and that db/db could be an ob/ob receptor, which was later proved. This factor was called leptin, i.e., a hormone produced by the ob/ob gene, that was responsible for communicating with the brain concerning the level of energy stored in adipose tissue in the form of fat[46-47].

b) Polygenic Models of Obesity

The body weight, adiposity and related metabolic traits shows significant variability in rodents, as with human beings[48].The high fat diet causes obesity and insulin resistance in inbred C57Bl/6J mice, but this strain remains non obese when fed on chow diet[49] Susceptibility to diet-induced obesity in this strain is polygenic and has been associated with hyperphagia and leptin resistance. AKR/J mice are also prone to diet induced obesity, however, unlike C57Bl/6J, AKR/J do not become hyperglycemic on a high-fat diet[50]. The SWR/J strain prefers carbohydrates and remains thin on high calorie diets[51]. Similarly, A/J mice are less prone to obesity[49]. As expected, these obesity-resistant mouse strains are less susceptible to hyperglycemia[49]. Obesity prone and resistant rats have also been bred in Sprague–Dawley and Fischer 344 genetic background[52] Together, these models facilitate the study of how diet and other environmental factors affect body weight, adiposity and metabolic disorders[53].

VI. Transgenic Models of Obesity

a) Pro-Opiomelanocortin and Melanocortin Receptor Knockouts

Adrenocorticotropin (ACTH), α, β- and γ melanocyte stimulating hormone (MSH), and the opioid β-endorphin are the Pro-opiomelanocortin (POMC) derived peptides. Leptin, a soluble hormone secreted from the adipocytes, acts on the POMC neurons of the hypothalamic arcuate nucleus[54-55]. The acute anorectic effects of leptin 4 appears due to the activation of POMC neurons and its activation might also be involved in the stimulation of metabolism by leptin[56]. Five melanocortin receptors have been cloned (MC1–5R). Two of these receptors (MC3R and MC4R) are expressed in the CNS in regions involved in energy homeostasis. The development of MC3R and MC4R knockouts help us to know the roles of the neurons expressing these receptors as there is no specific melanocortin receptor agonists. Melanocortin-4 receptor knockout (MC4RKO) mice exhibit the same phenotype observed in Ay/a mice, notably obesity, hyperphagia, increased longitudinal growth and in some cases the development of type 2 diabetes[56-57]. Mice in which the Pomc1 gene has been inactivated, exhibit obesity and hyperphagia and mice lacking a functional Mc3r gene also exhibit increased adiposity[58-59].

b) Neuropeptide Y Receptor Knockouts

Neuropeptide Y (NPY) is a 36-amino acid peptide neurotransmitter found in the brain. The genetic approaches to the study of the role of NPY in energy homeostasis have included knockouts of the NPY receptor genes Npy1r[60-61], Npy2r[62], and Npy5r[63]. It has been shown that there are at least six receptors for . Surprisingly, for the receptors of an orexigenic all of the NPY receptor knockout mice developed so far exhibit a mild late-onset obesity. The Npy1r knockout mouse exhibited a 27% increase in mature bodyweight in females. The Npy2r knockout mouse exhibited mild late-onset obesity in response to a HFD, th greater sensitivity in the females[63].The Npy5r knockout mouse exhibited mild obesity with increased adiposity and hyperphagia[64].

c) Peroxisome-Proliferator-Activated Receptors(PPAR)

The PPAR are members of the steroid/ thyroid/retinoid receptor superfamily that transactivate a variety of genes involved in the control of lipid metabolism65. The PPARα isoform is primarily expressed in liver, kidney, heart and skeletal muscle. The development of a mouse line with a disruption of the gene encoding PPARα has made it possible to determine the role of this receptor in vivo66. PPARα−/− mice develop late-onset obesity.

d) Steroid Receptor Knockouts

Estrogens are not just sex hormones, they play an important role in white adipose tissue regulation as estrogen replacement decreases white adipose tissue. ER knockouts exhibit an increase in white adipose tissue and reduced energy expenditure[67]. The ER knockout mice have helped to unravel the role of estrogen receptors in obesity[68]. Follicle stimulating hormone receptor is expressed on the granulose cells of the developing ovary[69]. Follicle stimulating hormone receptor knockouts (FORKO) retards ovarian development and causes chronic estrogen deficiency. Female FORKO develop obesity that is associated with an increased deposition of abdominal fat and that is reversed by estradiol treatment.

VII. β3-Adrenoreceptors

All three known subtypes of β-adrenergic receptors (β-ARs) are expressed in adipose tissue; however, the β3-AR appears to predominate in brown fat in the rodent. Mice lacking expression of the β3-AR (β3-KO) have normal body weight compared to WT mice, although they do exhibit modest increases in total body fat. Fed insulin and glucose levels and food intake are unchanged in the β3-KO mice; however, the increase in insulin and metabolic rate and decrease in glucose and food intake in response to a β3-AR agonist is eliminated. Compensatory mechanisms might operate in the β3-KO animals to maintain normal energy homeostasis and, in fact, the expression of β1-AR mRNA is upregulated in brown and white adipose tissue in these mice.

VIII. Conclusion

As the incidence of obesity is progressing at alarming rate worldwide, there is a great need for relevant experimental models to provide a better understanding of the pathophysiology of this epidemic to facilitate its therapy and prevention. The multifactorial etiology of obesity provides substantial alternatives for induction of obesity in the experimental models. The animal models may be grouped into genetic, dietary and neuroendocrine forms depending on the origin of the obesity. Although the ultimate model for this human malady is man himself; nevertheless these models provide in valuable insight into the physiological mechanisms that control the energy homeostasis. This information further catalysis the future prospective research for identification of potential therapeutic interventions for obesity. The key factor governing the choice of the model may be either environmental or genetic, depending on the characteristics to be targeted for research.

References Références Referencias

1. Spiegelman, B.M. and Flier, J.S. Obesity and the regulation of energy balance. Cell. 104:2001; 531-543.
2. Imai, Y and Ahima, R.A. Rodents as genetic models of obesity. Drug Discovery Today: Disease Models. 2:2005; 165-174.
3. Popkin, B.M. and Doak C. The obesity epidemic is a worldwide phenomenon. Nutr. Rev. 56:1998; 106-114.
4. Labib, M. The investigation and management of obesity. J. Clin. Pathol. 56:2003; 17–25.
5. Rosenthal, N and Brown, S. The mouse ascending: perspectives for human-disease models. Nature Cell Biology. 9:2007; 993-999.
6. NICE 2006 p.10–11.
7. Imaz, I.,et al.. Safety and effectiveness of the intragastric balloon for obesity. A meta-analysis. Obes Surg. 18:2008; 841–6.
8. York, D.A. Lessons from animal models of obesity. Endocrinology & Metabolism Clinics of North America. 25:1996; 781-800.
9. Aitman, et al. Progress and prospects in rat genetics: a community view. Nature Genetics. 40:2008; 516-522.
10. Diemen, V.V., et al. Experimental model to induce obesity in rats. Acta Cirurgica Brasileira. 21:2006; 425-429.
11. Reuter. T.Y. Diet-induced models for obesity and type 2 diabetes. Drug Discovery Today: Disease Models. 4:2007; 3-8.
12. Srinivasan, K., et al. Combination of high-fat diet-fed

and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol. Res. 52:2005; 313–320.
13. Gaiva, M.H., Couto, R.C., Oyama, L.M., Couto, G.E., Silveira, V.L., Riberio, E.B., and Nascimento, C.M. Polyunsaturated fatty acid-rich diets: effect on adipose tissue metabolism in rats. Br J Nutr. 86:2001; 371-7.
14. Lin, S., Thomas, T.C., Storlien, L.H., and Huang, X.F. Development of high fat diet induced obesity and leptin resistance in C57Bl/6J mice. Int J Obes Relat Metab Disord. 24:2000; 639-646.
15. Surwit, R.S., et al. Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. Metabolism. 44:1995; 645–651.
16. Sclafani, A., et al. Dietary obesity in adult rats: similarities to hypothalamic and human obesity syndromes. Physiol. Behav. 17:1976; 461–471.
17. Chang, S., et al. Metabolic differences between obesity-prone and obesity-resistant rats. Am. J. Physiol. 259:1990; R1103–R1110.
18. Farley, C., et al. Meal pattern analysis of diet-induced obesity in susceptible and resistant rats. Obes. Res. 11:2003;845–851.
19. Levin, B.E., et al. Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. Am. J. Physiol. 273:1997; R725–R730.
20. Zavaroni, I., et al. Effect of fructose feeding on insulin secretion and insulin action in the rat. Metabolism 29:1980; 970–973.
21. Park, O.J., et al. Mechanisms of fructose-induced hypertriglyceridemia in the rat. Biochem. J. 282:1992; 753–757.
22. Nakagawa, T., et al. A causal role to uric acid in fructose induced metabolic syndrome. Am. J. Physiol. Renal. Physiol. 2006:290; F625–F631.
23. Rothwell, N.J., et al. The cafeteria diets as a tool for studies of thermogenesis. J. Nutr. 118:1988; 925–928.
24. Prats, E., et al. Energy intake of rats fed a cafeteria diet. Physiol. Behav. 45:1989; 263–272.
25. Kretschmer, B.D., et al. Modulatory role of food, feeding regime and physical exercise on body weight and insulin resistance. Life Sci. 76:2005; 1553–1573.
26. Bunyan, J., Murrell, E.A., Shah, P.P. The induction of obesity in rodents by means of monosodium glutamate. Br J Nutr. 35:1976; 25-39.
27. Shivshankar, P., Devi, SC. Screening of stimulatory effects of dietary risk factors on mouse intestinal cell kinetics. World J Gastroenterol. 11:2005; 242-8.
28. Dolnikoff, M., et al. Decreased lipolysis and enhanced glycerol and glucose utilization by adipose tissue prior to development of obesity in monosodium glutamate (MSG) treated rats. Int J Obes Relat Metab Disord. 25: 2001; 426-433.
29. de Carvalho, P.P., Vargas, A.M., da Silva, J.L., Nunes, M.T., Machado, U.F. GLUT4 protein is differently modulated during development of obesity in monosodium glutamate-treated mice. Life Sci. 71:2002; 1917-28.
30. de Mello, M.A., de Souza, C.T, Braga, L.R., Santos, J.W., Ribeiro, I.A., Gobatto, C.A. Glucose tolerance and insulin action in monosodium glutamate (MSG) obese exercisetrained rats. Physiol Chem Phys Med NMR. 33:2001; 63-71.
31. de Souza, C.T., Nunes, W.M., Gobatto, C.A., de Mello, M.A. Insulin secretion in monosodium glutamate (MSG) obese rats submitted to aerobic exercise training. Physiol Chem Phys Med NMR. 35:2003; 43-53.
32. Tokuyama, K., Himms-Hagen, J. Adrenalectomy preventsobesity in glutamate-treated mice. Am J Physiol. 257:1989; E139-44.
33. Perello, M., Gaillard, R.C., Chisari, A., Spinedi, E., Adrenal enucleation in MSG-damaged hyperleptinemic male rats transiently restores adrenal sensitivity to leptin. Neuroendocrinology. 78:2003; 176-84.
34. Shimizu, Y., Yamazaki, M., Nakanish, K., Sakurai, M., Sanada, A., Takewaki, T., et al. Enhanced responses of the chorda tympani nerve to sugars in the ventromedial hypothalamic obese rat. J Neurophysiol. 90:2003;128-33.
35. Dube MG, Xu B, Kalra PS, Sninsky CA, Kalra SP. Disruption in neuropeptide Y and leptin signaling in obese ventromedial hypothalamic-lesioned rats. Brain Res. 816:1999; 38-46.
36. King, B.M., Frohman, L.A. Nonirritative lesions of VMH: effects on plasma insulin, obesity, and hyperreactivity. Am J Physiol. 248:1985; E669-75.
37. King, B.M., Frohman, L.A. Hypothalamic obesity: comparison of radio-frequency and electrolytic lesions in male and female rats. Brain Res Bull. 17:1986; 409-13.
38. King, B.M., Daigrepont, P.M., Michel, R.E., Zansler, C.A., Ahmed, J.I., Walker, A., et al. Hypothalamic obesity: comparison of radio-frequency and electrolytic lesions in weanling rats. Physiol Behav. 45:1989; 127-32.
39. King, B.M. Ventromedial hypothalamic obesity: a reexamination of the irritative hypothesis. Neurosci Biobehav Rev. 15:1991; 341-7.
40. Chu, S.C., Chou, Y.C., Liu, J.Y., Chen, C.H., Shyu, J.C., Chou, F.P. Fluctuation of serum leptin level in rats after ovariectomy and the influence of estrogen supplement. Life Sci. 64:1999; 2299-306.
41. Meli, R., Pacilio, M., Raso, G.M., Esposito, E., Coppola. A., Nasti, A., et al. Estrogen and raloxifene modulate leptin and its receptor in hypothalamus and adipose tissue from ovariectomized rats. Endocrinology. 145:2004; 3115-21.

42. Shimizu, H., Ohtani, K., Kato, Y., Tanaka, Y., Mori, M. Withdrawal of estrogen increases hypothalamic neuropeptide Y (NPY) mRNA expression in ovariectomized obese rat. Neurosci Lett. 204:1996; 81-84.
43. Pelleymounter, M.A., Baker, M.B., McCaleb, M. Does estradiol mediate leptin's effects on adiposity and body weight? Am J Physiol. 276:1999; E955-63.
44. Shimomura, K., Shimizu, H., Tsuchiya, T., Abe, Y., Uehara, Y., Mori, M. Is leptin a key factor which develops obesity by ovariectomy? Endocrinol J. 49:2002; 417-23.
45. Ainslie, D.A, Morris, M.J., Wittert, G., Turnbull, H., Proietto, J., Thorburn, A.W. Estrogen deficiency causes central leptin insensitivity and increased hypothalamic neuropeptide Y. Int J Obes Relat Metab Disord. 25(11):2001; 1680-8.
46. Chagnon, Y.C., Bouchard, C. Genetics of obesity: advances from rodents studies. TGI. 12: 1996.
47. Marques-Lopes, I., Marti, A., Moreno-Aliaga, M.J., Martinez, A. Aspectos geneticos da obesidade. Rev Nutr. 17:2004; 327-38.
48. Barsh, G.S., et al. Genetics of body-weight regulation. Nature 404:2000; 644–651.
49. West, D.B., et al. Dietary obesity in nine inbred mouse strains. Am. J. Physiol. 262:1992; R1025–R1032.
50. Rossmeisl, M., et al. Variation in type 2 diabetes-related traits in mouse strains susceptible to diet-induced obesity. Diabetes. 52: 2003; 1958–1966.
51. Smith, B.K., et al. Increased flavor preference and lick activity for sucrose and corn oil in SWR/J vs. AKR/J mice. Am. J. Physiol. Regul. Integr. Comp. Physiol. 281:2001; R596–R606.
52. Schemmel, R., et al. Dietary obesity in rats: body weight and body fat accretion in seven strains of rats. J. Nutr. 100:1970; 1041–1048.
53. Bell, C.G., et al. The genetics of human obesity. Nat. Rev. 6:2005; 221–234.
54. Leibel, R.L., et al. The molecular genetics of rodent single gene obesities. J. Biol. Chem. 272:1997: 31937–31940.
55. Cowley, M.A. et al. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. Nature 411:2001; 480–484.
56. Marie, L.S. et al. A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. Proc. Natl.Acad. Sci.U. S.A. 97:2000; 12339–12344.
57. Huszar, D. et al. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell 88:1997; 131–141.
58. Butler, A.A. et al. (2001) Melanocortin-4 receptor is required for acute homeostatic responses to increased dietary fat. Nat. Neurosci. 4:2001; 605–611.
59. Chen, A.S. et al. Inactivation of the mouse melanocortin-3 receptor results in increased fat mass and reduced lean body mass. Nat. Genet. 26:2000; 97–102.
60. Kushi, A. et al. Obesity and mild hyperinsulinemia found in neuropeptide Y-Y1 receptor-deficient mice. Proc. Natl.Acad. Sci.U. S.A. 1998:95; 15659–15664.
61. Pedrazzini,T. et al. Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. Nat. Med. 4:1998; 722–726.
62. Naveilhan, p, et al. Normal feeding behavior body weight and leptin response require the neuropeptide Y,Y2 receptor Nat. Med. 5, 1188-1193.
63. Marsh, D.J. et al. Role of the Y5 neuropeptide Y receptor in feeding and obesity. Nat. Med. 4:1998; 718–721.
64. Blomqvist, A.G. et al. Y-receptor subtypes – how many more? Trends Neurosci. 20:1997; 294–298.
65. Desvergne, B. et al. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocrinol. Rev. 20:1999; 649–688.
66. Lee, S.S.T. et al. Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. Mol. Cell. Biol. 15:1995; 3012–3022.
67. Heine, P.A. et al. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. Proc. Natl. Acad. Sci. U S A. 97: 2000; 12729–12734.
68. Stefan O M, and Kenneth S K. Estrogen receptors and endocrine diseases: lessons from estrogen receptor knockout mice. Anxiety and depression. 1:2001; 613-619.
69. Robker RL, Richards JS 1998 Hormonal control of the cell cycle in ovarian cells: proliferation versus differentiation. Biol Reprod 59:1998; 476–482.

Global Journals Inc. (US) Guidelines Handbook 2012

www.GlobalJournals.org

FELLOW OF INTERNATIONAL CONGRESS OF MEDICAL RESEARCH (FICMR)

- 'FICMR' title will be awarded to the person/institution after approval of Editor-in-Chief and Editorial Board. The title 'FICMR' can be added to name in the following manner:
  e.g. **Dr. Andrew Knoll, Ph.D., FICMR**

  Dr. Jhon Petter,M.D.,FICMR

- FICMR can submit two papers every year for publication without any charges. The paper will be sent to two peer reviewers. The paper will be published after the acceptance of peer reviewers and Editorial Board.
- Free unlimited Web-space will be allotted to 'FICMR 'along with subDomain to contribute and partake in our activities.
- A **professional email address** will be allotted free with unlimited email space.
- FICMR will be authorized to receive e-Journals -GJMR for the Lifetime.
- FICMR will be exempted from the registration fees of Seminar/Symposium/Conference/Workshop conducted internationally of GJMR (FREE of Charge).
- FICMR will be Honorable Guest of any gathering held.

ASSOCIATE OF INTERNATIONAL CONGRESS OF MEDICAL RESEARCH (AICMR)

- AICMR title will be awarded to the person/institution after approval of Editor-in-Chef and Editorial Board. The title 'AICMR' can be added to name in the following manner:
  eg. **Dr. Thomas Herry, Ph.D., AICMR**
- AICMR can submit one paper every year for publication without any charges. The paper will be sent to two peer reviewers. The paper will be published after the acceptance of peer reviewers and Editorial Board.
- Free 2GB Web-space will be allotted to 'FICMR' along with subDomain to contribute and participate in our activities.
- A professional email address will be allotted with free 1GB email space.

AUXILIARY MEMBERSHIPS

ANNUAL MEMBER

- Annual Member will be authorized to receive e-Journal GJMR for one year (subscription for one year).
- The member will be allotted free 1 GB Web-space along with subDomain to contribute and participate in our activities.
- A professional email address will be allotted free 500 MB email space.

PAPER PUBLICATION

- The members can publish paper once. The paper will be sent to two-peer reviewer. The paper will be published after the acceptance of peer reviewers and Editorial Board.

Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.Online Submission: There are three ways to submit your paper:

(A) (I) First, register yourself using top right corner of Home page then Login. If you are already registered, then login using your username and password.

(II) Choose corresponding Journal.

(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.

(B) If you are using Internet Explorer, then Direct Submission through Homepage is also available.

(C) If these two are not convenient, and then email the paper directly to dean@globaljournals.org.

Offline Submission: Author can send the typed form of paper by Post. However, online submission should be preferred.

Preferred Author Guidelines

MANUSCRIPT STYLE INSTRUCTION (Must be strictly followed)

Page Size: 8.27" X 11'"

- Left Margin: 0.65
- Right Margin: 0.65
- Top Margin: 0.75
- Bottom Margin: 0.75
- Font type of all text should be Swis 721 Lt BT.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, "Abstract" word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be three lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
- Numbering of Second Main Headings (Heading 2) must be in Alphabets, Italic, and Font Size of 10.

You can use your own standard format also.
Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

1. GENERAL

Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

Scope

The Global Journals Inc. (US) welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals Inc. (US) is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global

Journals Inc. (US) are being abstracted and indexed (in process) by most of the reputed organizations. Topics of only narrow interest will not be accepted unless they have wider potential or consequences.

2. ETHICAL GUIDELINES

Authors should follow the ethical guidelines as mentioned below for publication of research paper and research activities.

Papers are accepted on strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. If the paper once accepted by Global Journals Inc. (US) and Editorial Board, will become the copyright of the Global Journals Inc. (US).

Authorship: The authors and coauthors should have active contribution to conception design, analysis and interpretation of findings. They should critically review the contents and drafting of the paper. All should approve the final version of the paper before submission

The Global Journals Inc. (US) follows the definition of authorship set up by the Global Academy of Research and Development. According to the Global Academy of R&D authorship, criteria must be based on:

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the findings.

2) Drafting the paper and revising it critically regarding important academic content.

3) Final approval of the version of the paper to be published.

All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

Acknowledgements: Contributors to the research other than authors credited should be mentioned under acknowledgement. The specifications of the source of funding for the research if appropriate can be included. Suppliers of resources may be mentioned along with address.

Appeal of Decision: The Editorial Board's decision on publication of the paper is final and cannot be appealed elsewhere.

Permissions: It is the author's responsibility to have prior permission if all or parts of earlier published illustrations are used in this paper.

Please mention proper reference and appropriate acknowledgements wherever expected.

If all or parts of previously published illustrations are used, permission must be taken from the copyright holder concerned. It is the author's responsibility to take these in writing.

Approval for reproduction/modification of any information (including figures and tables) published elsewhere must be obtained by the authors/copyright holders before submission of the manuscript. Contributors (Authors) are responsible for any copyright fee involved.

3. SUBMISSION OF MANUSCRIPTS

Manuscripts should be uploaded via this online submission page. The online submission is most efficient method for submission of papers, as it enables rapid distribution of manuscripts and consequently speeds up the review procedure. It also enables authors to know the status of their own manuscripts by emailing us. Complete instructions for submitting a paper is available below.

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

To avoid postal delays, all transaction is preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

Complete support for both authors and co-author is provided.

4. MANUSCRIPT'S CATEGORY

Based on potential and nature, the manuscript can be categorized under the following heads:

Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5. STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also.Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

Papers: These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)Title should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, "Abstract" (less than 150 words) containing the major results and conclusions.

(c) Up to ten keywords, that precisely identifies the paper's subject, purpose, and focus.

(d) An Introduction, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; conclusions should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and to make suggestions to improve briefness.

It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

Structure

All manuscripts submitted to Global Journals Inc. (US), ought to include:

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals Inc. (US) have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Key Words

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the Harvard scheme of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals Inc. (US) homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals Inc. (US) recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals Inc. (US) recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

Color Charges: It is the rule of the Global Journals Inc. (US) for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals Inc. (US).

6.1 Proof Corrections

The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded

(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

As changes to proofs are costly, we inquire that you only correct typesetting errors. All illustrations are retained by the publisher. Please note that the authors are responsible for all statements made in their work, including changes made by the copy editor.

6.2 Early View of Global Journals Inc. (US) (Publication Prior to Print)

The Global Journals Inc. (US) are enclosed by our publishing's Early View service. Early View articles are complete full-text articles sent in advance of their publication. Early View articles are absolute and final. They have been completely reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after sending them. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the conventional way.

6.3 Author Services

Online production tracking is available for your article through Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The authors will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript.

6.4 Author Material Archive Policy

Please note that if not specifically requested, publisher will dispose off hardcopy & electronic information submitted, after the two months of publication. If you require the return of any information submitted, please inform the Editorial Board or dean as soon as possible.

6.5 Offprint and Extra Copies

A PDF offprint of the online-published article will be provided free of charge to the related author, and may be distributed according to the Publisher's terms and conditions. Additional paper offprint may be ordered by emailing us at: editor@globaljournals.org .

the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to Computer Science and Information Technology. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

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.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

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.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

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.

18. **Pick a good study spot:** To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

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

sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

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.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- 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

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

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

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

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.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to

shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- 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:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- 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
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- 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:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.
- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic

principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- 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.

Results:

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.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

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.

- 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

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- 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.

Administration Rules Listed Before Submitting Your Research Paper to Global Journals Inc. (US)

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals Inc. (US):

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.

- Do not give permission to anyone else to "PROOFREAD" your manuscript.

- Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

Criterion for Grading a Research Paper (Compilation) by Global Journals Inc. (US)

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals Inc. (US).

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

INDEX

A

antimicrobial · 31
azathioprine · 5, 6, 15, 17, 18

B

bilirubin · 5, 6, 10, 11, 12, 16, 17, 20
biochemical · 18, 32, 36
biomedical · 2

C

calcineurin · 5, 14, 15, 17, 18, 20
Calcineurin · 5, 15, 16, 19, 20
carcinogenic · 36
cardiovascular · 6, 16, 19, 39
cholesterol · 5, 6, 8, 9, 15, 17, 31, 35, 39
chronic · 18, 19, 40, 42
Corticosteroids · 5, 15
cyclosporine · 5, 6, 14, 15, 16, 17, 18, 19, 20
cytokine · 21, 22
Cytological · 1, 2, 3, 4
cytopathology · 3
Cytoplasmic · 25

E

epidemiological · 21, 23

G

gastrointestinal · 5, 14, 17
Genetics · 20, 21, 23, 42, XLV
glucose · 5, 6, 12, 13, 16, 20, 31, 33, 35, 36, 40, 42, 43
glycosides · 31

H

hemodynamics · 5
hepatocellular · 5, 16, 24
hirsutism · 5, 14, 17
histopathological · 1, 2, 3, 31, 35, 37
Histopathological · 3, 31, 32, 33, 34, 35, 36, 37, 38
homozygotes · 22, 23
Hormonal · XLVII
Hypercaloric · 39
hyperinsulinemic · 40
hyperplasias · 35
Hypertriglyceridemia · 15
hypothalamic · 40

I

immunity · 3
immunological · 2, 36
immunosuppressive · 5, 15, 17, 18, 20
Immunosuppressive · 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20

L

lepromatous · 2
Leptin · 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 41, XLV
lymphadenitis · 1, 2, 3
Lymphadenitis · 1, 2, 3, 4

M

melanocortin · 41, XLV, XLVI
methionine · 39
microbiological · 3
microscopy · 3
mofetil · 5, 6, 15, 17, 18, 19, 20

N

necrotic · 1, 2
neoplastic · 1
nephrotoxicity · 5, 6, 14, 15, 17, 18, 19
neuropsychiatric · 6
neurotoxic · 5
nutrients · 35, 39

O

Obesity · 23, 39, 40, 41, 42, 43, 44, XLVII
observations · 36
osteoporosis · 6
Ovariectomy · 41

P

parameters · 6, 7, 10, 32, 33, 35, 36
pathogenesis · 20, 21, 36
pathophysiology · 18, 42
phenotype · 40, 41
Phyllanthus · 31, 32, 33, 34, 35, 36, 37, 38
polymorphism · 21, 22, 23, 24, 25, 26, 28, 29
Polymorphism · 21, 22, 23, 24, 25, 26, 27, 28, 29, 30
prednisolone · 5, 6, 19
protein · 14, 21, 22, 23, 24, 25, 31, 33, 34, 35, 36, 43

S

saponin · 31, 32, 33, 34, 35, 36, 37, 38
saponins · 31, 35, 36, 37
sirolimus · 20
sodium · 18

T

tacrolimus · 5, 6, 15, 16, 17, 18, 19, 20
tetracycline · 14
toxic · 5, 16, 31, 32
Toxicity · 32, 37
Toxicological · 31, 32, 33, 34, 35, 36, 37, 38
tremor · 14, 17
triglyceride · 5, 6, 8, 9, 15, 17, 40
Tubercular · 1, 2, 3, 4
tuberculosis · 1, 2, 3

Global Journal of Medical Research

Visit us on the Web at www.GlobalJournals.org | www.MedicalResearchJournal.org
or email us at helpdesk@globaljournals.org

ISSN 09755888